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FINAL REPORT

Pubertal Toxicity Study of Vinclozolin, Flutamide and Phenobarbital in Male Sprague Dawley Rats and Methoxychlor, Ethinyl Estradiol and Phenobarbital in Female Sprague Dawley Rats when Administered in Corn Oil by Oral Gavage

Sponsor
National Toxicology Program (NTP)
National Institute of Environmental Health Sciences (NIEHS)
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Research Triangle Park, North Carolina 27709

Sponsor Study Numbers

Pubertal Vinclozolin Study:	RACB 20104
Pubertal Flutamide Study:	RACB 20105
Pubertal Phenobarbital Study:	RACB 20203
Pubertal Methoxychlor Study:	RACB 20103
Pubertal Ethinyl Estradiol Study:	RACB 20106

Testing Facility
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TherImmune Study Number: 7244-600

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Study Completion Date
May 28, 2004

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COMPLIANCE STATEMENT

Pubertal Toxicity Study of Vinclozolin, Flutamide and Phenobarbital in Male Sprague Dawley Rats and Methoxychlor, Ethinyl Estradiol and Phenobarbital in Female Sprague Dawley Rats when Administered in Corn Oil by Oral Gavage

This study was conducted according to a modification of the Health Effects Test Guidelines OPPTS 870.3800 Reproduction and Fertility Effects and in compliance with U.S. Food and Drug Administration (FDA) Good Laboratory Practice (GLP) Regulations for Nonclinical Laboratory Studies as set forth in Title 21 of the U.S. Code of Federal Regulations Part 58 with the following exceptions:

- The F0 Gestation Day 0 body weights, which were recorded by the breeder.
- The Feed Analysis, performed by PMI Nutrition International.
- Original vaginal cytology data analyses were misplaced and the slides were re-read.

Portions of the study performed by subcontractors were performed according to the protocol and GLP compliance was verified by their Quality Assurance Units (QAU).

The protocol, amendment, and deviations are presented in Appendix 18. There were no deviations from the aforementioned regulations that affected the quality or integrity of the study or the interpretation of the results in the report.

Study Director:

Gary W. Wolfe 5/28/04

Gary W. Wolfe, PhD, DABT Date

QUALITY ASSURANCE STATEMENT

Pubertal Toxicity Study of Vinclozolin, Flutamide and Phenobarbital in Male Sprague Dawley Rats and Methoxychlor, Ethinyl Estradiol and Phenobarbital in Female Sprague Dawley Rats when Administered in Corn Oil by Oral Gavage

This study, 7244-600 entitled "Pubertal Toxicity Study of Vinclozolin, Flutamide and Phenobarbital in Male Sprague Dawley Rats and Methoxychlor, Ethinyl Estradiol and Phenobarbital in Female Sprague Dawley Rats when Administered in Corn Oil by Oral Gavage" was inspected/audited by Quality Assurance in accordance with TherImmune Research Corporation Standard Operating Procedures, Health Effects Test Guidelines and FDA Good Laboratory Practice regulations. All findings were reported to the Study Director and Testing Facility Management.

		Date Reported	
Type of Audit	Date(s) Audited	Study Director	Management
Protocol Audit	August 19, 26, 2002	August 26, 2002	August 26, 2002
Dose Administration	December 19, 2002	December 20, 2002	December 20, 2002
Draft Final Report & Raw Data	January 6-9, 12-13, 15-16 & 18-19, 2004	January 19, 2004	January 19, 2004
Final Report Post Audit	May 17-18, 2004	May 19, 2004	May 19, 2004

Action has been taken in response to all items listed by Quality Assurance. It is concluded that the final report accurately reflects the TherImmune Research Corporation's Standard Operating Procedures and the raw data for this study.

Pathology Associates, A Division of Charles River, performed the histopathology evaluation of this study under the purview of their QAU. The respective appendix contains the corresponding quality assurance statement.

Taconic Anmed performed the serology analysis of this study under the purview of their QAU. The respective appendix contains the corresponding quality assurance statement.

Midwest Research Institute performed the bulk chemical analysis of the Phenobarbital and Ethinyl Estradiol for this study under the purview of their QAU. Additionally, They also performed the test article analysis of the Phenobarbital and Ethinyl Estradiol for this study under the purview of their QAU. The respective appendixes contain the corresponding quality assurance statements.

Battelle performed the bulk chemical analysis of the Vinclozolin and Flutamide for this study under the purview of their QAU. Additionally, They also performed the test article analysis of the Vinclozolin and Flutamide for this study under the purview of their QAU. The respective appendixes contain the corresponding quality assurance statements.

RTI International performed the bulk chemical analysis of the Methoxychlor for this study under the purview of their QAU. Additionally, They also performed the test article analysis of the Methoxychlor for this study under the purview of their QAU. The respective appendixes contain the corresponding quality assurance statements.

Richard Hughes, Jr.
Quality Assurance Auditor

5-28-04
Date

SIGNATURE PAGE

Pubertal Toxicity Study of Vinclozolin, Flutamide and Phenobarbital in Male Sprague Dawley Rats and Methoxychlor, Ethinyl Estradiol and Phenobarbital in Female Sprague Dawley Rats when Administered in Corn Oil by Oral Gavage

This report was prepared and reviewed by:

Gary W. Wolfe — 5/28/04

Study Director
Gary W. Wolfe, PhD, DABT — Date

Rita V. Patel — 5/28/04

Toxicologist
Rita V. Patel, MS — Date

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Eias A. Zahalka — 5/28/04

Director of Toxicology
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Sponsor Review:

Jack B. Bishop — 5/25/04

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SUMMARY

Pubertal Toxicity Study of Vinclozolin, Flutamide and Phenobarbital in Male Sprague Dawley Rats and Methoxychlor, Ethinyl Estradiol and Phenobarbital in Female Sprague Dawley Rats when Administered in Corn Oil by Oral Gavage

The purpose of this study was to examine the sensitivity of the pubertal assay to an estrogen (Methoxychlor), anti-androgen (Vinclozolin and Flutamide), and a thyroid-active agent (Phenobarbital) in intact juvenile/peripubertal male and female rats. Fifty-five time-mated female (F_0) Sprague Dawley Rats were received from Charles River Laboratories and allowed to litter at TherImmune. After weaning on Postnatal Day (PND) 21, 135 F_1 males and 135 F_1 females were assigned to male and female cohort studies, respectively. The F_1 males were dosed with corn oil (0 mg/kg), or the test articles: Phenobarbital (25, 50, and 100 mg/kg/day), Vinclozolin (10, 30, and 100 mg/kg/day), or Flutamide (25 and 50 mg/kg/day), once daily via oral gavage from PND 23-53/54. The F_1 females were dosed with corn oil (0 mg/kg), or the test articles: Ethinyl Estradiol (0.0025 and 0.0050 mg/kg/day), Methoxychlor (12.5, 25, and 50 mg/kg/day), or Phenobarbital (25, 50 and 100 mg/kg/day), once daily via oral gavage from PND 22-42/43.

Parameters evaluated include mortality and clinical observations, body weights, day of preputial separation, day of vaginal opening, estrous cyclicity, gross pathology, organ weights and histopathology.

The following parameters were affected by treatment:

Males

Test Article	Concentration (mg/kg/day)	Body Weight	Age of Preputial Separation	Organ Weights	Histology
Phenobarbital	25	-	-	↑ liver	-
	50	-	-	↑ liver	-
	100	↓	↑	↑ liver ↓pituitary	-
Vinclozolin	10	-	↑	-	-
	30	-	↑	↓repro. organs ↑ testis,	-
	100	-	↑	↓repro. organs ↑ testis	-
Flutamide	25	-	↑	↓repro. organs ↑ testis, ↓ kidneys	-
	50	-	↑	↓repro. organs ↑ testis, ↓ kidneys, ↑adrenal glands	testis lesions

- = no effect; ↑ = higher than control means; ↓ = lower than control means
repro. = reproductive

Females

Test Article	Concentration (mg/kg/day)	Age at Vaginal Opening	Age at First Estrus	Irregular Estrous Cycles	Organ Weights
Phenobarbital	25	-	-	-	↑ liver ↑ adrenal
	50	-	-	-	↑ liver ↑ adrenal
	100	↑	-	-	↑ liver ↑ adrenal ↓ thyroid
Methoxychlor	12.5	↓	↓	-	-
	25	↓	↓	X	-
	50	↓	↓	X	↓ liver ↓ ovary ↓ pituitary
Ethinyl Estradiol	0.0025	-	-	-	↑ adrenal
	0.005	↓	↓	X	↑ adrenal

- = no effect; ↑ = higher than control means; ↓ = lower than control means; X =present
repro. = reproductive

The lowest observable effect level (LOEL) for an endocrine effect was 25 mg/kg/day for Flutamide, 10 mg/kg/day for Vinclozolin, 100 mg/kg/day for Phenobarbital, 12.5 mg/kg/day for Methoxychlor, and 0.005 mg/kg/day for Ethinyl Estradiol.

STUDY PERSONNEL AND TEST SITES

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Chemicals:	TherImmune Research Corporation 18761 North Frederick Avenue, Suite A Gaithersburg, MD 20879

STUDY TIMETABLE

Study Initiation Date:	August 13, 2002
Experimental Start Date: [Gestation Day 1 Body weights]	October 30, 2002
Receipt of Animals:	November 7, 2002
Randomization of F_1 Animals:	December 12 and 13, 2002
First Day of Dosing:	
F_1 Females:	December 13, 2002
F_1 Males:	December 14, 2002
Last Day of Dosing:	
F_1 Females:	January 3, 2003
F_1 Males:	January 14, 2003
Necropsy:	
F_1 Females:	January 2 and 3, 2003
F_1 Males:	January 13 and 14, 2003
Experimental Completion Date: [Histology Analysis]	May 27, 2004

INTRODUCTION

Proposed Investigations/Rationale for Dose Selection

The purpose of this study was to obtain data on proposed procedures and to evaluate the lowest observable effect level of Vinclozolin, Methoxychlor, Flutamide, Ethinyl Estradiol, and Phenobarbital on pubertal development in the intact juvenile/peripubertal male and female rats. Puberty is a time of rapid interactive endocrine and morphological changes, and numerous pharmaceutical and environmental agents have been shown to alter the timing of pubertal development in mammals. This study was designed to detect agents that have antithyroid, estrogenic, androgenic, or antiandrogenic activity, or alter puberty by changing follicle stimulating hormone (FSH), luteinizing hormone (LH), prolactin, growth hormone (GH), or hypothalamic function (Stoker *et al*., 2000 and Goldman *et al.*, 2000). The Sprague Dawley rat was selected as the test system due to its established quality as a breeder and the availability of historical control data for reference.

Vinclozolin, 3-(3,5-dichlorophenyl)-5-methyl-5-vinylox-azolidine-2,4-dione, is a fungicide used on fruits, vegetables, turfgrass, and ornamental plants (U.S. EPA, 1998). *In vivo*, Vinclozolin inhibits androgen receptor (AR)-dependent gene expression (Kelce *et al*., 1997) and produces a spectrum of anatomical defects. Administration of Vinclozolin (400 mg/kg) to rats on Gestation Day (GD) 14 through PND 3 resulted in effects similar to those caused by Flutamide, a well-known AR antagonist. These effects included reduced anogenital distance (AGD); persistent nipples; cleft phallus; hypospadias; reduced weights of the ventral prostate, seminal vesicles, and epididymis; and, reduced sperm count (Gray *et al*., 1994; 1999a). Exposing weanling male rats to the antiandrogenic pesticides p,p'- DDE or Vinclozolin delayed pubertal development as indicated by delayed preputial separation and increased body weight at puberty. In contrast, to delays associated with exposure to estrogenic substances, antiandrogens do not inhibit food consumption or retard growth (Anderson *et al*., 1995b).

Flutamide (4'-nitro-3'-trifluoromethyl-isobutyranilide) is a potent non-steroidal androgen-receptor antagonist that has been used therapeutically to treat androgen-dependent prostrate cancer (Delaere and Van Thillo, 1991; Murphy *et al*., 1991) and as a tool to study male reproductive development. Studies in rats have demonstrated that pre- or postnatal Flutamide (6.25 to 50 mg/kg) exposure alters androgen-dependent reproductive development (Imperato-McGinley *et al.*, 1992; Kassim *et al*., 1997) and has been shown to produce decreased reproductive organ weights, feminization of male external genitalia, altered androgen-dependent testicular descent, and retention of nipples when male offspring are exposed *in utero* (Imperato-McGinley *et al*., 1992).

Methoxychlor has been used for nearly 50 years for insect and larval control. Its advantage over DDT is that the Methoxychlor is more readily metabolized and excreted by mammalian systems (Kapoor *et al*., 1970) and is therefore less likely to undergo bioconcentration than is DDT. This metabolism also yields mono- and bis-hydroxy metabolites of Methoxychlor (Bulger *et al*., 1978), which helps explain both the uterotrophic effects noted earlier for Methoxychlor (Tullner, 1961) and the observations that Methoxychlor *in vivo* reduced the uterine uptake of radiolabeled estradiol (Welch *et al*., 1969). Treatment of rats with Methoxychlor at 5, 50, or 150 mg/kg for the week before and the week after birth until PND 7 resulted in unchanged anogenital distance, accelerated vaginal opening, and delayed prepuce separation; at 50 and 150 mg/kg, it disrupted adult estrous cyclicity and reduced epididymal sperm counts and testis weights.

As cited in Goodman and Gilman's Pharmacological Basis of Therapeutics (1996), estrogens are among the most commonly prescribed drugs in the United States. The two major uses are as components of combination oral contraceptives and hormone replacement therapy. The pharmacological considerations for estrogen use in oral contraceptives, as opposed to postmenopausal hormone replacement, are substantially different, primarily because of the doses used. Historically, conjugated estrogens have been the most common agents for postmenopausal use, and 625 µg/kg/day is effective in most women. In contrast, most combination oral contraceptives in current use employ 20 to 35 µg/day of Ethinyl Estradiol. Conjugated estrogens

and Ethinyl Estradiol differ widely in their oral potencies; for example, a dose of 625 µg of conjugated estrogens generally is considered equivalent to 5 to 10 µg of Ethinyl Estradiol.

Several authors have demonstrated estrogenic responses to Ethinyl Estradiol in rodents. Laws *et al.* (2000) showed that *in vivo* studies indicated the 3-day uterotrophic assay in prepubertal rats was best for detecting estrogenic activity when compared with all other models, based upon the dose-response data for Ethinyl Estradiol (0.01-0.1 mg/kg, oral), 4-tert-octylphenol (50-200 mg/kg, oral), and 4-nonylphenol (25-100 mg/kg, oral). Although oral doses of Ethinyl Estradiol (0.01 mg/kg) induced a significant increase in uterine weight in the prepubertal rat, this dose was ineffective for stimulating a similar response in ovariectomized adult rats. The age of vaginal opening was advanced following oral exposure from postnatal days 21-35 to Ethinyl Estradiol (0.01 mg/kg). Ethinyl Estradiol advanced the day of vaginal opening by 6.0 ± 0.18 days (30.6 days in control vs. 24.6 days in treated groups). In addition, the number of 4-5 day estrous cycles was reduced during a 25-day exposure to Ethinyl Estradiol (0.01 mg/kg). Advanced vaginal opening was also demonstrated by Odum *et al*. (1997) using doses of 2-400 µg/kg/day, and Singh and Kamboj (1980) using doses of 5 µg/kg/day for 5 days. Singh and Kamboj (1980) also showed an advance in the appearance of cornified vaginal cells.

Phenobarbital is a commonly prescribed antiepileptic agent whose hepatotoxicity and effects on the thyroid have been established. Endpoints of reproductive and developmental toxicity have not been as well explored, although some data are available. Gupta *et al*. (1980) evaluated the reproductive and developmental toxicity of Phenobarbital in male rats. Males were exposed *in utero* from GD 12-19 (40 mg/kg/day administered to the dam). Treatment-related effects included reduced anogenital distance; delayed testes descent; decreased seminal vesicles weight; and reduced fertility. Both serum testosterone and luteinizing hormone were decreased in the exposed males. However, the age at onset of puberty (i.e., age at preputial separation) was not affected by Phenobarbital exposure. In a similar study (Gupta and Yaffe, 1981), female rats were exposed to Phenobarbital *in utero* (40 mg/kg/day administered to the dam) or immediately following birth (20 mg/kg/day on PND 1-8). Phenobarbital treatment resulted in delayed vaginal

opening (34.6 ± 1.2 days in the control vs. 37.5 ± 1.2 days in the treated group); disruptions in the estrous cycle (only 40% of treated rats displayed normal estrous cyclicity, compared to 91% of control rats); and impaired fertility (50% of treated rats were fertile, and 100% of the control rats were fertile). These effects were observed during critical periods for neuroendocrine development: GD 17-20 and PND 1-8 (Gupta and Yaffe, 1981).

Based on the above-cited literature, the hypotheses of the current study were:

1. Ethinyl Estradiol and Methoxychlor administration to juvenile female rats will result in advanced vaginal opening, advanced first estrous and onset of estrous cycles, and/or persistent vaginal estrus

2. Administration of Vinclozolin and Flutamide to juvenile male rats will result in delayed preputial separation, decreased reproductive organ weights, altered external genitalia, and/or retention of nipples

3. Phenobarbital treatment will result in delayed vaginal opening and irregular estrous cyclicity in juvenile female rats, reduced reproductive organ weights, and possibly delayed preputial separation in juvenile males. Phenobarbital treatment may also cause increased thyroid weights and follicular cell hyperplasia, particularly in the males.

METHODS AND MATERIALS

TEST AND CONTROL ARTICLES

Neat Materials

The neat test and control articles used in this study are described in Text Table 1A and 1B.

Text Table 1A: Neat Test and Control Articles

Name	Lot No.	Supplier	Purity	Date Received
Corn Oil	N/A	TherImmune	100%	N/A
Vinclozolin	102996	Battelle	100.1%	07/12/2001
Flutamide	109H0952	Battelle	99.3%	07/12/2001
Methoxychlor	124F0575	Research Triangle Institute	95%	07/12/2001
Ethinyl Estradiol	45H0716	Midwest Research Institute	99.7%	07/13/2001
Phenobarbital	Q10645	Midwest Research Institute	100%	07/18/2002

Information on the methods of synthesis and stability, data on the composition, and other characteristics that define the test articles are on file with the Sponsor. Test article analyses are presented in Appendix 1.

Text Table 1B: Neat Test and Control Articles- Storage Condition

Name	Lot No.	Storage Condition
Corn Oil	N/A	Room temperature protected from light
Vinclozolin	102996	Room temperature protected from light
Flutamide	109H0952	Room temperature protected from light
Methoxychlor	124F0575	Room temperature protected from light
Ethinyl Estradiol	45H0716	Room temperature protected from light
Phenobarbital	Q10645	Room temperature protected from light

Two 5 g reserve samples of each bulk test article were taken prior to use on the study, and stored at –20 °C for possible future re-analysis. One 5 g sample of each bulk test article was taken within 30 days of the receipt, and sent on dry ice to the appropriate NTP subcontractor for purity and stability testing. Samples of the bulk test articles and their formulations were sent for analysis to the following NTP subcontractors: Flutamide and Vinclozolin were sent to Battelle, Columbus, Ohio, Ethinyl Estradiol and Phenobarbital were sent to MRI, Kansas City, Missouri, and Methoxychlor was sent to RTI International, Research Triangle Park, North Carolina.

Prior to the start of the study, the dose-formulation stability study for each test article was conducted by the Sponsor. The Vinclozolin dose formulation (2.0 mg/mL in corn oil) was stable for 42 days at 25°C, 5°C, and -20°C. The Methoxychlor dose formulation (1.82 mg/mL in corn oil) was stable for 30 days under refrigerated conditions (2 to 5°C) and for 23 days under ambient (23 to 28°C) conditions. The Flutamide dose formulation (10 mg/mL in corn oil) was stable for 42 days at 5°C or –20°C, with –20°C being preferable. The Ethinyl Estradiol dose formulation (1.0 µg/mL in corn oil) was stable for up to 14 days under refrigerated conditions (approximately 5°C). The Phenobarbital dose formulation (2.5 mg/mL in corn oil) was stable for up to 14 days (refrigerated at approximately 5°C). All formulations were stored in sealed amber glass bottles and protected from light. Under conditions that simulate animal dosing (room temperature, exposed to air and light), the dosage formulations showed no appreciable loss.

Dose Formulations

Dose formulations of Vinclozolin, Methoxychlor, Ethinyl Estradiol, Flutamide, and Phenobarbital were prepared every ten days. For each dose level, an appropriate amount of test article was accurately weighed into a pre-calibrated beaker using a stainless steel spatula. A sufficient quantity of corn oil was added to achieve the desired final volume and formulation was mixed using a magnetic stir bar for at least 10 minutes and sonicated, if necessary, to ensure complete dissolution. A table of test articles, concentrations, dose levels and group numbers is given in Text Tables 3A and 3B. Formulations were dispensed in daily aliquots that were stored

in amber glass bottles with Teflon®-coated lids, and kept protected from light in a refrigerator set to maintain 2-8 °C until used for dosing. Formulations were stirred prior to and during dosing. Formulation procedures are presented in Appendix 3.

Since this was conducted as a "blind" study, the vials containing the daily aliquots were not labeled explicitly with the test article name and concentration. Instead, the group number, mix number and a one-letter code were used to uniquely identify each vial. This prevented the animal room technicians from knowing the identity of the dosing compound. Group identification was assigned separately for males and females.

Dosage Analysis

Every time a new mix was prepared, three 35 mL archival samples from each dose level of each test article were collected and stored at TherImmune, protected from light in a refrigerator at 2-8°C. One set of samples was forwarded on ice packs to the NTP subcontractors for dose concentration analysis at the following intervals: initial, middle, and final formulations (Mix 1, 2, and 4). Samples of the corn oil were also forwarded for analysis. Additionally, 35 mL homogeneity samples were collected from the top, middle, and bottom of the low and high dose formulations for each test article from mix 1. Samples were stored in a refrigerator at 2-8°C, protected from light and forwarded to the NTP subcontractors on ice packs for analysis. Analytical methods are described in the dose formulation concentration analysis reports presented in Appendix 2.

TEST ANIMALS AND HUSBANDRY

F_0 Animals

Fifty-five time-mated female (F_0) Sprague Dawley Rats (Crl: CD® (SD) IGS BR) were received (9 to 10 weeks old) from Charles River Laboratories, Raleigh, North Carolina, on November 7, 2002 (GD 8). The animals were received with unique tail identification numbers. For the purpose of this study, the day of mating was considered as GD 0, and the day of littering was

considered as PND 0. For all animals, the day of positive mating was October 30, 2002 (GD 0). Throughout the study, the F_0 dams were identified by tail mark and cage label, and housed one per cage. GD 0 body weights (between 200 and 225 g) were recorded and provided by the supplier. Animals were acclimated to laboratory conditions for two weeks and released from quarantine by a staff veterinarian. During the acclimation period, one dam was sent to Taconic Anmed (Rockville, Maryland) for serological testing. The results of theses tests are presented in Appendix 14. F_0 dams were never exposed to the test articles.

F_1 Animals

The litters (F_1 animals) delivered on November 21, 2002 were selected for the juvenile male and female cohort studies. On PND 4, the selected litters were culled to 10 pups/litter, using computer-generated random numbers. The targeted litter size after culling was 5 males and 5 females. When less than 5 pups/sex were available in a litter, additional pups from the opposite sex (same litter) were selected to achieve a litter size of 10 pups. Culled pups were euthanized and discarded without necropsy on PND 4. Throughout the lactation period, the pups were not individually identified. At weaning on PND 21, 135 males and 135 females were selected for the study, identified individually by tail tattoo and cage label, and housed 3 per cage (sexes separated) for the remainder of the study. Animals not selected on PND 21 were euthanized and discarded without necropsy on PND 24.

TherImmune's Institutional Animal Care and Use Committee (IACUC) approved this protocol and found it to be in accordance with provisions of the USDA Animal Welfare Act, the PHS Policy on Humane Care and Use of Laboratory Animals and the US Interagency Research Animal Committee Principles for the Utilization and Care of Research Animals.

Husbandry

Animal husbandry was provided as described in Text Table 2.

Feed and water were provided *ad libitum*, unless otherwise noted. The feed was analyzed by the vendor for concentrations of nutrients, heavy metals, aflatoxins, nitrosamines, chlorinated hydrocarbons, organophosphates, PCBs, nitrates, nitrites, BHA, BHT, total bacterial plates, genistein, daidzein, glycite, metabolizable energy content, coliforms, *E coli* and *Salmonella*. Results of this analysis are presented in Appendix 15. A 71g sample of feed was collected from the same lot number used in the study and stored at room temperature at TherImmune, for possible future analysis. Water and bedding analyses/certification requirements are on file at TherImmune. No contaminants were known to be present in the diet, water, or bedding at levels that might have interfered with achieving the objectives of the study.

Environmental controls were set to maintain animal room conditions as shown in Text Table 2. Actual temperature and relative humidity in the animal room were monitored continuously by a computerized system and manually recorded at least once daily. All environmental parameters were maintained within the protocol requirements except for deviations detailed in Appendix 18. While humidity was frequently outside the protocol specified range (40-50%) it was within the acceptable range (30-70%) specified in the *Guide for the Care and Use of Laboratory Animals* (National Academic Press, 1996) and did not influence the health of the animals and/or the outcome of the study.

Text Table 2: Husbandry

Feed	Purina Certified 5002, Lot no. 082802 3B
Water[a]	Filtered tap water via an automatic watering system.
Bedding[b]	Sani-Chip® Certified Hardwood Bedding, autoclaved prior to use
Caging	Polycarbonate cages, 19" x 10½" x 8"
Racks	Stainless steel racks
Animals Per Cage	One (F_0 dams), 3 (F_1 animals)
Temperature Range	Set to maintain 22 ± 2 °C or 68 to 75.2 °F
Humidity Range	Set to maintain 40 to 50%
Light Cycle[c]	14-hour light/10-hour dark
Air Changes	Minimum of 10 air changes per hour

a = The water was routinely analyzed for contaminants and specific microbes.
b = The bedding was analyzed by the manufacturer for acceptable levels of heavy metals, aflatoxins, bacteria, yeasts, molds, and organophosphates prior to certification.
c = Light cycles were interrupted when necessary for study related events.

EXPERIMENTAL DESIGN

Group Assignment and Doses

Animals were assigned to groups as shown in Text Table 3A and 3B.

Text Table 3A: Study Design, F_1 Males

Group	Treatment	Test Article Dosage (mg/kg/day)	Test Article Concentration (mg/mL)	Males	
				N	Animal Numbers
1	Corn Oil	0	0	15	9026-9040
2	Phenobarbital	25	10	15	9041-9055
3	Phenobarbital	50	20	15	9056-9070
4	Phenobarbital	100	40	15	9071-9085
5	Vinclozolin	10	4	15	9086-9100
6	Vinclozolin	30	12	15	9101-9115
7	Vinclozolin	100	40	15	9116-9130
8	Flutamide	25	10	15	9131-9145
9	Flutamide	50	20	15	9146-9160

N = number of animals per group

Text Table 3B: Study Design, F_1 Females

Group	Treatment	Test Article Dosage (mg/kg/day)	Test Article Concentration (mg/mL)	Females	
				N	Animal Numbers
1	Corn Oil	0	0	15	9161-9175
2	Ethinyl Estradiol	0.0025	0.001	15	9176-9190
3	Ethinyl Estradiol	0.005	0.002	15	9191-9205
4	Methoxychlor	12.5	5	15	9206-9220
5	Methoxychlor	25	10	15	9221-9235
6	Methoxychlor	50	20	15	9236-9250
7	Phenobarbital	25	10	15	9251-9265
8	Phenobarbital	50	20	15	9266-9280
9	Phenobarbital	100	40	15	9281-9295

N = number of animals per group

Animals were accepted into the randomization pool based on PND 21 body weights and physical examinations. The following procedure was performed separately for males and females: after

collecting PND 21 body weights, the weanlings were assigned to 15 different blocks (9 animals/block), ranging from the heaviest to the lightest (block number 1 being the heaviest). Using random card draw, the weanlings were then assigned to study groups, such that each group received exactly one pup from each of the 15 weight blocks. At randomization the mean body weight for each group was not statistically different from the control mean. During the randomization process, each study animal was assigned a unique number and identified by tail tattoo and cage label. Randomization was performed on PND 21 for females and PND 22 for males.

Dose Administration

Animals were given the appropriate dose formulation as shown in Text Table 4.

Text Table 4: Dose Administration

Route of Administration	Oral Gavage
Days Dosed	Males: SD 23 through termination (PND 53-54) Females: SD 22 through termination (PND 42-43)
Dose Volume	2.5 mL/kg/day, based on most recent body weight
Equipment	1-2 inches, stainless steel, 18 gauge, gavage needle with a 2.25 mm ball, and 1 cc syringe
Test Article Conditions	Formulations were removed from the refrigerator at least 30 min prior to dosing. Formulations were stirred continuously during dosing.

Dosing was performed each day between 7:00 and 10:00 AM, with one exception: female dosing ended at 10:37 AM on PND 42.

OBSERVATIONS

F_0 dams were observed twice daily for signs of mortality and general health. Body weights were collected at littering and on PND 21.

In addition, on PND 0, 4, 7, 14 and 21, the litters were observed for the following: number of live pups, number of dead pups, number of males, total body weights of males, number of females, and total body weights of females. Individual pup body weights were collected on PND 4, 7, 14 and 21, although the pups were not uniquely identified until PND 21.

Following randomization, F_1 animals were observed as shown in Text Table 5.

Cage-side observations included observation for mortality, moribundity, general health and signs of toxicity. Clinical observations included evaluation of skin and fur characteristics, eye and mucous membranes, respiratory, circulatory, autonomic and central nervous systems, and somatomotor and behavior patterns.

Text Table 5: F_1 Animal Observations

Procedure	Frequency of Testing	
	F_1 Males	**F_1 Females**
Cage-side Observations	≥ 2 Daily	≥ 2 Daily
Clinical Observations	At randomization, on PND 23 and weekly thereafter	At randomization, on PND 22 and weekly thereafter
Body Weights	At randomization, on PND 23 and daily thereafter	At randomization, on PND 22 and daily thereafter
Preputial Separation	Daily, starting on PND 23 until complete separation or termination, whichever came first	NA
Vaginal Opening	NA	Daily, starting on PND 22 until complete opening
Estrous Cyclicity	NA	Starting on the day of vaginal opening, daily until termination

Note: A partial preputial separation was recorded, however, the day of complete separation was the endpoint used in the analysis for the age of preputial separation.

TERMINATION, NECROPSY AND HISTOPATHOLOGY

Termination

F_0 dams that did not litter, and the dams that delivered on November 20, 2002 and their litters, were euthanized and discarded without necropsy. Remaining F_0 females were terminated without necropsy on PND 21. F_1 pups culled on PND 4 were terminated without necropsy. F_0 dams were euthanized by carbon dioxide inhalation and the pups were euthanized by sodium pentobarbital injection. F_1 animals not selected for study on PND 21 were terminated by carbon dioxide inhalation on PND 24 and discarded without necropsy. Treated F_1 males and females were terminated on PND 53-54 or PND 42-43, respectively, by carbon dioxide inhalation and exsanguination.

Necropsy

Scheduled necropsies were conducted under the supervision of a veterinary pathologist. A gross necropsy, which included examination of the external surface of the body, all orifices, the cranial, thoracic, and abdominal cavities, and their contents, was performed. One 100 mg/kg/day Flutamide-treated male was found dead on PND 24; a gross necropsy was performed and protocol specified tissues were collected, but organs weights were not collected.

For all F_1 males that survived to the terminal necropsy, the following organs were weighed as soon as possible after dissection: adrenal glands (paired), epididymides (right and paired weights), kidneys (paired), levator ani plus bulbocavernosus muscles, liver, pituitary, seminal vesicles plus coagulating glands (with and without fluid), testes (paired), thyroid and parathyroid (post-fixation), ventral and dorsal-lateral prostate (separately). After weighing, the following tissues were placed in Bouin's fixative and transferred to 70% ethanol within 24-48 hours: adrenal glands (paired), epididymides (paired), kidneys (paired), liver, pituitary, testes (paired), gross lesions. Thyroid and parathyroid (with attached portion of the trachea) were fixed in 10% neutral buffered formalin, then the attached portion of trachea was removed and the thyroid/parathyroid weights were collected.

For all F_1 females, the following organs were weighed as soon as possible after dissection: adrenal glands (paired), kidneys (paired), liver, ovaries (paired), pituitary, thyroid and parathyroid (post-fixation, as described for males), uterus and cervix (with and without luminal fluid). Small tissues, as well as tissues that contained fluid, were weighed immediately to prevent partial drying prior to weighing. After weighing, the following tissues were placed in Bouin's fixative and transferred to 70% ethanol within 24-48 hours: adrenal glands (paired), kidneys (paired), liver, ovaries (paired), pituitary, uterus and cervix, gross lesions.

Histopathology

Preserved tissues were transferred to PAI, Frederick, Maryland. The following tissues were embedded in paraffin, sectioned, stained with hematoxylin and eosin, and examined microscopically by the study pathologist: thyroid, testes, epididymides, and gross lesions from all males, as well as the ovaries, uterus, cervix, thyroid and gross lesions from all females. The methods are described in the Pathology Report, presented in Appendix 17.

STATISTICAL ANALYSES

Data from the treated F_1 animals were analyzed by Analytical Sciences Inc. (Durham, North Carolina).

All endpoints (age and weight at vaginal opening/preputial separation, body weights, estrous cycle length, and organ weights at necropsy) with the exception of a few related to vaginal cytology (number of cycling females and number of females with regular cycles) were analyzed using ANOVA or ANCOVA to determine if there was a dose effect. For endpoints that were analyzed with an ANOVA, Levene's test was used to assess whether the assumption of equal variance across dose groups was tenable. For those endpoints where the Levene's test was rejected at the 0.1 level, no p-values for the overall ANOVA are reported.

Organ weights were analyzed using ANCOVA with terminal body weight as the covariate. In fitting the ANCOVA models, appropriate statistical tests were carried to ascertain the validity of

a constant slope for each endpoint. The hypothesis of constant slope was rejected only in a handful of cases (ovaries, uterus and cervix without fluid for ethynil estradiol and pituary for vinclozolin). However, caution should be used in interpreting the results from the ANCOVA model given that the covariate (terminal body weight) could be impacted by the dose treatment.

Animals in each treated group were compared with animals in the control group using appropriate multiple comparison procedures. The Dunnett's (1955) test was used for all endpoints that were analyzed with an ANCOVA model or an ANOVA models in which the assumption of homogeneity of variance was not rejected. When the Levene's test was rejected at the 0.1 level, Dunn's test (nonparametric) for multiple comparisons with a control was used. For number of cycling females and number of females with regular cycles, multiple comparisons with the control were performed using an unadjusted chi-square test.

RECORD RETENTION

All original study records, including all original data sheets, and original final report will be archived by the TherImmune. Preserved tissues, blocks, slides, and paper data associated with histological processing will be maintained at the Archive facility at PAI. Copies of the final report and computer printouts generated in the statistical analysis will be forwarded to the contracting agency, the N.I.E.H.S., Research Triangle Park, North Carolina. Study data generated by the Sponsor will be archived by the Sponsor. Data generated by analytical subcontractors will be archived by the subcontractors.

RESULTS

DOSE FORMULATION RESULTS

Bulk Test Article

The results of bulk test article analyses are presented in Appendix 1 and summarized in Text Table 6.

Text Table 6: Bulk Test Article Analyses

Test Article	Average Purity (%)
Vinclozolin	100.1
Flutamide	99.3
Phenobarbital	99.4
Methoxychlor	99.2
Ethinyl Estradiol	98.8

FORMULATION ANALYSES

The results of formulation analyses are presented in Appendix 2.

All analyses of Flutamide and Methoxychlor formulations were within ± 10% of the target concentrations. Out of specification results are presented in Text Table 7.

Text Table 7: Formulation Analyses

Test Article	Target Concentration (mg/mL)	Mix 1	Mix 2	Mix 3	Mix 4
Vinclozolin	4	126.6%	-	-	-
	12	113.2	-	-	-
Phenobarbital	10	-	-	87.0%	-
	20	-	82.8%	-	-
	40	-	-	80.3	77.2
Ethinyl Estradiol	0.001	< LOQ	350.0%	-	183.8
	0.002	45.0%	332.5%	111.2%	112.1%

LOQ = Limit Of Quantification; - = Within Specifications or Analysis Not Required.

The high and low concentrations of all dose formulations were homogeneous with the exception of Ethinyl Estradiol. Ethinyl estradiol analysis was a problem throughout the study. Results varied from below the limit of quantification to 350% of target. As the same mixing procedure was used each time and the target concentrations were very close to the limits of detection of assay, in spite of the variable results we believe the test article was properly formulated.

DETERMINATION OF PEROXIDE IN CORN OIL.

The average peroxide value in the corn oil was 2.45 Meq, which was within the acceptable range (< 5Meq).

F_0 GENERATION RESULTS

Of the 55 F_0 females received for this study, 53 of them had viable litters, one was used for serological testing, and one did not litter. Littering took place over a two-day period, as follows: 18 litters were delivered on November 20, 2002 and 35 litters were delivered on November 21, 2002. The dam that did not litter plus the dams that delivered on November 20,2002 and their litters were terminated on PND 5. Dams littering on November 21, 2002 were allowed to raise their litters to PND 21. On PND 4, these litters were culled to 10 pups/litter, with an equal number of males and females wherever possible. On PND 21, 135 male and 135 female weanlings were selected for the F_1 generation study. F_0 dams were terminated on December 12, 2002. Data recorded during the lactation period are presented in Appendices 4-6.

F_1 GENERATION RESULTS

All findings described in this report as "increased" or "decreased" were statistically significant as compared to the control group.

MORTALITY AND CLINICAL OBSERVATIONS

Data are presented in Table 1 and Appendix 7.

Treatment with Vinclozolin, Flutamide, Methoxychlor or Ethinyl Estradiol had no effect on mortality or clinical observations.

One 100 mg/kg/day Phenobarbital-treated male was found dead on PND 24 (the second day of treatment). Treatment with Phenobarbital affected clinical observations in the 100 mg/kg/day dose group males and females for the first few days of treatment. Minimal to severe ataxia was recorded between PND 23-26 in all surviving males and females, prostration was noted in 3 males and 8 females on PND 23-24, and languid behavior was noted in one male on PND 24.

BODY WEIGHT AND BODY WEIGHT GAINS

Data are presented in Tables 2 and 3 and in Appendix 8.

Males

Male body weight data are presented in Figures 1-3.

Treatment with 100 mg/kg/day Phenobarbital affected male body weights. Although males gained weight daily except on SD 54, body weight gains were lower than controls resulting in significantly lower mean body weights on SD 32, 34, 37, 38, and 40-54 (11.3% lower than control on PND 54).

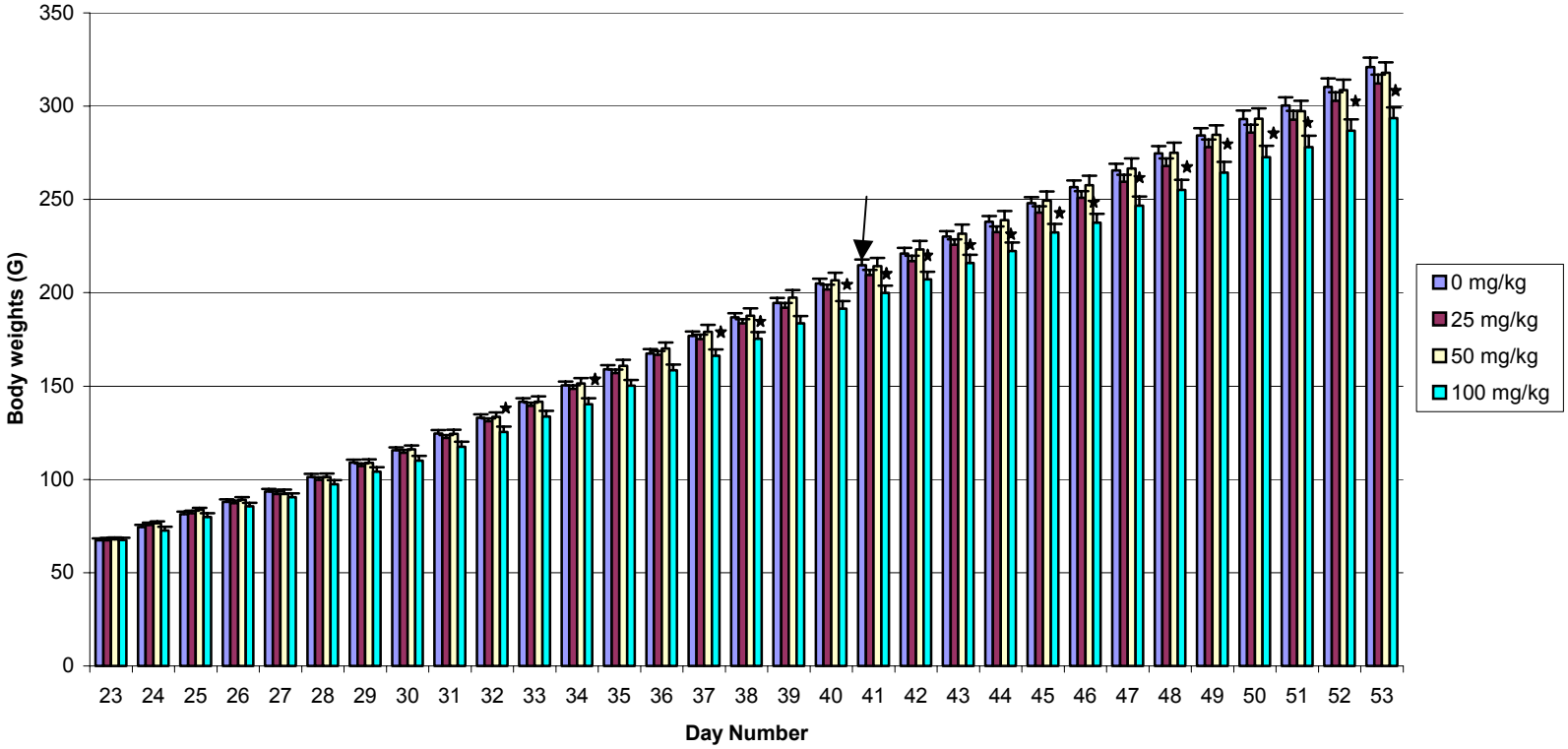
Treatment with Vinclozolin, Flutamide, 25 mg/kg/day Phenobarbital, or 50 mg/kg/day Phenobarbital had no effect on mean body weight. There were significant differences in mean body weight gain among groups, but not consistent enough in magnitude or direction to affect mean body weights and not considered to be test-article related.

Females

Female body weight data are presented in Figures 4-6.

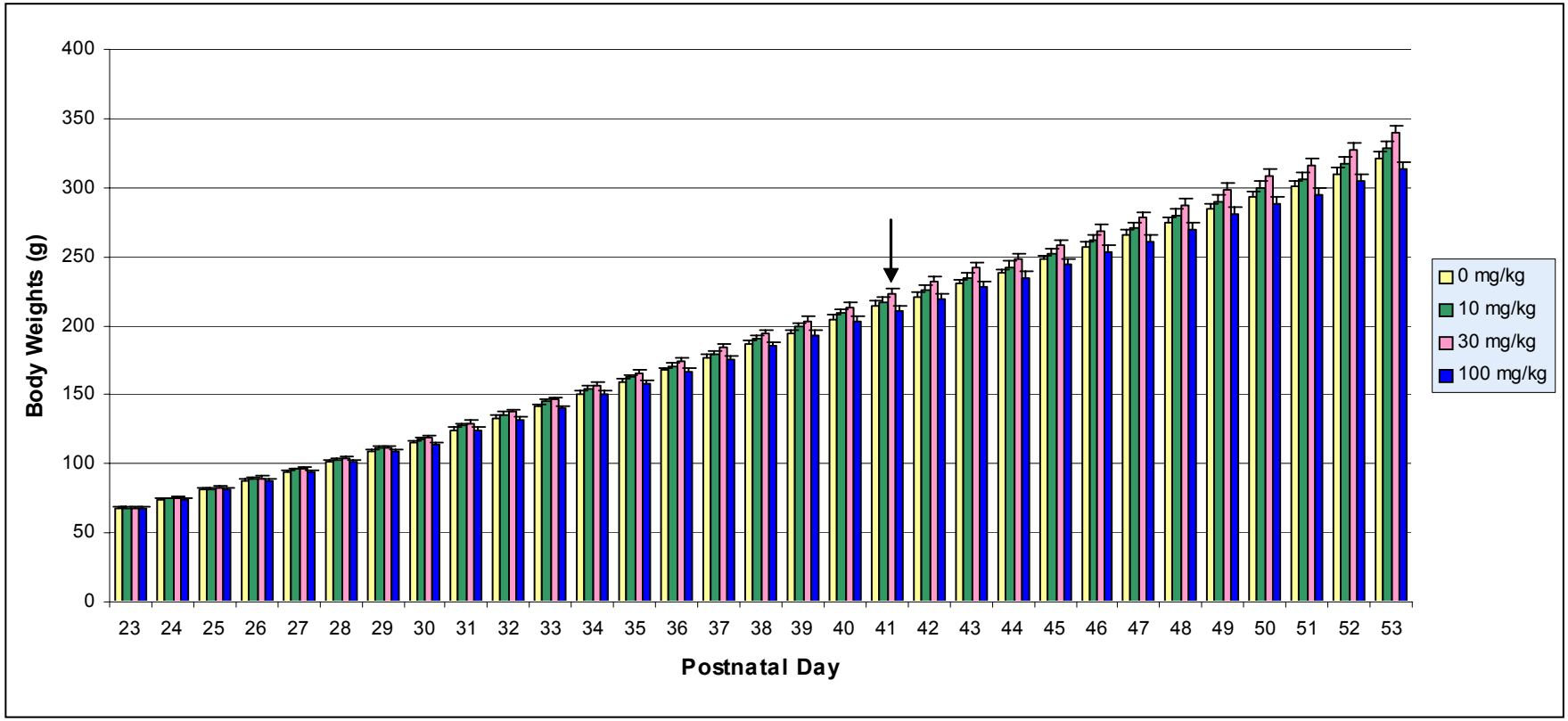
Treatment with Ethinyl Estradiol, Methoxychlor, or Phenobarbital had no effect on female body weights. No consistent or meaningful changes were observed in the body weight gains of the treated females as compared to the control females.

Figure 1: Phenobarbital- Male Body Weights



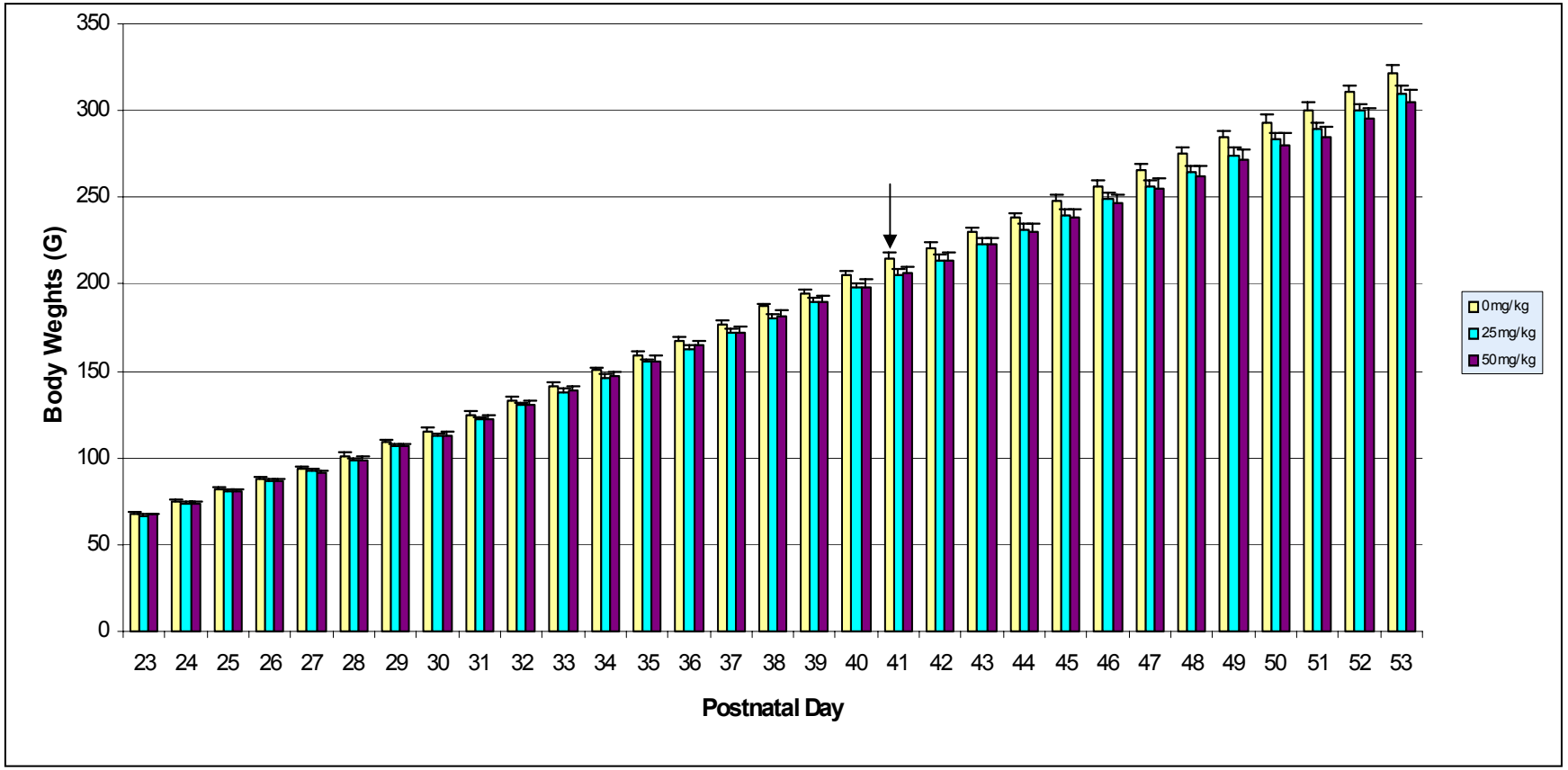
= Mean age of control animals at preputial separation.
Statistical Significance for comparison of dosed groups to control (* = P<0.05, **=P<0.01) using Dunnett's Test

Figure 2: Vinclozolin – Male Body Weights



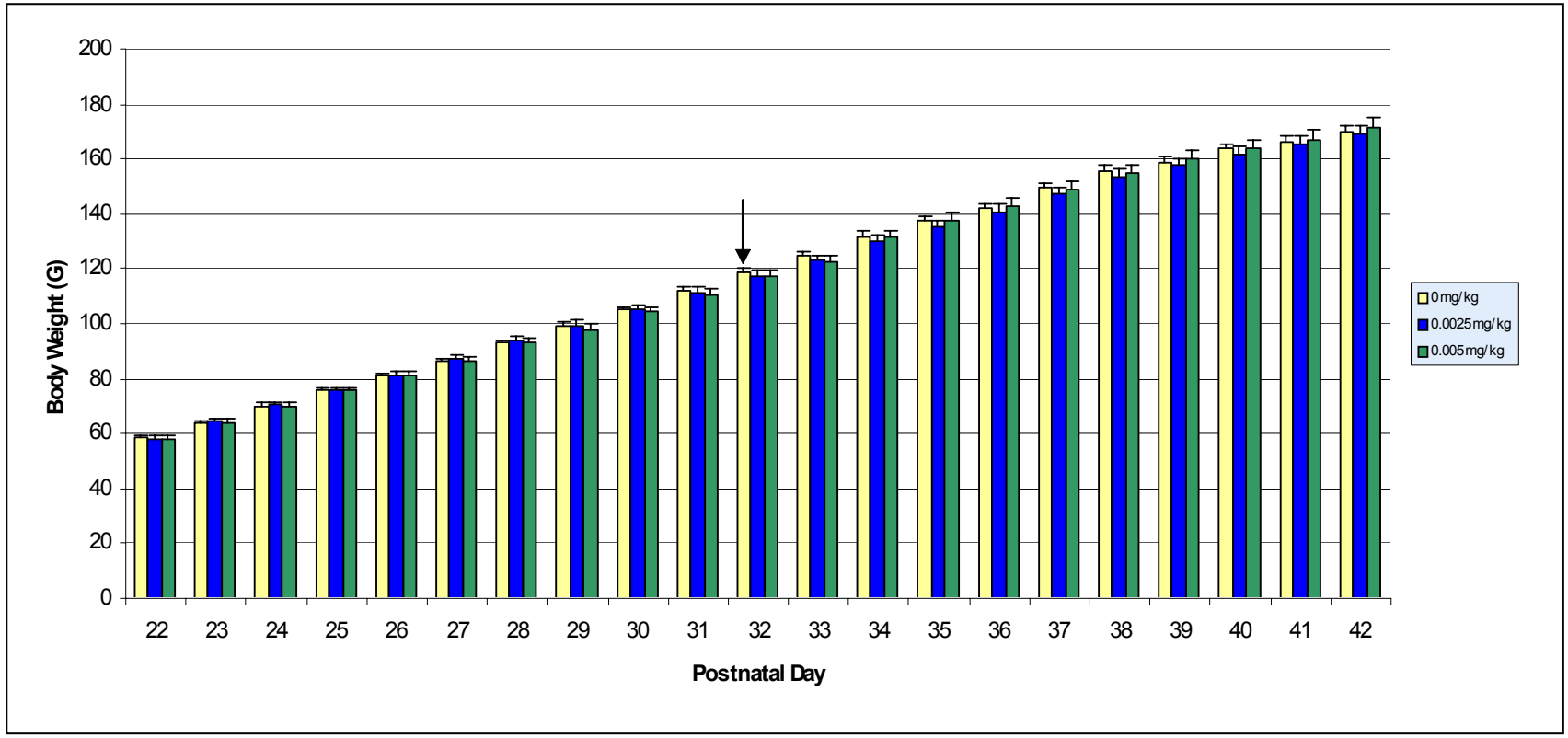
↓ = Mean age of control animals at preputial separation.

Figure 3: Flutamide – Male Body Weights



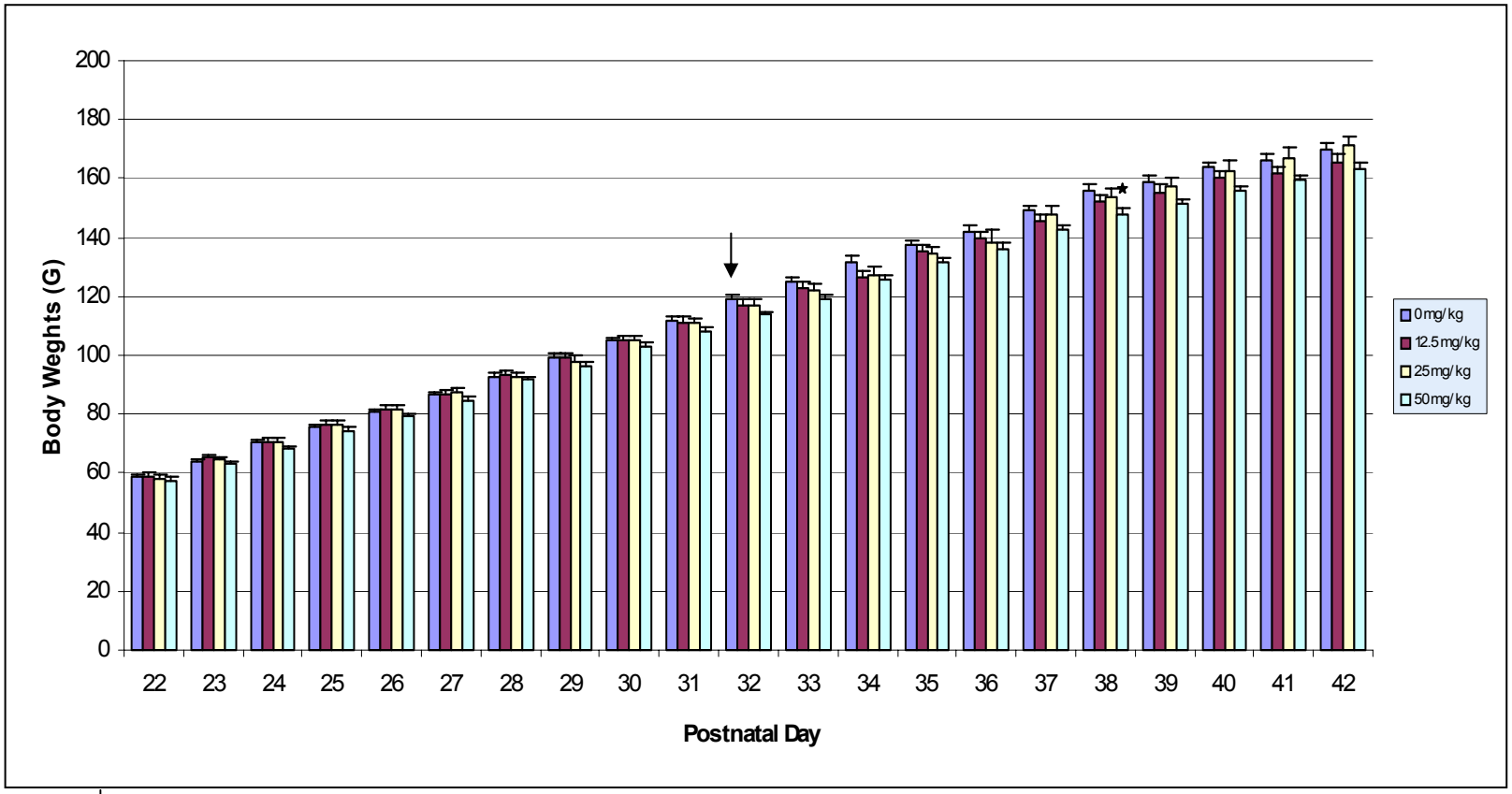
↓ = Mean age of control animals at preputial separation.

Figure 4: Ethynil Estradiol – Female Body Weights



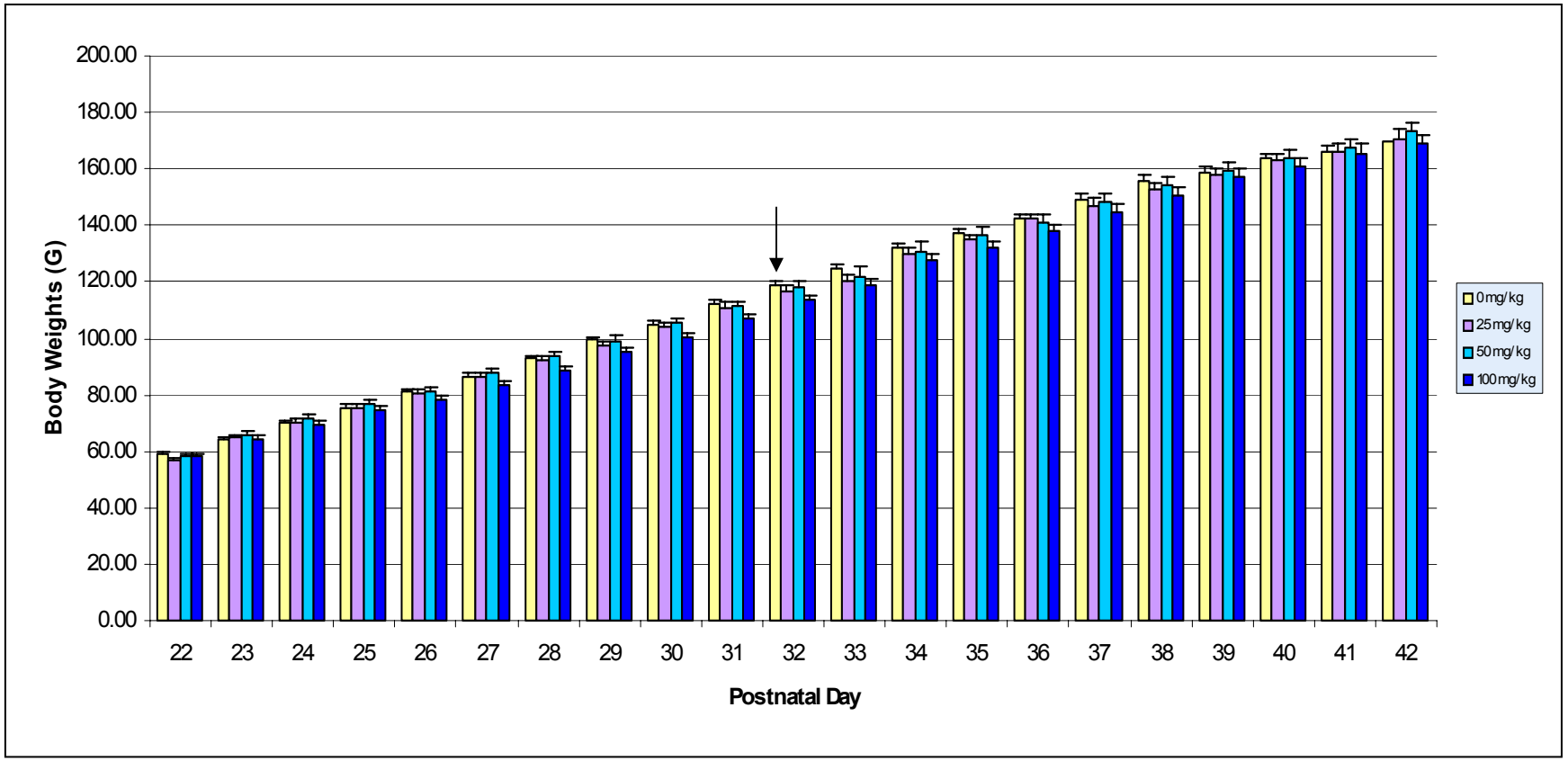
↓ = Mean age of control animals at vaginal opening.

Figure 5: Methoxychlor – Female Body Weights



↓ = Mean age of control animals at vaginal opening.
★ Statistical Significance for comparison of dosed groups to control (* = P<0.05, **=P<0.01) using Dunnett's Test

Figure 6: Phenobarbital – Female Body Weights



↓ = Mean age of control animals at vaginal opening.

PREPUTIAL SEPARATION

Data are presented in Table 4 and Appendix 9.

The mean day and weight at preputial separation (PPS) were PND 41.1 and 213.63 g, respectively, in control males. For statistical purposes, males that did not exhibit PPS by the time of termination were assigned PND 54 as the day of PPS.

Age at PPS was affected by treatment with all three test articles. Phenobarbital treatment showed the least effect, with only the highest dose, 100 mg/kg/day, being significantly different than control (PND 43.6). The age of PPS increased with increasing dose of Vinclozolin - PND 42.6, 43.9, and 47.7 for 10, 30, and 100 mg/kg/day males, respectively. Flutamide treatment had a dramatic effect on PPS, with only six 50 mg/kg/day and one 100 mg/kg/day males achieving PPS prior to termination on PND 54. Mean body weight at PPS was increased in the Vinclozolin and Flutamide-treated males in direct relationship to their increased age. Comparison of body weight gains and pubertal development in males is given in Text Table 8.

Text Table 8: Comparison of Body Weight Gain and Pubertal Development in Males

	Phenobarbital (mg/kg/day)			
Parameter	**0**	**25**	**50**	**100**
Age at preputial separation (days)	41.1 ± 0.38	41.5 ± 0.36	41.1 ± 0.39	**43.6 (14) ± 0.54[a]
Body weight at preputial separation (g)	213.63 ± 4.522	213.44 ± 3.609	215.78 ± 3.748	219.81 (14) ± 4.903
Body weight at PND 23 (g)	67.41 ± 0.995	67.32 ± 1.151	67.84 ± 0.998	67.53 ± 1.116
Body weight at PND 53 (g)	320.99 ± 5.006	312.19 ± 4.734	317.86 ± 5.656	**293.56 (14) ± 5.790[a]
Body weight as % of control at PND 53 (%)	.	97.26	99.02	91.45
Body weight gain PND 23-53 (g)	253.6 ± 4.799	245.3 ± 5.106	243.1 ± 4.295	238.2 ± 6.191

Mean ± SE (n = 15 unless specified in parenthesis by the mean)
[a] Significant treatment effect by ANOVA and statistical significance for comparison of dosed groups to control (* = P<0.05, **=P<0.01) by Dunnett's Test.
. – Not Applicable

	Vinclozolin (mg/kg/day)			
Parameter	**0**	**10**	**30**	**100**
Age at preputial separation (days)	41.1 ± 0.376	* 42.6 ± 0.434[a]	**43.9 ± 0.336[a]	**47.7 ± 0.319[a]
Body weight at preputial separation (g)	213.63 ± 4.522	231.29 ± 5.120	**248.59 ± 4.902[a]	**267.83 ± 6.046[a]
Body weight at PND 23 (g)	67.41 ± 0.995	67.65 ± 0.812	68.08 ± 1.132	67.91 ± 1.020
Body weight at PND 53 (g)	320.99 ± 5.006	328.37 ± 5.673	339.25 ± 5.247	313.17 ± 5.850
Body weight as % of control at PND 53 (%)	.	102.30	105.69	97.56
Body weight gain PND 23-53 (g)	253.6 ± 4.799	244.9 ± 4.374	250.0 ± 5.208	226.2 ± 4.904

Mean ± SE (n = 15 unless specified in parenthesis by the mean)
[a]Significant treatment effect by ANOVA and statistical significance for comparison of dosed groups to control (* = P<0.05, **=P<0.01) by Dunnett's Test.
. – Not Applicable

Text Table 8: Comparison of Body Weight Gain and Pubertal Development in Males (continued)

Parameter	Flutamide (mg/kg/day)		
	0	25	50
Age at preputial separation (days)	41.1 ± 0.38	**53.3 (6) ± 0.33[a]	**54.0 (1) ± 0.0[a]
Body weight at preputial separation (g)	213.63 ± 4.522	**318.57 (6) ± 8.237[a]	**311.00 (1) ± 0.0[a]
Body weight at PND 23 (g)	67.41 ± 0.995	66.93 ± 0.850	67.06 ± 1.054
Body weight at PND 53 (g)	320.99 ± 5.006	310.03 ± 4.332	305.21 ± 6.811
Body weight as % of control at PND 53 (%)	.	96.59	95.08
Body weight gain PND 23-53 (g)	253.6 ± 4.799	260.7 ± 5.543	271.2 ± 5.020

Mean ± SE (n = 15 unless specified in parenthesis by the mean)
[a]Significant treatment effect by ANOVA and statistical significance for comparison of dosed groups to control (* = P<0.05, **=P<0.01) by Dunnett's Test.
. – Not Applicable

VAGINAL OPENING

Data are presented in Table 5 and Appendix 10.

The mean day and weight at vaginal opening (VO) were PND 31.9 and 117.58 g, respectively, in control females. All three test articles affected the age at VO. Ethinyl Estradiol advanced the age at VO to PND 28.4 at 0.005 mg/kg/day. Methoxychlor advanced the age at VO in a dose-related fashion to PND 27.9, 27.0 and 26.5 in 12.5, 25, and 50 mg/kg/day females, respectively. The highest dose of Phenobarbital, 100 mg/kg/day, delayed VO to PND 34.5. Body weight at VO was decreased in Ethinyl Estradiol and Methoxychlor-treated females in relationship to their decreased age, but not statistically different in Phenobarbital-treated females. Comparison of body weight gains and pubertal development in females is given in Text Table 9.

Text Table 9: Comparison of Body Weight Gain and Pubertal Development in Females

Parameter	Methoxychlor (mg/kg/day)			
	0	12.5	25	50
Age at vaginal opening (days)	31.9 ± 0.322	**27.9 ± 0.215[a]	**27.0 ± 0.195[a]	**26.5 ± 0.13[a]
Body weight at vaginal opening (g)	117.58 ± 2.482	**92.41 ± 2.255[a]	**87.48 ± 2.214[a]	**82.02 ± 1.217[a]
Body weight at PND 22 (g)	58.87 ± 0.755	58.97 ± 1.003	58.32 ± 1.151	57.47 ± 0.998
Body weight at PND 42 (g)	169.83 ± 2.002	165.79 ± 2.330	171.03 ± 3.441	163.54 ± 1.823
Body weight as % of control at PND 42 (%)	.	97.62	100.71	96.30
Body weight gain PND 22-42 (g)	111.0 ± 1.769	110.8 ± 2.355	113.5 ± 3.080	106.8 ± 1.717

Mean ± SE (n = 15 unless specified in parenthesis by the mean)
[a]Significant treatment effect by ANOVA and statistical significance for comparison of dosed groups to control (* = P<0.05, **=P<0.01) by Dunnett's Test.
. – Not Applicable

Parameter	Ethinyl Estradiol (mg/kg/day)		
	0	0.0025	0.005
Age at vaginal opening (days)	31.9 ± 0.32	31.1 ± 0.63	**28.4 ± 0.22[a]
Body weight at vaginal opening (g)	117.58 ± 2.482	112.05 ± 3.967	**95.20 ± 1.797[a]
Body weight at PND 22 (g)	58.87 ± 0.755	58.17 ± 0.884	58.03 ± 0.999
Body weight at PND 42 (g)	169.83 ± 2.002	169.01 ± 2.808	171.55 ± 3.728
Body weight as % of control at PND 42 (%)	.	99.52	101.01
Body weight gain PND 22-42 (g)	111.0 ± 1.769	112.7 ± 2.576	106.1 ± 1.500

Mean ± SE (n = 15 unless specified in parenthesis by the mean)
[a]Significant treatment effect by ANOVA and statistical significance for comparison of dosed groups to control (* = P<0.05, **=P<0.01) by Dunnett's Test.
. – Not Applicable

Text Table 9: Comparison of Body Weight Gain and Pubertal Development in Females (continued)

	Phenobarbital (mg/kg/day)			
Parameter	**0**	**25**	**50**	**100**
Age at vaginal opening (days)	31.9 ± 0.32	33.1 ± 0.42	32.8 ± 0.53	**34.5 ± 0.67[a]
Body weight at vaginal opening (g)	117.58 ± 2.482	122.63 ± 3.311	120.67 ± 4.548	128.04 ± 5.107
Body weight at PND 22 (g)	58.87 ± 0.755	56.70 ± 0.769	57.96 ± 1.070	58.14 ± 0.966
Body weight at PND 42 (g)	169.83 ± 2.002	170.39 ± 3.703	173.11 ± 3.357	169.15 ± 3.128
Body weight as % of control at PND 42 (%)	.	100.33	101.93	99.60
Body weight gain PND 22-42 (g)	111.0 ± 1.769	113.7 ± 3.234	115.1 ± 2.725	111.0 ± 2.786

Mean ± SE (n = 15 unless specified in parenthesis by the mean)
[a]Significant treatment effect by ANOVA and statistical significance for comparison of dosed groups to control (* = P<0.05, **=P<0.01) by Dunnett's Test.
. – Not Applicable

ESTROUS CYCLICITY

Data are presented in Table 6 and Appendix 11.

The mean age at first estrus, cycle length, percentage cycling, and percentage cycling normally are shown in Text Table 10. Treatment with Ethinyl Estradiol and Methoxychlor affected age at first estrus, cycle length, and/or the percentage of regular cycles. Although 100 mg/kg/day Phenobarbital-treatment appears to affect the percentage of females cycling, this is due to 3 females (9287, 9290, 9292) with unclear cycles. If these females are excluded from evaluation, 92% of females in this group were cycling.

Text Table 10: Estrous Cyclicity Data

Test Article	Dosage (mg/kg/day)	Age at First Estrus (PND)	Cycle Length (days)	Cycling (%)	Regular Cycles (%)
Vehicle	0	33.4	4.9	100	80
Ethinyl Estradiol	0.0025	32.5	5.1	93	67
	0.005	28.7*	5.1	100	20**
Methoxychlor	12.5	30.9*	5.2	100	80
	25	30.7*	6.2*	100	27**
	50	28.6*	5.9*	100	20**
Phenobarbital	25	35.6	4.9	100	80
	50	34.0	5.0	93	80
	100	34.5	5.3	73*	67

* = statistically significant $p \leq .05$, ** = statistically significant $p \leq .01$

GROSS PATHOLOGY

Data are presented in Table 7 and Appendix 12.

Males

Treatment with Flutamide produced size reductions in the seminal vesicles, coagulating glands, ventral prostate, dorsolateral prostate, epididymides, and/or levator ani plus bulbocavernosus muscles and an increase in testis size. The incidence of these findings was confined to or slightly higher in the 50 mg/kg/day males.

Treatment with Vinclozolin resulted in a higher incidence of kidney dilation: 6/15 males in the 100 mg/kg/day group, 4/15 males in the 30 mg/kg/day group and 3/15 males in the 10 mg/kg/day group.

Treatment with Phenobarbital had no effect on gross pathology.

Females

There were no gross observations in females that were considered to be related to treatment.

ORGAN WEIGHTS

Data are presented in Table 8 and in Appendix 13.

Males

Body weight and organ weights at necropsy are presented in Text Table 11.

Phenobarbital treatment resulted in a dose-related higher liver weights at all doses and lower pituitary weight at 100 mg/kg/day. Observed differences in kidney and ventral prostate weights at 100 mg/kg/day are most likely due to lower body weight and delayed age of sexual maturation and not a direct test-article effect.

Vinclozolin treatment resulted in lower weights of seminal vesicles, prostate, and levator ani plus bulbocavernosus muscles, and higher adrenal weights at 100 mg/kg/day. Testis weight was higher than control for 30 and 100 mg/kg/day males.

Flutamide treatment also resulted in lower weights of testosterone-sensitive tissues including seminal vesicles, prostate, epididymides, and levator ani plus bulbocavernosus muscles, while testis weights were higher at 25 and 50 mg/kg/day. Liver weights were higher and kidney weights were lower at 25 and 50 mg/kg/day. Adrenal weight was higher than control in 50 mg/kg/day Flutamide-treated males.

Text Table 11: Body Weight and Organ Weights at Necropsy - Males (continued)

Parameter	Phenobarbital (mg/kg/day)			
	0	25	50	100
Terminal Body Weight (g)	324.18 ± 5.027	315.81 ± 4.780	322.71 ± 5.789	**298.27 (14) ± 5.153[b]
Adrenal Glands, Paired (g)	0.04927 ± .002135	0.05323 ± .002243	0.04787 ± .002981	0.05256 (14) ± .002905
Adrenal Glands, Paired (g) (ANCOVA mean)[a]	0.04893 ± .002643	0.05322 ± .002578	0.04759 ± .002623	0.05323 (14) ± .002911
Dorsolateral Prostate (g)	0.26575 ± .011858	0.22301 ± .014107	0.25227 ± .021030	0.21192 (13) ± .013477
Dorsolateral Prostate (g) (ANCOVA mean)[a]	0.26272 ± .015833	0.22295 ± .015451	0.24976 ± .015714	0.21839 (13) ± .018162
Epididymides, Paired (g)	0.52313 ± .016801	0.52643 ± .023714	0.53801 ± .020924	0.50856 (14) ± .025601
Epididymides, Paired (g) (ANCOVA mean)[a]	0.52205 ± .022445	0.52639 ± .021894	0.53711 ± .022276	0.51073 (14) ± .024719
Kidneys, Paired (g)	3.11392 ± .048012	2.90428 ± .061123	3.06683 ± .065461	**2.81193 (14) ± .083957[b]
Kidneys, Paired (g) (ANCOVA mean)[a]	3.04396 ± .051112	2.90201 ± .049856	3.00874 ± .050725	2.95157 (14) ± .056290
Levator Ani Plus Bulbocavernosus Muscles (g)	0.59862 ± .035631	0.63485 ± .043294	0.58468 ± .043846	0.58501 (14) ± .028037
Levator Ani Plus Bulbocavernosus Muscles (g) (ANCOVA mean)[a]	0.59001 ± .039217	0.63457 ± .038254	0.57753 ± .038920	0.60219 (14) ± .043190
Liver (g)	16.61980 ± .414274	18.13560 ± .496956	**19.59220 ± .585405[b]	**19.8170 (14) ± .677245[b]
Liver (g) (ANCOVA mean)[a]	15.91920 ± .358643	**18.1128 ± .349834[c]	**19.0105 ± .355929[c]	**21.21540 (14) ± .394979[c]
Pituitary (g)	0.01041 ± .000365	0.00955 ± .000461	0.01003 ± .000418	* 0.00873 (14) ± .000331[b]
Pituitary (g) (ANCOVA mean)[a]	0.01042 ± .000409	0.00955 ± .000399	0.01003 ± .000406	* 0.00871 (14) ± .000451[c]
Right Epididymis (g)	0.25189 ± .007466	0.25883 ± .014121	0.26270 ± .013686	0.25520 (14) ± .015511
Right Epididymis (g) (ANCOVA mean)[a]	0.25228 ± .013316	0.25884 ± .012989	0.26303 ± .013216	0.25441 (14) ± .014665

Mean ± SE (n = 15 unless specified in parenthesis by the mean)

[a]Mean ± SE has been adjusted for body weight at necropsy using ANCOVA.

[b]Significant treatment effect by ANOVA and statistical significance for comparison of dosed groups to control (* = P<0.05, **=P<0.01) by Dunnett's Test.

[c]Significant treatment effect by ANCOVA and statistical significance for comparison of dosed groups to control (* = P<0.05, **=P<0.01) by Dunnett's Test.

Text Table 11: Body Weight and Organ Weights at Necropsy - Males (continued)

Parameter	Phenobarbital (mg/kg/day)			
	0	25	50	100
Seminal Vesicles and Coagulating glands, with fluid(g)	0.69805 ± .045150	0.77617 ± .035129	0.68632 ± .059840	0.58574 (14) ± .038317
Seminal Vesicles and Coagulating glands, with fluid (g) (ANCOVA mean)[a]	0.68813 ± .046656	0.77584 ± .045510	0.67809 ± .046303	0.60553 (14) ± .051383
Seminal Vesicles and Coagulating glands, without fluid (g)	0.41088 ± .025802	0.45304 ± .023274	0.39272 ± .027148	0.36340 (14) ± .029119
Seminal Vesicles and Coagulating glands, without fluid (g) (ANCOVA mean)[a]	0.40358 ± .026642	0.45280 ± .025988	0.38666 ± .026440	0.37796 (14) ± .029341
Testes, Paired (Grams)	2.70352 ± .041039	2.74459 ± .034174	2.81499 ± .060105	2.65386 (14) ± .072035
Testes, Paired (g) (ANCOVA mean)[a]	2.65840 ± .047018	2.74313 ± .045863	2.77752 ± .046662	2.74393 (14) ± .051782
Thyroid/Parathyroid, Post Fixation (grams)	0.02165 ± .001256	0.02045 ± .000869	0.02189 ± .000794	0.02323 (14) ± .001309
Thyroid/Parathyroid, Post Fixation (g) (ANCOVA mean)[a]	0.02160 ± .001101	0.02045 ± .001074	0.02185 ± .001092	0.02332 (14) ± .001212
Ventral Prostate (Grams)	0.23843 ± .013510	0.19983 ± .013573	0.21057 ± .013437	**0.17921 (14) ± .011430[b]
Ventral Prostate (g) (ANCOVA mean)[a]	0.23116 ± .012642	0.19959 ± .012332	0.20454 ± .012547	0.19372 (14) ± .013923

Mean ± SE (n = 15 unless specified in parenthesis by the mean)

[a]Mean ± SE has been adjusted for body weight at necropsy using ANCOVA.

[b]Significant treatment effect by ANOVA and statistical significance for comparison of dosed groups to control (* = P<0.05, **=P<0.01) by Dunnett's Test.

[c]Significant treatment effect by ANCOVA and statistical significance for comparison of dosed groups to control (* = P<0.05, **=P<0.01) by Dunnett's Test.

Text Table 11: Body Weight and Organ Weights at Necropsy - Males

Parameter	Vinclozolin (mg/kg/day)			
	0	10	30	100
Terminal Body Weight (g)	324.18 ± 5.027	332.56 ± 6.112	* 343.99 ± 5.750[b]	317.08 ± 5.448
Adrenal Glands, Paired (g)	0.04927 ± .002135	0.05312 ± .002528	0.05177 ± .002297	0.05627 ± .001981
Adrenal Glands, Paired (g) (ANCOVA mean)[a]	0.04986 ± .002191	0.05277 ± .002180	0.05014 ± .002301	* 0.05766 ± .002267[c]
Dorsolateral Prostate (g)	0.26575 ± .011858	0.24932 ± .014290	0.24642 ± .008573	**0.18895 ± .007196 [b]
Dorsolateral Prostate (g) (ANCOVA mean)[a]	0.26652 ± .010994	0.24886 ± .010937	0.24429 ± .011545	**0.19076 ± .011373[c]
Epididymides, Paired (g)	0.52313 ± .016801	0.58333 ± .020179	0.51642 ± .023740	0.45851 ± .015936
Epididymides, Paired (g) (ANCOVA mean)[a]	0.52685 ± .019328	0.58114 ± .019230	0.50618 ± .020299	0.46723 ± .019996
Kidneys, Paired (g)	3.11392 ± .048012	3.02505 ± .069358	3.14487 ± .055835	2.95730 ± .042097
Kidneys, Paired (g) (ANCOVA mean)[a]	3.14712 ± .042640	3.00548 ± .042423	3.05332 ± .044780	3.03522 ± .044113
Levator Ani Plus Bulbocavernosus Muscles (g)	0.59862 ± .035631	0.62068 ± .032690	0.62008 ± .022220	**0.45897 ± .037204 [b]
Levator Ani Plus Bulbocavernosus Muscles (g) (ANCOVA mean)[a]	0.59770 ± .033003	0.62122 ± .032835	0.62260 ± .034659	* 0.45682 ± .034143[c]
Liver (g)	16.61980 ± .414274	17.08590 ± .523925	18.07300 ± .457382	16.38420 ± .538874
Liver (g) (ANCOVA mean)[a]	17.00750 ± .263041	16.85730 ± .261701	17.00400 ± .276244	17.29410 ± .272125
Pituitary (g)	0.01041 ± .000365	0.01016 ± .000410	0.01021 ± .000290	0.00947 ± .000414
Pituitary (g) (ANCOVA mean)[a]	0.01047 ± .000374	0.01012 ± .000372	0.01004 ± .000393	0.00962 ± .000387
Right Epididymis (g)	0.25189 ± .007466	0.28631 ± .011063	0.25524 ± .012479	0.22400 ± .010342
Right Epididymis (g) (ANCOVA mean)[a]	0.25358 ± .010519	0.28532 ± .010465	0.25058 ± .011047	0.22797 ± .010882

Mean ± SE (n = 15 unless specified in parenthesis by the mean)

[a]Mean ± SE has been adjusted for body weight at necropsy using ANCOVA.

[b]Significant treatment effect by ANOVA and statistical significance for comparison of dosed groups to control (* = P<0.05, **=P<0.01) by Dunnett's Test.

[c]Significant treatment effect by ANCOVA and statistical significance for comparison of dosed groups to control (* = P<0.05, **=P<0.01) by Dunnett's Test.

Text Table 11: Body Weight and Organ Weights at Necropsy - Males (continued)

Parameter	Vinclozolin (mg/kg/day)			
	0	10	30	100
Seminal Vesicles and Coagulating glands, with fluid(g)	0.69805 ± .045150	0.67235 ± .040422	0.62576 ± .029288	**0.45636 ± .026981[b]
Seminal Vesicles and Coagulating glands, with fluid (g) (ANCOVA mean)[a]	0.70918 ± .034857	0.66579 ± .034680	0.59506 ± .036607	**0.48249 ± .036061[c]
Seminal Vesicles and Coagulating glands, without fluid (g)	0.41088 ± .025802	0.40970 ± .020047	0.37417 ± .012722	**0.29803 ± .014735[b]
Seminal Vesicles and Coagulating glands, without fluid (g) (ANCOVA mean)[a]	0.41642 ± .018390	0.40643 ± .018296	0.35888 ± .019313	**0.31104 ± .019025[c]
Testes, Paired (g)	2.70352 ± .041039	2.81745 ± .031426	**3.00937 ± .037069[b]	**2.97171 ± .048928[b]
Testes, Paired (g) (ANCOVA mean)[a]	2.71865 ± .037394	2.80853 ± .037204	**2.96767 ± .039271[c]	**3.00721 ± .038686[c]
Thyroid/Parathyroid, Post Fixation (g)	0.02165 ± .001256	0.01923 ± .000828	0.02189 ± .000845	0.02101 ± .000854
Thyroid/Parathyroid, Post Fixation (g) (ANCOVA mean)[a]	0.02159 ± .000977	0.01926 ± .000972	0.02206 ± .001026	0.02087 ± .001011
Ventral Prostate (g)	0.23843 ± .013510	0.20364 ± .016725	0.21021 ± .013379	* 0.18338 ± .014043[b]
Ventral Prostate (g) (ANCOVA mean)[a]	0.24041 ± .014567	0.20247 ± .014493	0.20474 ± .015299	* 0.18803 ± .015070[c]

Mean ± SE (n = 15 unless specified in parenthesis by the mean)
[a]Mean ± SE has been adjusted for body weight at necropsy using ANCOVA.
[b]Significant treatment effect by ANOVA and statistical significance for comparison of dosed groups to control (* = P<0.05, **=P<0.01) by Dunnett's Test.
[c]Significant treatment effect by ANCOVA and statistical significance for comparison of dosed groups to control (* = P<0.05, **=P<0.01) by Dunnett's Test.

Text Table 11: Body Weight and Organ Weights at Necropsy - Males (continued)

Parameter	Flutamide (mg/kg/day)		
	0	25	50
Terminal Body Weight (g)	324.18 ± 5.027	313.49 ± 4.523	308.69 ± 6.675
Adrenal Glands, Paired (g)	0.04927 ± .002135	0.051800 ± .001562	**0.05916 ± .002285[b]
Adrenal Glands, Paired (g) (ANCOVA mean)[a]	0.04922 ± .002103	0.05181 ± .002046	**0.05919 ± .002079[c]
Dorsolateral Prostate (g)	0.26575 ± .011858	**0.12383 ± .007379[b]	**0.10189 ± .007076[b]
Dorsolateral Prostate (g) (ANCOVA mean)[a]	0.26297 ± .009243	**0.12445 ± .008990[c]	**0.10404 ± .009138[c]
Epididymides, Paired (g)	0.52313 ± .016801	**0.34428 ± .016145[b]	**0.35045 ± .016320[b]
Epididymides, Paired (g) (ANCOVA mean)[a]	0.52239 ± .017110	**0.34445 ± .016642[c]	**0.35102 ± .016915[c]
Kidneys, Paired (g)	3.11392 ± .048012	**2.71213 ± .052210[b]	**2.76443 ± .074129[b]
Kidneys, Paired (g) (ANCOVA mean)[a]	3.05519 ± .048267	**2.72534 ± .046947[c]	**2.80995 ± .047716[c]
Levator Ani Plus Bulbocavernosus Muscles (g)	0.59862 ± .035631	**0.38779 ± .018250[b]	**0.38493 ± .023846[b]
Levator Ani Plus Bulbocavernosus Muscles (g) (ANCOVA mean)[a]	0.60114 ± .027988	**0.38722 ± .027223[c]	**0.38297 ± .027669[c]
Liver (g)	16.61980 ± .414274	16.733900 ± .495116	17.33660 ± .457025
Liver (g) (ANCOVA mean)[a]	16.01600 ± .264420	* 16.86970 ± .257192[c]	**17.8046 ± .261405[c]
Pituitary (g)	0.01041 ± .000365	0.011030 ± .000544	0.01101 ± .000498
Pituitary (g) (ANCOVA mean)[a]	0.01036 ± .000494	0.01104 ± .000480	0.01105 ± .000488
Right Epididymis (g)	0.25189 ± .007466	**0.16875 ± .009692[b]	**0.16479 ± .010204[b]
Right Epididymis (g) (ANCOVA mean)[a]	0.24974 ± .009481	**0.16924 ± .009222[c]	**0.16646 ± .009373[c]

Mean ± SE (n = 15 unless specified in parenthesis by the mean)

[a]Mean ± SE has been adjusted for body weight at necropsy using ANCOVA.

[b]Significant treatment effect by ANOVA and statistical significance for comparison of dosed groups to control (* = P<0.05, **=P<0.01) by Dunnett's Test.

[c]Significant treatment effect by ANCOVA and statistical significance for comparison of dosed groups to control (* = P<0.05, **=P<0.01) by Dunnett's Test.

Text Table 11: Body Weight and Organ Weights at Necropsy - Males (continued)

Parameter	Flutamide (mg/kg/day)		
	0	25	50
Seminal Vesicles and Coagulating glands, with fluid (g)	0.69800 ± .045150	* 0.17400 ± .017629[d]	* 0.13667 ± .011083[d]
Seminal Vesicles and Coagulating glands, with fluid (g) (ANCOVA mean)[a]	0.69253 ± .029697	**0.17607 ± .028885[c]	**0.13969 ± .029358[c]
Seminal Vesicles and Coagulating glands, without fluid (g)	0.41088 ± .025802	**0.14489 ± .014215[b]	**0.11647 ± .009418[b]
Seminal Vesicles and Coagulating glands, without fluid (g) (ANCOVA mean)[a]	0.40815 ± .018522	**0.14551 ± .018016[c]	**0.11859 ± .018311[c]
Testes, Paired (g)	2.70267 ± .041039	* 2.99933 ± .053884[d]	* 3.49533 ± .130636[d]
Testes, Paired (g) (ANCOVA mean)[a]	2.67219 ± .086126	* 3.00533 ± .083772[c]	**3.51951 ± .085144[c]
Thyroid/Parathyroid, Post Fixation (g)	0.02165 ± .001256	0.021150 ± .001077	0.02037 ± .001162
Thyroid/Parathyroid, Post Fixation (g) (ANCOVA mean)[a]	0.02138 ± .001204	0.02121 ± .001171	0.02058 ± .001190
Ventral Prostate (g)	0.23843 ± .013510	**0.10616 ± .006672[b]	**0.08347 ± .009508[b]
Ventral Prostate (g) (ANCOVA mean)[a]	0.23502 ± .010484	**0.10693 ± .010198[c]	**0.08612 ± .010365[c]

Mean ± SE (n = 15 unless specified in parenthesis by the mean)

[a]Mean ± SE has been adjusted for body weight at necropsy using ANCOVA.

[b]Significant treatment effect by ANOVA and statistical significance for comparison of dosed groups to control (* = P<0.05, **=P<0.01) by Dunnett's Test.

[c]Significant treatment effect by ANCOVA and statistical significance for comparison of dosed groups to control (* = P<0.05, **=P<0.01) by Dunnett's Test.

[d]Significant treatment effect by ANOVA and statistical significance for comparison of dosed groups to control (* = P<0.05, **=P<0.01) by Dunn's Test. Dunn's Test was used for pair-wise comparisons because HOV test failed.

Females

Body weight and organ weights at necropsy are presented in Text Table 12.

The only significant effect of Ethinyl Estradiol treatment was mean adrenal gland weight that was higher than control means at both 0.0025 and 0.005 mg/kg/day.

Affects of Methoxychlor on adrenal gland and kidney weights were not dose related and not considered to be a treatment effect. Liver, ovary, and pituitary weights were lower than control in 50 mg/kg/day treated females.

Phenobarbital treatment resulted in a dose-related higher liver weights and higher adrenal gland weights at 25, 50 and 100 mg/kg/day. Thyroid weight was higher than control in 100 mg/kg/day females.

Text Table 12: Body Weight and Organ Weights at Necropsy - Females (continued)

Parameter	Ethinyl Estradiol (mg/kg/day)		
	0	0.0025	0.005
Endocrine Status[d]			
Diestrus	7	5	4
Proestrus	1	2	2
Estrus	5	7	5
Metestrus	1	1	4
Not cycling	0	1	0
Terminal Body Weight (g)	171.10 ± 1.953	169.91 ± 2.541	173.24 ± 3.358
Adrenal Glands, Paired (g)	0.04136 ± 0.001746	**0.0501 ± 0.002059[b]	**0.0487 ± 0.001271[b]
Adrenal Glands, Paired (g) (ANCOVA mean)[a]	0.04133 ± .001728	**0.04999 ± .001734[c]	**0.04887 ± .001737[c]
Kidneys, Paired (g)	1.72900 ± 0.028952	1.71570 ± 0.035149	1.69916 ± 0.029806
Kidneys, Paired (g) (ANCOVA mean)[a]	1.73114 ± .026057	1.72582 ± .026151	1.68691 ± .026196
Liver (g)	8.66695 ± 0.146905	8.41119 ± 0.162899	8.75587 ± 0.158135
Liver (g) (ANCOVA mean)[a]	8.68006 ± .111601	8.47325 ± .112000	8.68070 ± .112194
Ovaries, Paired (g)	0.09299 ± 0.004155	0.09435 ± 0.004259	0.09869 ± 0.004581
Ovaries, Paired (g) (ANCOVA mean)[a]	0.09297 ± .004386	0.09427 ± .004402	0.09879 ± .004410
Pituitary (g)	0.00861 ± 0.000529	0.00816 ± 0.000564	0.00934 ± 0.000453
Pituitary (g) (ANCOVA mean)[a]	0.00863 ± .000495	0.00826 ± .000497	0.00922 ± .000498

Mean ± SE (n = 15 unless specified in parenthesis by the mean)
[a]Mean ± SE has been adjusted for body weight at necropsy using ANCOVA.
[b]Significant treatment effect by ANOVA and Statistical Significance for comparison of dosed groups to control (* = P<0.05, **=P<0.01) by Dunnett's Test.
[c]Significant treatment effect by ANCOVA and Statistical Significance for comparison of dosed groups to control (* = P<0.05, **=P<0.01) by Dunnett's Test.
[d]Number of females in each stage of the estrous cycle at necropsy, as characterized by vaginal cytology.

Text Table 12: Body Weight and Organ Weights at Necropsy - Females (continued)

Parameter	Ethinyl Estradiol (mg/kg/day)		
	0	0.0025	0.005
Thyroid/Parathyroid, Post Fixation (g)	0.01649 ± 0.001048	0.01687 ± 0.000759	0.01679 ± 0.001072
Thyroid/Parathyroid, Post Fixation (g) (ANCOVA mean)[a]	0.01653 ± .000942	0.01703 ± .000946	0.01660 ± .000947
Uterus and Cervix without Fluid (g)	0.35533 ± 0.027628	0.38714 ± 0.023646	0.36026 ± 0.020087
Uterus and Cervix without Fluid (g) (ANCOVA mean)[a]	0.35545 ± .024258	0.38771 ± .024345	0.35956 ± .024387
Uterus and Cervix, with Fluid (g)	0.41943 ± 0.049172	0.43567 ± 0.037224	0.39721 ± 0.033864
Uterus and Cervix, with Fluid (g) (ANCOVA mean)[a]	0.41953 ± .041110	0.43617 ± .041258	0.39660 ± .041329

Mean ± SE (n = 15 unless specified in parenthesis by the mean)
[a]Mean ± SE has been adjusted for body weight at necropsy using ANCOVA.
[b]Significant treatment effect by ANOVA and Statistical Significance for comparison of dosed groups to control (* = P<0.05, **=P<0.01) by Dunnett's Test.
[c]Significant treatment effect by ANCOVA and Statistical Significance for comparison of dosed groups to control (* = P<0.05, **=P<0.01) by Dunnett's Test.
[d]Number of females in each stage of the estrous cycle at necropsy, as characterized by vaginal cytology.

Text Table 12: Body Weight and Organ Weights at Necropsy - Females

Parameter	Methoxychlor (mg/kg/day)			
	0	12.5	25	50
Endocrine Status[d]				
Diestrus	7	5	11	4
Proestrus	1	1	0	4
Estrus	5	5	3	2
Metestrus	1	4	1	5
Not cycling	0	0	0	0
Terminal Body Weight (g)	171.10 ± 1.953	167.85 ± 2.126	172.53 ± 3.009	165.59 ± 1.761
Adrenal Glands, Paired (g)	0.04136 ± 0.001746	* 0.04865 ± 0.001334[b]	0.04627 ± 0.002196	0.04476 ± 0.002252
Adrenal Glands, Paired (g) (ANCOVA mean)[a]	0.04167 ± .001907	* 0.04841 ± .001903[c]	0.04683 ± .001931	0.04413 ± .001941
Kidneys, Paired (g)	1.72900 ± 0.028952	1.66866 ± 0.029096	1.66640 ± 0.033034	1.66618 ± 0.032442
Kidneys, Paired (g) (ANCOVA mean)[a]	1.71116 ± .022037	1.68244 ± .021985	* 1.63461 ± .022312[c]	1.70203 ± .022421
Liver (g)	8.66695 ± 0.146905	8.12279 ± 0.218546	8.39611 ± 0.248415	* 7.89805 ± 0.168019[b]
Liver (g) (ANCOVA mean)[a]	8.53798 ± .121895	8.22243 ± .121610	8.16621 ± .123419	8.15729 ± .124018
Ovaries, Paired (g)	0.09299 ± 0.004155	0.09529 ± 0.004337	0.08504 ± 0.003972	* 0.07888 ± 0.003368[b]
Ovaries, Paired (g) (ANCOVA mean)[a]	0.09250 ± .003987	0.09567 ± .003978	0.08417 ± .004037	0.07986 ± .004057
Pituitary (g)	0.00861 ± 0.000529	0.00790 ± 0.000469	0.00771 ± 0.000323	* 0.00692 ± 0.000538[b]
Pituitary (g) (ANCOVA mean)[a]	0.00863 ± .000479	0.00789 ± .000478	0.00774 ± .000485	* 0.00689 ± .000488[b]

Mean ± SE (n = 15 unless specified in parenthesis by the mean)
[a]Mean ± SE has been adjusted for body weight at necropsy using ANCOVA.
[b]Significant treatment effect by ANOVA and Statistical Significance for comparison of dosed groups to control (* = P<0.05, **=P<0.01) by Dunnett's Test.
[c]Significant treatment effect by ANCOVA and Statistical Significance for comparison of dosed groups to control (* = P<0.05, **=P<0.01) by Dunnett's Test.
[d]Number of females in each stage of the estrous cycle at necropsy, as characterized by vaginal cytology.

Text Table 12: Body Weight and Organ Weights at Necropsy - Females

Parameter	Methoxychlor (mg/kg/day)			
	0	12.5	25	50
Thyroid/Parathyroid, Post Fixation (g)	0.01649 ± 0.001048	0.01725 ± 0.000628	0.01851 ± 0.000869	0.01693 ± 0.000934
Thyroid/Parathyroid, Post Fixation (g) (ANCOVA mean)[a]	0.01643 ± .000893	0.01730 ± .000891	0.01840 ± .000904	0.01706 ± .000908
Uterus and Cervix without Fluid (g)	0.35533 ± 0.027628	0.34717 ± 0.022768	0.33103 ± 0.018269	0.33157 ± 0.023957
Uterus and Cervix without Fluid (g) (ANCOVA mean)[a]	0.35464 ± .023729	0.34770 ± .023673	0.32980 ± .024026	0.33296 ± .024142
Uterus and Cervix, with Fluid (g)	0.41943 ± 0.049172	0.37781 ± 0.037327	0.35801 ± 0.023003	0.43732 ± 0.055971
Uterus and Cervix, with Fluid (g) (ANCOVA mean)[a]	0.41818 ± .043841	0.37878 ± .043739	0.35579 ± .044389	0.43982 ± .044605

Mean ± SE (n = 15 unless specified in parenthesis by the mean)
[a]Mean ± SE has been adjusted for body weight at necropsy using ANCOVA.
[b]Significant treatment effect by ANOVA and Statistical Significance for comparison of dosed groups to control (* = P<0.05, **=P<0.01) by Dunnett's Test.
[c]Significant treatment effect by ANCOVA and Statistical Significance for comparison of dosed groups to control (* = P<0.05, **=P<0.01) by Dunnett's Test.
[d]Number of females in each stage of the estrous cycle at necropsy, as characterized by vaginal cytology.

Text Table 12: Body Weight and Organ Weights at Necropsy - Females (continued)

Parameter	Phenobarbital (mg/kg/day)			
	0	25	50	100
Endocrine Status[d]				
Diestrus	7	4	7	5
Proestrus	1	2	2	2
Estrus	5	6	3	6
Metestrus	1	3	3	1
Not cycling	0	0	1	3
Terminal Body Weight (g)	171.10 ± 1.953	171.36 ± 3.543	175.37 ± 3.151	171.46 ± 2.753
Adrenal Glands, Paired (g)	0.04136 ± .001746	**0.05203 ± .002524[b]	**0.05261 ± .001560[b]	**0.05182 ± .001970[b]
Adrenal Glands, Paired (g) (ANCOVA mean)[a]	0.04128 ± .001994	**0.05197 ± .001993[c]	**0.05282 ± .002010[c]	**0.05176 ± .001992[c]
Kidneys, Paired (g)	1.72900 ± .028952	1.95411 ± .209151	1.74191 ± .073924	1.74029 ± .067031
Kidneys, Paired (g) (ANCOVA mean)[a]	1.73311 ± .117595	1.95735 ± .117525	1.73166 ± .118552	1.74319 ± .117502
Liver (g)	8.66695 ± .146905	* 9.78521 ± .312736[b]	**10.7576 ± .372795[b]	**11.32150 ± .330428[b]
Liver (g) (ANCOVA mean)[a]	8.76602 ± .192284	**9.86320 ± .192169[c]	**10.5107 ± .193849[c]	**11.3913 0 ± .192132[c]
Ovaries, Paired (g)	0.09299 ± .004155	0.09769 ± .004014	0.10069 ± .003386	0.09420 ± .004302
Ovaries, Paired (g) (ANCOVA mean)[a]	0.09313 ± .004007	0.09780 ± .004005	0.10033 ± .004040	0.09430 ± .004004
Pituitary (g)	0.00861 ± .000529	0.00889 ± .000951	0.00790 ± .000520	0.00832 ± .000362 (14)
Pituitary (g) (ANCOVA mean)[a]	0.00864 ± .000637	0.00891 ± .000636	0.00785 ± .000641	0.00833 ± .000658 (14)

Mean ± SE (n = 15 unless specified in parenthesis by the mean)
[a]Mean ± SE has been adjusted for body weight at necropsy using ANCOVA.
[b]Significant treatment effect by ANOVA and Statistical Significance for comparison of dosed groups to control (* = P<0.05, **=P<0.01) by Dunnett's Test.
[c]Significant treatment effect by ANCOVA and Statistical Significance for comparison of dosed groups to control (* = P<0.05, **=P<0.01) by Dunnett's Test.
[d]Number of females in each stage of the estrous cycle at necropsy, as characterized by vaginal cytology.

Text Table 12: Body Weight and Organ Weights at Necropsy - Females (continued)

Parameter	Phenobarbital (mg/kg/day)			
	0	25	50	100
Thyroid/Parathyroid, Post Fixation (g)	0.01649 ± .001048	0.01765 ± .000992	0.01813 ± .001149	* 0.01999 ± .000688[b]
Thyroid/Parathyroid, Post Fixation (g) (ANCOVA mean)[a]	0.01647 ± .000993	0.01764 ± .000993	0.01818 ± .001001	* 0.01998 ± .000993[c]
Uterus and Cervix without Fluid (g)	0.35533 ± .027628	0.35147 ± .018169	0.34745 ± .020805	0.35189 ± .023022
Uterus and Cervix without Fluid (g) (ANCOVA mean)[a]	0.35565 ± .022902	0.35172 ± .022888	0.34667 ± .023088	0.35211 ± .022884
Uterus and Cervix, with Fluid (g)	0.41943 ± .049172	0.40701 ± .039135	0.39323 ± .037337	0.40873 ± .036177
Uterus and Cervix, with Fluid (g) (ANCOVA mean)[a]	0.42021 ± .041172	0.40762 ± .041147	0.39128 ± .041507	0.40928 ± .041139

Mean ± SE (n = 15 unless specified in parenthesis by the mean)

[a]Mean ± SE has been adjusted for body weight at necropsy using ANCOVA.

[b]Significant treatment effect by ANOVA and Statistical Significance for comparison of dosed groups to control (* = P<0.05, **=P<0.01) by Dunnett's Test.

[c]Significant treatment effect by ANCOVA and Statistical Significance for comparison of dosed groups to control (* = P<0.05, **=P<0.01) by Dunnett's Test.

[d]Number of females in each stage of the estrous cycle at necropsy, as characterized by vaginal cytology.

HISTOPATHOLOGY

The Histopathology Report is presented in Appendix 17.

As this was a blinded study, the test article information was disclosed after the processing and histological evaluation of the tissues was completed.

The majority of the gross lesions concerned the accessory sex glands of the males, and dilation of the kidneys of both males and females, which corresponded to hydronephrosis of variable degree. Hydronephrosis was observed in both males and females throughout all groups, except in the 25 mg/kg/day Flutamide-treated males and 50 mg/kg/day Methoxychlor-treated females, and considered an incidental finding.

Microscopic findings observed at each group in both males and females were incidental and expected in animals of similar age and environment, except for 50 mg/kg/day Flutamide treated males. Although 5 animals were microscopically normal in this group, changes in the remaining 10 males included luminal dilation of the testicular tubules, degeneration of the germinal epithelium and edema. Four of these males were also observed with sperm granulomas in the epididymis.

DISCUSSION

This study tested several hypotheses about the effects of endocrine disruptors on pubertal Sprague Dawley rats. Vinclozolin and Flutamide are both androgen receptor antagonists and were expected to delay preputial separation, reduce the weight of androgen sensitive reproductive organs, alter external genitalia, and cause retained nipples when administered to juvenile male Sprague Dawley rats (PND 23-53/54). Treatment did delay preputial separation and reduce the weight of androgen sensitive reproductive organs as expected. Vinclozolin and Flutamide treatment also resulted in higher testis weights and testis lesions (50 mg/kg/day Flutamide only). Treatment did not alter external genitalia or cause retained nipples. The lowest observable effect levels (LOEL) for an endocrine effect were the lowest doses tested, 25 mg/kg/day for Flutamide and 10 mg/kg/day for Vinclozolin.

Phenobarbital is known to be hepatotoxic and affect thyroid function. Treating juvenile males with Phenobarbital was expected to delay preputial separation and reduce the weight of reproductive organs. Treatment did delay preputial separation (100 mg/kg/day), as expected, but did not affect reproductive organ weights. Liver weights were increased in 50 and 100 mg/kg/day Phenobarbital-treated males. In juvenile females, Phenobarbital treatment (PND 22-42/43) was expected to result in delayed vaginal opening and irregular estrous cycling. Treatment did delay vaginal cycling (100 mg/kg/day), but did not disrupt estrous cycling. Treatment in females also increased liver, adrenal and thyroid (100 mg/kg/day) weights. The lowest observable effect level (LOEL) for an endocrine effect was 100 mg/kg/day for Phenobarbital (males and females). No endocrine effect was observed at 25 mg/kg/day.

Methoxychlor and Ethinyl Estradiol are both estrogen receptor agonists and were expected to result in advanced vaginal opening, advanced first estrus and onset of estrous cycling, and/or persistent vaginal estrus. Treatment with Methoxychlor or Ethinyl Estradiol (0.005 mg/kg/day) advanced vaginal opening and first estrus, and disrupted estrous cycling. Ethinyl Estradiol

treatment also resulted in increased adrenal weights. Methoxychlor (50 mg/kg/day) treatment resulted in increased liver, ovary and pituitary weights. The lowest observable effect levels (LOEL) for an endocrine effect were 12.5 mg/kg/day for Methoxychlor and 0.005 mg/kg/day for Ethinyl Estradiol. 12.5 mg/kg/day was the lowest dose tested for Methoxychlor. No endocrine effect was observed at 0.0025 mg/kg/day Ethinyl Estradiol.

CONCLUSIONS

Juvenile male Sprague Dawley rats were treated with Phenobarbital, Vinclozolin, or Flutamide from PND 23 until necropsy on PND 53 or 54. Parameters evaluated included mortality and clinical observations, body weights, day of preputial separation, gross pathology, organ weights and histopathology. The following parameters were affected by treatment.

Test Article	Concentration (mg/kg/day)	Body Weight	Age of Preputial Separation	Organ Weights	Histology
Phenobarbital	25	-	-	↑ liver	-
	50	-	-	↑ liver	-
	100	↓	↑	↑ liver ↓pituitary	-
Vinclozolin	10	-	↑	-	-
	30	-	↑	↓repro. organs ↑ testis,	-
	100	-	↑	↓repro. organs ↑ testis	-
Flutamide	25	-	↑	↓repro. organs ↑ testis, ↓ kidneys	-
	50	-	↑	↓repro. organs ↑ testis, ↓ kidneys, ↑adrenal glands	testis lesions

- = no effect; ↑ = higher than control means; ↓ = lower than control means
repro. = reproductive

Juvenile female Sprague Dawley rats were treated with Phenobarbital, Methoxychlor or Ethinyl Estradiol from PND 22 until necropsy on PND 42 or 43. Parameters evaluated included mortality and clinical observations, body weights, day of vaginal opening, estrous cyclicity, gross pathology, organ weights and histopathology. The following parameters were affected by treatment.

Test Article	Concentration (mg/kg/day)	Age at Vaginal Opening	Age at First Estrus	Irregular Estrous Cycles	Organ Weights
Phenobarbital	25	-	-	-	↑ liver ↑ adrenal
	50	-	-	-	↑ liver ↑ adrenal
	100	↑	-	-	↑ liver ↑ adrenal ↓ thyroid
Methoxychlor	12.5	↓	↓	-	-
	25	↓	↓	X	-
	50	↓	↓	X	↓ liver ↓ ovary ↓ pituitary
Ethinyl Estradiol	0.0025	-	-	-	↑ adrenal
	0.005	↓	↓	X	↑ adrenal

- = no effect; ↑ = higher than control means; ↓ = lower than control means; X = present

The lowest observable effect level (LOEL) for an endocrine effect was 25 mg/kg/day for Flutamide, 10 mg/kg/day for Vinclozolin, 100 mg/kg/day for Phenobarbital, 12.5 mg/kg/day for Methoxychlor, and 0.005 mg/kg/day for Ethinyl Estradiol.

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ABBREVIATIONS

Not all abbreviations listed are used in this report

↑	greater than control
↓	less than control
>	greater than
<	less than
≥	greater than or equal to
≤	less than or equal to
~	approximately
°	degree
%	percent
C	Celsius
F	Fahrenheit
L	liter
mL	milliliter
µL	microliter
g	gram
kg	kilogram
mg	milligram
µg	microgram
ng	nanogram
pg	picogram
cm	centimeter
mm	millimeter
µm	micrometer
sec	second
min	minute
h	hour
d	day
wk	week
rpm	revolutions per minute
NBF	neutral buffered formalin
MRI	Midwest Research Institute
PAI	Pathology Associates, A Division of Charles River
S.E.	standard deviation
RSD	relative standard deviation
TK	toxicokinetic
PK	pharmacokinetic
AUC	area under the curve
C_{max}	maximum concentration
$t_{1/2}$	half-life
SD	study day
GD	gestation day
PND	post-natal day
i.p.	intraperitoneal
i.v.	intravenous
s.c.	subcutaneous
i.m.	intramuscular
EPA	Environmental Protection Agency
FDA	Food and Drug Administration
GLP	Good Laboratory Practices
GMP	Good Manufacturing Practices
IACUC	Institutional Animal Care and Use Committee
ICH	International Conference on Harmonization
MHLW	Ministry of Health, Labor and Welfare
NIEHS	National Institute of Environmental Health Sciences
NTP	National Toxicology Program
OECD	Organization for Economic Co-operation and Development
PHS	Public Health Service
QA	Quality Assurance
QAU	Quality Assurance Unit
SOP	Standard Operating Procedures
USDA	United States Department of Agriculture
LCA	Laboratory Corporation of America
RTI	Research Triangle Institute
PPS	Preputial separation
VO	Vaginal opening

Table 1
F_1 Mortality and Clinical Observations
Pubertal Toxicity Study of Vinclozolin, Flutamide and Phenobarbital in Male Sprague Dawley Rats and Methoxychlor, Ethinyl Estradiol and Phenobarbital in Female Sprague Dawley Rats when Administered in Corn Oil by Oral Gavage

Postnatal Day

Sex: Male

	Group 1	Group 2	Group 3	Group 4	Group 5	Group 6	Group 7	Group 8	Group 9
Mortality									
Found Dead									
Number of Observations	.	.	.	1	.	.	.	.	.
Number of Animals	.	.	.	1	.	.	.	.	.
Days from - to	.	.	.	24 24	.	.	.	.	.
Terminal Kill									
Number of Observations	15	15	15	14	15	15	15	15	15
Number of Animals	15	15	15	14	15	15	15	15	15
Days from - to	53 54	53 54	53 54	53 54	53 54	53 54	53 54	53 54	53 54
Clinical Observations									
Ataxia									
Number of Observations	.	.	.	51	.	.	.	.	.
Number of Animals	.	.	.	14	.	.	.	.	.
Days from - to	.	.	.	23 26	.	.	.	.	.
Languid									
Number of Observations	.	.	.	1	.	.	.	.	.
Number of Animals	.	.	.	1	.	.	.	.	.
Days from - to	.	.	.	24 24	.	.	.	.	.

. = No data
Dose Level: Group 1 - 0 mg/kg/day; Group 2 - 25 mg/kg/day Phenobarbital; Group 3 - 50 mg/kg/day Phenobarbital
Group 4 - 100 mg/kg/day Phenobarbital; Group 5 - 10 mg/kg/day Vinclozolin; Group 6 - 30 mg/kg/day Vinclozolin
Group 7 - 100 mg/kg/day Vinclozolin; Group 8 - 25 mg/kg/day Flutamide; Group 9 - 50 mg/kg/day Flutamide

Table 1 (continued)
F_1 Mortality and Clinical Observations
Pubertal Toxicity Study of Vinclozolin, Flutamide and Phenobarbital in Male Sprague Dawley Rats and Methoxychlor, Ethinyl Estradiol and Phenobarbital in Female Sprague Dawley Rats when Administered in Corn Oil by Oral Gavage

Postnatal Day

Sex: Male

	Group 1	Group 2	Group 3	Group 4	Group 5	Group 6	Group 7	Group 8	Group 9
Clinical Observations									
Prostration									
Number of Observations	.	.	.	3	.	.	.	.	.
Number of Animals	.	.	.	3	.	.	.	.	.
Days from - to	.	.	.	23 23	.	.	.	.	.

. = No data
Dose Level: Group 1 - 0 mg/kg/day; Group 2 - 25 mg/kg/day Phenobarbital; Group 3 - 50 mg/kg/day Phenobarbital
Group 4 - 100 mg/kg/day Phenobarbital; Group 5 - 10 mg/kg/day Vinclozolin; Group 6 - 30 mg/kg/day Vinclozolin
Group 7 - 100 mg/kg/day Vinclozolin; Group 8 - 25 mg/kg/day Flutamide; Group 9 - 50 mg/kg/day Flutamide

Table 1 (continued)
F_1 Mortality and Clinical Observations
Pubertal Toxicity Study of Vinclozolin, Flutamide and Phenobarbital in Male Sprague Dawley Rats and Methoxychlor, Ethinyl Estradiol and Phenobarbital in Female Sprague Dawley Rats when Administered in Corn Oil by Oral Gavage

Postnatal Day

Sex: Female

	Group 1	Group 2	Group 3	Group 4	Group 5	Group 6	Group 7	Group 8	Group 9
Mortality									
Terminal Kill									
Number of Observations	15	15	15	15	15	15	15	15	15
Number of Animals	15	15	15	15	15	15	15	15	15
Days from - to	42 43	42 43	42 43	42 43	42 43	42 43	42 43	42 43	42 43
Clinical Observations									
Ataxia									
Number of Observations	.	.	.	.	.	.	.	.	55
Number of Animals	.	.	.	.	.	.	.	.	15
Days from - to	.	.	.	.	.	.	.	.	22 26
Missing anatomy, Tip of tail									
Number of Observations	.	.	.	.	.	.	2	.	.
Number of Animals	.	.	.	.	.	.	1	.	.
Days from - to	.	.	.	.	.	.	36 42	.	.
Prostration									
Number of Observations	.	.	.	.	.	.	.	.	8
Number of Animals	.	.	.	.	.	.	.	.	8
Days from - to	.	.	.	.	.	.	.	.	23 24

. = No data
Dose Level: Group 1 - 0 mg/kg/day; Group 2 - 0.0025 mg/kg/day Ethinyl Estradiol; Group 3 - 0.005 mg/kg/day Ethinyl Estradiol
Group 4 - 12.5 mg/kg/day Methoxychlor; Group 5 - 25 mg/kg/day Methoxychlor; Group 6 - 50 mg/kg/day Methoxychlor
Group 7 - 25 mg/kg/day Phenobarbital; Group 8 - 50 mg/kg/day Phenobarbital; Group 9 - 100 mg/kg/day Phenobarbital

Table 2
F_1 Summary of Body Weights (Grams)
Pubertal Toxicity Study of Vinclozolin, Flutamide and Phenobarbital in Male Sprague Dawley Rats and Methoxychlor, Ethinyl Estradiol and Phenobarbital in Female Sprague Dawley Rats when Administered in Corn Oil by Oral Gavage

		Postnatal Day							
Group Sex		23	24	25	26	27	28	29	30
1M	Mean	67.41	74.5	81.41	88.03	93.61	101.37	109.01	115.59
	S.E.	0.995	1.145	1.318	1.318	1.32	1.551	1.551	1.603
	N	(15)	(15)	(15)	(15)	(15)	(15)	(15)	(15)
2M	Mean	67.32	75.61	81.9	87.3	92.23	99.77	107.25	114.33
	S.E.	1.151	1.176	1.288	1.278	1.282	1.382	1.323	1.476
	N	(15)	(15)	(15)	(15)	(15)	(15)	(15)	(15)
3M	Mean	67.84	76.46	83.67	89.12	92.21	101.23	108.9	116.19
	S.E.	0.998	1.089	1.136	1.388	2.32	1.851	1.88	2.023
	N	(15)	(15)	(15)	(15)	(15)	(15)	(15)	(15)
4M	Mean	67.53	72.62	79.88	85.54	90.52	97.44	104.13	110.15
	S.E.	1.116	2.052	1.978	1.914	2.065	2.198	2.38	2.393
	N	(15)	(14)	(14)	(14)	(14)	(14)	(14)	(14)
	P	0.987	0.259	0.338	0.397	0.693	0.38	0.213	0.123

Dose Level: Group 1 - 0 mg/kg/day Group 2 - 25 mg/kg/day Phenobarbital Group 3 - 50 mg/kg/day Phenobarbital
Group 4 - 100 mg/kg/day Phenobarbital
M = Male P = P-value from ANOVA
Statistical Significance: * = P<0.05 ** = P<0.01

Table 2 (continued)
F_1 Summary of Body Weights (Grams)
Pubertal Toxicity Study of Vinclozolin, Flutamide and Phenobarbital in Male Sprague Dawley Rats and Methoxychlor, Ethinyl Estradiol and Phenobarbital in Female Sprague Dawley Rats when Administered in Corn Oil by Oral Gavage

		Postnatal Day							
Group Sex		31	32	33	34	35	36	37	38
1M	Mean	124.65	133.01	141.71	150.51	159.12	167.57	176.91	186.91
	S.E.	1.767	1.853	1.847	1.927	2.075	2.334	2.398	2.268
	N	(15)	(15)	(15)	(15)	(15)	(15)	(15)	(15)
2M	Mean	122.3	131.12	139.49	148.53	156.94	166.77	175.33	183.71
	S.E.	1.516	1.595	1.664	1.855	1.86	2.02	2.266	2.159
	N	(15)	(15)	(15)	(15)	(15)	(15)	(15)	(15)
3M	Mean	124.41	133.61	141.72	151.38	160.85	170.22	179.21	187.69
	S.E.	2.241	2.381	2.729	2.909	3.228	3.207	3.575	3.961
	N	(15)	(15)	(15)	(15)	(15)	(15)	(15)	(15)
4M	Mean	117.53	*125.53	133.76	*140.31	150.24	158.48	*166.26	*175.44
	S.E.	2.754	2.707	3.05	3.259	2.987	3.104	3.438	3.61
	N	(14)	(14)	(14)	(14)	(14)	(14)	(14)	(14)
	P	0.077	0.047	0.074	0.014	0.033	0.023	0.02	0.03

Dose Level: Group 1 - 0 mg/kg/day Group 2 - 25 mg/kg/day Phenobarbital Group 3 - 50 mg/kg/day Phenobarbital
Group 4 - 100 mg/kg/day Phenobarbital
M = Male P = P-value from ANOVA
Statistical Significance: * = P<0.05 ** = P<0.01

Table 2 (continued)
F_1 Summary of Body Weights (Grams)
Pubertal Toxicity Study of Vinclozolin, Flutamide and Phenobarbital in Male Sprague Dawley Rats and Methoxychlor, Ethinyl Estradiol and Phenobarbital in Female Sprague Dawley Rats when Administered in Corn Oil by Oral Gavage

		Postnatal Day							
Group Sex		39	40	41	42	43	44	45	46
1M	Mean	194.56	204.97	214.68	221.04	230.14	238.04	248.01	256.57
	S.E.	2.567	2.579	3.04	3.08	2.927	3.018	3.195	3.623
	N	(15)	(15)	(15)	(15)	(15)	(15)	(15)	(15)
2M	Mean	191.99	201.73	209.6	216.95	225.71	232.52	242.91	250.89
	S.E.	2.454	2.613	2.685	2.828	2.948	3.084	3.362	3.537
	N	(15)	(15)	(15)	(15)	(15)	(15)	(15)	(15)
3M	Mean	197.33	206.68	214.27	223.15	231.69	238.98	249.32	257.61
	S.E.	4.093	3.961	4.434	4.615	4.836	4.789	4.888	5.161
	N	(15)	(15)	(15)	(15)	(15)	(15)	(15)	(15)
4M	Mean	183.64	*191.54	*199.88	*207.14	*215.91	*222.39	*232.31	**237.51
	S.E.	3.901	3.946	3.929	4.006	4.399	4.56	4.592	4.802
	N	(14)	(14)	(14)	(14)	(14)	(14)	(14)	(14)
	P	0.035	0.012	0.019	0.019	0.027	0.018	0.021	0.007

Dose Level: Group 1 - 0 mg/kg/day Group 2 - 25 mg/kg/day Phenobarbital Group 3 - 50 mg/kg/day Phenobarbital
Group 4 - 100 mg/kg/day Phenobarbital
M = Male P = P-value from ANOVA
Statistical Significance: * = P<0.05 ** = P<0.01

Table 2 (continued)
F_1 Summary of Body Weights (Grams)
Pubertal Toxicity Study of Vinclozolin, Flutamide and Phenobarbital in Male Sprague Dawley Rats and Methoxychlor, Ethinyl Estradiol and Phenobarbital in Female Sprague Dawley Rats when Administered in Corn Oil by Oral Gavage

Group Sex		Postnatal Day 47	48	49	50	51	52	53	54
1M	Mean	265.57	274.67	284.29	293.03	300.39	310.29	320.99	327.89
	S.E.	3.524	3.86	3.965	4.557	4.401	4.544	5.006	4.417
	N	(15)	(15)	(15)	(15)	(15)	(15)	(15)	(7)
2M	Mean	259.47	267.99	278.07	285.85	292.81	302.83	312.19	316.91
	S.E.	3.717	4.007	4.081	4.167	4.601	4.62	4.734	6.767
	N	(15)	(15)	(15)	(15)	(15)	(15)	(15)	(7)
3M	Mean	266.62	275.02	284.59	293.35	297.35	308.68	317.86	322.83
	S.E.	5.324	5.501	5.207	5.548	5.594	5.538	5.656	8.609
	N	(15)	(15)	(15)	(15)	(15)	(15)	(15)	(7)
4M	Mean	*246.69	*255.14	*264.41	*272.61	*278.00	**286.87	**293.56	**290.89
	S.E.	4.933	5.302	5.716	6.212	6.069	6.02	5.79	4.093
	N	(14)	(14)	(14)	(14)	(14)	(14)	(14)	(7)
	P	0.01	0.015	0.015	0.022	0.02	0.011	0.003	0.002

Dose Level: Group 1 - 0 mg/kg/day Group 2 - 25 mg/kg/day Phenobarbital Group 3 - 50 mg/kg/day Phenobarbital
Group 4 - 100 mg/kg/day Phenobarbital
M = Male P = P-value from ANOVA
Statistical Significance: * = P<0.05 ** = P<0.01

Table 2 (continued)
F_1 Summary of Body Weights (Grams)
Pubertal Toxicity Study of Vinclozolin, Flutamide and Phenobarbital in Male Sprague Dawley Rats and Methoxychlor, Ethinyl Estradiol and Phenobarbital in Female Sprague Dawley Rats when Administered in Corn Oil by Oral Gavage

		Postnatal Day							
Group Sex		23	24	25	26	27	28	29	30
1M	Mean	67.41	74.50	81.41	88.03	93.61	101.37	109.01	115.59
	S.E.	1.00	1.15	1.32	1.32	1.32	1.55	1.55	1.60
	N	(15)	(15)	(15)	(15)	(15)	(15)	(15)	(15)
5M	Mean	67.65	74.75	82.09	88.85	96.05	102.52	111.45	118.17
	S.E.	0.81	0.81	0.79	1.00	1.08	1.27	1.31	1.30
	N	(15)	(15)	(15)	(15)	(15)	(15)	(15)	(15)
6M	Mean	68.08	75.25	82.27	89.54	96.41	104.05	111.63	118.92
	S.E.	1.13	1.06	1.30	1.50	1.38	1.61	1.65	1.60
	N	(15)	(15)	(15)	(15)	(15)	(15)	(15)	(15)
7M	Mean	67.91	74.05	81.20	87.17	93.83	101.11	108.69	113.96
	S.E.	1.02	1.19	1.27	1.48	1.56	1.79	1.80	1.52
	N	(15)	(15)	(15)	(15)	(15)	(15)	(15)	(15)
	P	0.967	0.880	0.903	0.626	0.325	0.535	0.417	0.086

Dose Level: Group 1 - 0 mg/kg/day Group 5 - 10 mg/kg/day Vinclozolin Group 6 - 30 mg/kg/day Vinclozolin
Group 7 - 100 mg/kg/day Vinclozolin
M = Male P = P-value from ANOVA
Statistical Significance: * = P<0.05 ** = P<0.01

Table 2 (continued)
F_1 Summary of Body Weights (Grams)
Pubertal Toxicity Study of Vinclozolin, Flutamide and Phenobarbital in Male Sprague Dawley Rats and Methoxychlor, Ethinyl Estradiol and Phenobarbital in Female Sprague Dawley Rats when Administered in Corn Oil by Oral Gavage

		Postnatal Day							
Group Sex		31	32	33	34	35	36	37	38
1M	Mean	124.65	133.01	141.71	150.51	159.12	167.57	176.91	186.91
	S.E.	1.77	1.85	1.85	1.93	2.08	2.33	2.40	2.27
	N	(15)	(15)	(15)	(15)	(15)	(15)	(15)	(15)
5M	Mean	128.05	135.83	144.88	154.11	162.53	170.75	179.69	190.48
	S.E.	1.73	1.84	1.98	2.33	2.33	2.45	2.57	2.83
	N	(15)	(15)	(15)	(15)	(15)	(15)	(15)	(15)
6M	Mean	129.15	137.79	146.51	156.69	165.34	174.13	183.94	194.29
	S.E.	1.92	1.90	2.02	2.38	2.44	2.44	2.73	2.88
	N	(15)	(15)	(15)	(15)	(15)	(15)	(15)	(15)
7M	Mean	124.11	131.71	140.06	149.93	158.16	166.84	174.99	185.30
	S.E.	1.93	2.15	2.23	2.51	2.51	2.66	3.05	3.29
	N	(15)	(15)	(15)	(15)	(15)	(15)	(15)	(15)
	P	0.152	0.124	0.110	0.137	0.130	0.154	0.114	0.127

Dose Level: Group 1 - 0 mg/kg/day Group 5 - 10 mg/kg/day Vinclozolin Group 6 - 30 mg/kg/day Vinclozolin
Group 7 - 100 mg/kg/day Vinclozolin
M = Male P = P-value from ANOVA
Statistical Significance: * = P<0.05 ** = P<0.01

Table 2 (continued)
F_1 Summary of Body Weights (Grams)
Pubertal Toxicity Study of Vinclozolin, Flutamide and Phenobarbital in Male Sprague Dawley Rats and Methoxychlor, Ethinyl Estradiol and Phenobarbital in Female Sprague Dawley Rats when Administered in Corn Oil by Oral Gavage

		Postnatal Day							
Group Sex		39	40	41	42	43	44	45	46
1M	Mean	194.56	204.97	214.68	221.04	230.14	238.04	248.01	256.57
	S.E.	2.57	2.58	3.04	3.08	2.93	3.02	3.20	3.62
	N	(15)	(15)	(15)	(15)	(15)	(15)	(15)	(15)
5M	Mean	199.01	209.04	217.38	225.75	234.51	242.58	251.71	261.82
	S.E.	3.12	3.21	3.38	3.64	3.55	3.92	3.93	4.19
	N	(15)	(15)	(15)	(15)	(15)	(15)	(15)	(15)
6M	Mean	203.37	213.53	223.31	231.95	241.57	248.16	258.39	268.71
	S.E.	3.17	3.09	3.20	3.52	3.66	3.68	3.94	4.23
	N	(15)	(15)	(15)	(15)	(15)	(15)	(15)	(15)
7M	Mean	193.39	202.75	210.88	218.99	228.31	235.07	244.12	253.15
	S.E.	3.25	3.66	3.84	4.02	4.12	4.38	4.25	4.77
	N	(15)	(15)	(15)	(15)	(15)	(15)	(15)	(15)
	P	0.092	0.091	0.078	0.062	0.054	0.089	0.069	0.063

Dose Level: Group 1 - 0 mg/kg/day Group 5 - 10 mg/kg/day Vinclozolin Group 6 - 30 mg/kg/day Vinclozolin
Group 7 - 100 mg/kg/day Vinclozolin
M = Male P = P-value from ANOVA
Statistical Significance: * = P<0.05 ** = P<0.01

Table 2 (continued)
F_1 Summary of Body Weights (Grams)
Pubertal Toxicity Study of Vinclozolin, Flutamide and Phenobarbital in Male Sprague Dawley Rats and Methoxychlor, Ethinyl Estradiol and Phenobarbital in Female Sprague Dawley Rats when Administered in Corn Oil by Oral Gavage

		Postnatal Day							
Group Sex		47	48	49	50	51	52	53	54
1M	Mean	265.57	274.67	284.29	293.03	300.39	310.29	320.99	327.89
	S.E.	3.52	3.86	3.97	4.56	4.40	4.54	5.01	4.42
	N	(15)	(15)	(15)	(15)	(15)	(15)	(15)	(7)
5M	Mean	270.39	280.20	289.89	299.51	306.17	317.29	328.37	338.61
	S.E.	4.43	4.55	4.85	5.05	4.99	5.33	5.67	10.35
	N	(15)	(15)	(15)	(15)	(15)	(15)	(15)	(7)
6M	Mean	277.75	287.28	298.49	308.17	315.96	327.75	339.25	353.03
	S.E.	4.10	4.51	4.46	4.76	4.92	5.05	5.25	8.92
	N	(15)	(15)	(15)	(15)	(15)	(15)	(15)	(7)
7M	Mean	261.31	270.03	280.28	287.86	294.35	304.25	313.17	312.14
	S.E.	4.84	5.09	5.11	5.42	5.52	5.91	5.85	10.58
	N	(15)	(15)	(15)	(15)	(15)	(15)	(15)	(7)
	P	0.051	0.055	0.042	0.033	0.023	0.016	0.010	0.024

Dose Level: Group 1 - 0 mg/kg/day Group 5 - 10 mg/kg/day Vinclozolin Group 6 - 30 mg/kg/day Vinclozolin
Group 7 - 100 mg/kg/day Vinclozolin
M = Male P = P-value from ANOVA
Statistical Significance: * = P<0.05 ** = P<0.01

Table 2 (continued)
F_1 Summary of Body Weights (Grams)
Pubertal Toxicity Study of Vinclozolin, Flutamide and Phenobarbital in Male Sprague Dawley Rats and Methoxychlor, Ethinyl Estradiol and Phenobarbital in Female Sprague Dawley Rats when Administered in Corn Oil by Oral Gavage

Group Sex		Postnatal Day 23	24	25	26	27	28	29	30
1M	Mean	67.41	74.50	81.41	88.03	93.61	101.37	109.01	115.59
	S.E.	0.995	1.145	1.318	1.318	1.32	1.551	1.551	1.603
	N	(15)	(15)	(15)	(15)	(15)	(15)	(15)	(15)
8M	Mean	66.93	73.83	80.53	86.52	92.42	98.94	106.62	112.74
	S.E.	0.85	0.923	0.918	1.13	1.165	1.177	1.455	1.33
	N	(15)	(15)	(15)	(15)	(15)	(15)	(15)	(15)
9M	Mean	67.06	73.49	80.36	86.07	91.68	98.81	106.75	112.90
	S.E.	1.054	1.015	1.21	1.339	1.433	1.504	1.677	1.775
	N	(15)	(15)	(15)	(15)	(15)	(15)	(15)	(15)
	P	0.938	0.783	0.792	0.525	0.579	0.367	0.483	0.366

Dose Level: Group 1 - 0 mg/kg/day Group 8 - 25 mg/kg/day Flutamide Group 9 - 50 mg/kg/day Flutamide
M = Male P = P-value from ANOVA
Statistical Significance: * = P<0.05 ** = P<0.01

Table 2 (continued)
F_1 Summary of Body Weights (Grams)
Pubertal Toxicity Study of Vinclozolin, Flutamide and Phenobarbital in Male Sprague Dawley Rats and Methoxychlor, Ethinyl Estradiol and Phenobarbital in Female Sprague Dawley Rats when Administered in Corn Oil by Oral Gavage

Group Sex		Postnatal Day 31	32	33	34	35	36	37	38
1M	Mean	124.65	133.01	141.71	150.51	159.12	167.57	176.91	186.91
	S.E.	1.767	1.853	1.847	1.927	2.075	2.334	2.398	2.268
	N	(15)	(15)	(15)	(15)	(15)	(15)	(15)	(15)
8M	Mean	121.75	129.97	137.57	146.39	154.87	162.52	171.71	180.59
	S.E.	1.432	1.673	1.854	2.092	2.3	2.39	2.493	2.565
	N	(15)	(15)	(15)	(15)	(15)	(15)	(15)	(15)
9M	Mean	122.43	130.02	138.26	147.45	155.88	164.50	172.14	181.82
	S.E.	1.955	2.286	2.498	2.581	2.891	3.027	3.171	3.526
	N	(15)	(15)	(15)	(15)	(15)	(15)	(15)	(15)
	P	0.469	0.459	0.332	0.403	0.445	0.392	0.331	0.260

Dose Level: Group 1 - 0 mg/kg/day Group 8 - 25 mg/kg/day Flutamide Group 9 - 50 mg/kg/day Flutamide
M = Male P = P-value from ANOVA
Statistical Significance: * = P<0.05 ** = P<0.01

Table 2 (continued)
F_1 Summary of Body Weights (Grams)
Pubertal Toxicity Study of Vinclozolin, Flutamide and Phenobarbital in Male Sprague Dawley Rats and Methoxychlor, Ethinyl Estradiol and Phenobarbital in Female Sprague Dawley Rats when Administered in Corn Oil by Oral Gavage

Group Sex		Postnatal Day 39	40	41	42	43	44	45	46
1M	Mean	194.56	204.97	214.68	221.04	230.14	238.04	248.01	256.57
	S.E.	2.567	2.579	3.04	3.08	2.927	3.018	3.195	3.623
	N	(15)	(15)	(15)	(15)	(15)	(15)	(15)	(15)
8M	Mean	189.69	198.03	205.25	214.09	223.20	231.05	240.18	248.67
	S.E.	2.765	3	3.258	3.224	3.31	3.491	3.487	3.533
	N	(15)	(15)	(15)	(15)	(15)	(15)	(15)	(15)
9M	Mean	189.81	198.59	205.87	214.09	222.46	229.69	238.20	246.39
	S.E.	3.737	4.033	4.324	4.45	4.57	4.931	5.185	5.498
	N	(15)	(15)	(15)	(15)	(15)	(15)	(15)	(15)
	P	0.447	0.258	0.127	0.307	0.275	0.278	0.207	0.228

Dose Level: Group 1 - 0 mg/kg/day Group 8 - 25 mg/kg/day Flutamide Group 9 - 50 mg/kg/day Flutamide
M = Male P = P-value from ANOVA
Statistical Significance: * = P<0.05 ** = P<0.01

Table 2 (continued)
F_1 Summary of Body Weights (Grams)
Pubertal Toxicity Study of Vinclozolin, Flutamide and Phenobarbital in Male Sprague Dawley Rats and Methoxychlor, Ethinyl Estradiol and Phenobarbital in Female Sprague Dawley Rats when Administered in Corn Oil by Oral Gavage

Group Sex		Postnatal Day 47	48	49	50	51	52	53	54
1M	Mean	265.57	274.67	284.29	293.03	300.39	310.29	320.99	327.89
	S.E.	3.524	3.86	3.965	4.557	4.401	4.544	5.006	4.417
	N	(15)	(15)	(15)	(15)	(15)	(15)	(15)	(7)
8M	Mean	256.55	264.61	274.61	283.38	289.27	299.99	310.03	313.84
	S.E.	3.755	3.773	3.858	4.231	4.286	4.212	4.332	7.2
	N	(15)	(15)	(15)	(15)	(15)	(15)	(15)	(7)
9M	Mean	254.90	261.95	271.54	280.41	284.47	295.26	305.21	*306.01
	S.E.	5.725	5.73	6.181	6.256	6.436	6.478	6.811	7.203
	N	(15)	(15)	(15)	(15)	(15)	(15)	(15)	(7)
	P	0.200	0.126	0.158	0.199	0.092	0.123	0.126	0.076

Dose Level: Group 1 - 0 mg/kg/day Group 8 - 25 mg/kg/day Flutamide Group 9 - 50 mg/kg/day Flutamide
M = Male P = P-value from ANOVA
Statistical Significance: * = P<0.05 ** = P<0.01

Table 2 (continued)
F_1 Summary of Body Weights (Grams)
Pubertal Toxicity Study of Vinclozolin, Flutamide and Phenobarbital in Male Sprague Dawley Rats and Methoxychlor, Ethinyl Estradiol and Phenobarbital in Female Sprague Dawley Rats when Administered in Corn Oil by Oral Gavage

		Postnatal Day						
Group Sex		22	23	24	25	26	27	28
1F	Mean	58.87	64.26	70.27	75.57	80.94	86.63	92.89
	S.E.	0.755	0.762	0.807	0.925	1.033	0.944	1.037
	N	(15)	(15)	(15)	(15)	(15)	(15)	(15)
2F	Mean	58.17	64.58	70.75	75.62	81.48	87.06	93.81
	S.E.	0.884	0.84	0.946	1.052	1.23	1.308	1.471
	N	(15)	(15)	(15)	(15)	(15)	(15)	(15)
3F	Mean	58.03	64.23	70.17	75.59	81.17	86.37	93.11
	S.E.	0.999	1.054	1.065	1.215	1.366	1.487	1.551
	N	(15)	(15)	(15)	(15)	(15)	(15)	(15)
	P	0.774	0.954	0.900	0.999	0.952	0.928	0.883

Dose Level: Group 1 - 0 mg/kg/day Group 2 - 0.0025 mg/kg/day Ethinyl Estradiol Group 3 - 0.005 mg/kg/day Ethinyl Estradiol
F = Female P = P-value from ANOVA
Statistical Significance: * = P<0.05 ** = P<0.01

Table 2 (continued)
F_1 Summary of Body Weights (Grams)
Pubertal Toxicity Study of Vinclozolin, Flutamide and Phenobarbital in Male Sprague Dawley Rats and Methoxychlor, Ethinyl Estradiol and Phenobarbital in Female Sprague Dawley Rats when Administered in Corn Oil by Oral Gavage

		Postnatal Day						
Group Sex		29	30	31	32	33	34	35
1F	Mean	99.50	104.96	112.10	119.06	124.73	131.91	137.22
	S.E.	1.169	1.151	1.24	1.425	1.456	1.57	1.847
	N	(15)	(15)	(15)	(15)	(15)	(15)	(15)
2F	Mean	99.59	105.10	111.25	117.61	123.04	129.94	135.40
	S.E.	1.626	1.59	1.928	1.983	2.065	2.134	2.456
	N	(15)	(15)	(15)	(15)	(15)	(15)	(15)
3F	Mean	97.96	104.19	110.77	117.39	122.32	131.35	137.76
	S.E.	1.758	1.926	2.097	2.269	2.769	2.623	2.709
	N	(15)	(15)	(15)	(15)	(15)	(15)	(15)
	P	0.704	0.909	0.869	0.802	0.722	0.801	0.762

Dose Level: Group 1 - 0 mg/kg/day Group 2 - 0.0025 mg/kg/day Ethinyl Estradiol Group 3 - 0.005 mg/kg/day Ethinyl Estradiol
F = Female P = P-value from ANOVA
Statistical Significance: * = P<0.05 ** = P<0.01

Table 2 (continued)
F_1 Summary of Body Weights (Grams)
Pubertal Toxicity Study of Vinclozolin, Flutamide and Phenobarbital in Male Sprague Dawley Rats and Methoxychlor, Ethinyl Estradiol and Phenobarbital in Female Sprague Dawley Rats when Administered in Corn Oil by Oral Gavage

Group Sex		Postnatal Day 36	37	38	39	40	41	42	43
1F	Mean	142.27	149.29	156.01	158.89	163.83	166.39	169.83	169.06
	S.E.	1.647	1.797	1.91	2.112	1.804	2.219	2.002	2.891
	N	(15)	(15)	(15)	(15)	(15)	(15)	(15)	(7)
2F	Mean	140.89	147.29	153.35	157.89	161.71	165.49	169.01	165.84
	S.E.	2.38	2.391	2.783	2.602	2.597	2.628	2.808	3.267
	N	(15)	(15)	(15)	(15)	(15)	(15)	(15)	(7)
3F	Mean	142.87	149.01	154.78	160.03	163.69	166.74	171.55	166.63
	S.E.	3.018	3.155	3.312	3.086	3.407	3.78	3.728	3.648
	N	(15)	(15)	(15)	(15)	(15)	(15)	(15)	(7)
	P	0.839	0.832	0.789	0.847	0.824	0.953	0.823	0.774

Dose Level: Group 1 - 0 mg/kg/day Group 2 - 0.0025 mg/kg/day Ethinyl Estradiol Group 3 - 0.005 mg/kg/day Ethinyl Estradiol
F = Female P = P-value from ANOVA
Statistical Significance: * = P<0.05 ** = P<0.01

Table 2 (continued)
F_1 Summary of Body Weights (Grams)
Pubertal Toxicity Study of Vinclozolin, Flutamide and Phenobarbital in Male Sprague Dawley Rats and Methoxychlor, Ethinyl Estradiol and Phenobarbital in Female Sprague Dawley Rats when Administered in Corn Oil by Oral Gavage

Group Sex		Postnatal Day 22	23	24	25	26	27	28
1F	Mean	58.87	64.26	70.27	75.57	80.94	86.63	92.89
	S.E.	0.76	0.76	0.81	0.93	1.03	0.94	1.04
	N	(15)	(15)	(15)	(15)	(15)	(15)	(15)
4F	Mean	58.97	65.36	70.90	76.60	81.85	87.07	93.51
	S.E.	1.00	0.91	1.05	1.23	1.40	1.40	1.54
	N	(15)	(15)	(15)	(15)	(15)	(15)	(15)
5F	Mean	58.32	64.45	70.46	76.26	81.74	87.31	92.84
	S.E.	1.15	1.18	1.26	1.36	1.48	1.61	1.60
	N	(15)	(15)	(15)	(15)	(15)	(15)	(15)
6F	Mean	57.47	63.13	68.28	74.48	79.25	84.92	91.57
	S.E.	1.00	1.04	1.11	1.06	1.20	1.25	1.27
	N	(15)	(15)	(15)	(15)	(15)	(15)	(15)
	P	0.695	0.465	0.325	0.582	0.466	0.579	0.789

Dose Level: Group 1 - 0 mg/kg/day Group 4 - 12.5 mg/kg/day Methoxychlor Group 5 - 25 mg/kg/day Methoxychlor
Group 6 - 50 mg/kg/day Methoxychlor
F = Female P = P-value from ANOVA
Statistical Significance: * = P<0.05 ** = P<0.01

Table 2 (continued)
F_1 Summary of Body Weights (Grams)
Pubertal Toxicity Study of Vinclozolin, Flutamide and Phenobarbital in Male Sprague Dawley Rats and Methoxychlor, Ethinyl Estradiol and Phenobarbital in Female Sprague Dawley Rats when Administered in Corn Oil by Oral Gavage

		Postnatal Day						
Group Sex		29	30	31	32	33	34	35
1F	Mean	99.50	104.96	112.10	119.06	124.73	131.91	137.22
	S.E.	1.17	1.15	1.24	1.43	1.46	1.57	1.85
	N	(15)	(15)	(15)	(15)	(15)	(15)	(15)
4F	Mean	99.31	104.99	110.81	117.12	123.04	126.72	135.25
	S.E.	1.76	1.92	2.21	2.28	2.21	1.90	2.39
	N	(15)	(15)	(15)	(15)	(15)	(15)	(15)
5F	Mean	97.91	105.07	110.77	117.10	122.03	126.97	134.63
	S.E.	1.78	1.82	2.05	2.11	1.97	3.17	2.26
	N	(15)	(15)	(15)	(15)	(15)	(15)	(15)
6F	Mean	96.45	102.63	107.95	113.63	119.05	125.97	131.53
	S.E.	1.35	1.45	1.35	1.42	1.35	1.52	1.60
	N	(15)	(15)	(15)	(15)	(15)	(15)	(15)
	P	0.471	0.654	0.406	0.227	0.160	0.200	0.275

Dose Level: Group 1 - 0 mg/kg/day Group 4 - 12.5 mg/kg/day Methoxychlor Group 5 - 25 mg/kg/day Methoxychlor
Group 6 - 50 mg/kg/day Methoxychlor
F = Female P = P-value from ANOVA
Statistical Significance: * = P<0.05 ** = P<0.01

Table 2 (continued)
F_1 Summary of Body Weights (Grams)
Pubertal Toxicity Study of Vinclozolin, Flutamide and Phenobarbital in Male Sprague Dawley Rats and Methoxychlor, Ethinyl Estradiol and Phenobarbital in Female Sprague Dawley Rats when Administered in Corn Oil by Oral Gavage

		Postnatal Day							
Group Sex		36	37	38	39	40	41	42	43
1F	Mean	142.27	149.29	156.01	158.89	163.83	166.39	169.83	169.06
	S.E.	1.65	1.80	1.91	2.11	1.80	2.22	2.00	2.89
	N	(15)	(15)	(15)	(15)	(15)	(15)	(15)	(7)
4F	Mean	139.78	145.27	152.11	155.37	160.01	161.65	165.79	163.84
	S.E.	2.17	2.17	2.61	2.70	2.80	2.67	2.33	3.14
	N	(15)	(15)	(15)	(15)	(15)	(15)	(15)	(7)
5F	Mean	138.37	147.85	153.99	157.57	162.73	167.13	171.03	164.47
	S.E.	3.95	2.98	2.81	2.89	3.22	3.12	3.44	2.56
	N	(15)	(15)	(15)	(15)	(15)	(15)	(15)	(7)
6F	Mean	136.35	142.53	*147.99	151.46	155.85	159.50	163.54	165.50
	S.E.	1.58	1.80	1.72	1.66	1.74	1.84	1.82	3.47
	N	(15)	(15)	(15)	(15)	(15)	(15)	(15)	(7)
	P	(c)	0.163	0.099	0.149	0.116	0.103	0.128	0.628

Dose Level: Group 1 - 0 mg/kg/day Group 4 - 12.5 mg/kg/day Methoxychlor Group 5 - 25 mg/kg/day Methoxychlor
Group 6 - 50 mg/kg/day Methoxychlor
F = Female P = P-value from ANOVA
Statistical Significance: * = P<0.05 ** = P<0.01

Table 2 (continued)
F_1 Summary of Body Weights (Grams)
Pubertal Toxicity Study of Vinclozolin, Flutamide and Phenobarbital in Male Sprague Dawley Rats and Methoxychlor, Ethinyl Estradiol and Phenobarbital in Female Sprague Dawley Rats when Administered in Corn Oil by Oral Gavage

Group Sex		Postnatal Day 22	23	24	25	26	27	28
1F	Mean	58.87	64.26	70.27	75.57	80.94	86.63	92.89
	S.E.	0.76	0.76	0.81	0.93	1.03	0.94	1.04
	N	(15)	(15)	(15)	(15)	(15)	(15)	(15)
7F	Mean	56.70	64.64	70.13	75.31	80.56	86.23	92.42
	S.E.	0.77	0.98	1.12	1.17	1.18	1.24	1.38
	N	(15)	(15)	(15)	(15)	(15)	(15)	(15)
8F	Mean	57.96	66.01	71.83	76.84	81.53	87.63	93.45
	S.E.	1.07	1.24	1.23	1.32	1.42	1.55	1.75
	N	(15)	(15)	(15)	(15)	(15)	(15)	(15)
9F	Mean	58.14	64.35	69.26	74.41	78.07	83.65	88.61
	S.E.	0.97	1.43	1.49	1.39	1.51	1.55	1.58
	N	(15)	(15)	(15)	(15)	(15)	(15)	(15)
	P	0.399	0.670	0.492	0.568	0.258	0.203	0.092

Dose Level: Group 1 - 0 mg/kg/day Group 7 - 25 mg/kg/day Phenobarbital Group 8 - 50 mg/kg/day Phenobarbital
Group 9 - 100 mg/kg/day Phenobarbital
F = Female P = P-value from ANOVA
Statistical Significance: * = P<0.05 ** = P<0.01

Table 2 (continued)
F_1 Summary of Body Weights (Grams)
Pubertal Toxicity Study of Vinclozolin, Flutamide and Phenobarbital in Male Sprague Dawley Rats and Methoxychlor, Ethinyl Estradiol and Phenobarbital in Female Sprague Dawley Rats when Administered in Corn Oil by Oral Gavage

Group Sex		Postnatal Day 29	30	31	32	33	34	35
1F	Mean	99.50	104.96	112.10	119.06	124.73	131.91	137.22
	S.E.	1.17	1.15	1.24	1.43	1.46	1.57	1.85
	N	(15)	(15)	(15)	(15)	(15)	(15)	(15)
7F	Mean	97.44	104.21	111.07	116.97	120.57	130.15	134.83
	S.E.	1.41	1.48	1.73	1.97	1.74	1.95	2.06
	N	(15)	(15)	(15)	(15)	(15)	(15)	(15)
8F	Mean	98.87	105.44	111.25	118.30	121.56	130.82	136.32
	S.E.	1.89	1.88	2.01	2.18	3.67	3.48	3.13
	N	(15)	(15)	(15)	(15)	(15)	(15)	(15)
9F	Mean	95.15	100.21	106.99	113.40	119.17	127.55	131.91
	S.E.	1.58	1.65	1.65	1.99	2.07	2.29	2.53
	N	(15)	(15)	(15)	(15)	(15)	(15)	(15)
	P	0.203	0.084	0.149	0.173	0.412	0.629	0.443

Dose Level: Group 1 - 0 mg/kg/day Group 7 - 25 mg/kg/day Phenobarbital Group 8 - 50 mg/kg/day Phenobarbital
Group 9 - 100 mg/kg/day Phenobarbital
F = Female P = P-value from ANOVA
Statistical Significance: * = P<0.05 ** = P<0.01

Table 2 (continued)
F_1 Summary of Body Weights (Grams)
Pubertal Toxicity Study of Vinclozolin, Flutamide and Phenobarbital in Male Sprague Dawley Rats and Methoxychlor, Ethinyl Estradiol and Phenobarbital in Female Sprague Dawley Rats when Administered in Corn Oil by Oral Gavage

		Postnatal Day							
Group Sex		36	37	38	39	40	41	42	43
1F	Mean	142.27	149.29	156.01	158.89	163.83	166.39	169.83	169.06
	S.E.	1.65	1.80	1.91	2.11	1.80	2.22	2.00	2.89
	N	(15)	(15)	(15)	(15)	(15)	(15)	(15)	(7)
7F	Mean	142.23	146.95	152.75	157.84	162.93	165.74	170.39	166.51
	S.E.	2.03	2.56	2.53	2.55	2.69	3.02	3.70	4.66
	N	(15)	(15)	(15)	(15)	(15)	(15)	(15)	(7)
8F	Mean	140.79	148.48	154.14	159.40	163.66	167.56	173.11	167.57
	S.E.	2.97	2.99	3.13	3.24	3.14	3.21	3.36	4.42
	N	(15)	(15)	(15)	(15)	(15)	(15)	(15)	(7)
9F	Mean	137.71	144.95	150.31	156.85	160.81	165.54	169.15	168.56
	S.E.	2.30	2.41	2.85	3.22	3.32	3.41	3.13	3.98
	N	(15)	(15)	(15)	(15)	(15)	(15)	(15)	(7)
	P	0.460	0.622	0.486	0.921	0.864	0.964	0.818	0.972

Dose Level: Group 1 - 0 mg/kg/day Group 7 - 25 mg/kg/day Phenobarbital Group 8 - 50 mg/kg/day Phenobarbital
Group 9 - 100 mg/kg/day Phenobarbital
F = Female P = P-value from ANOVA
Statistical Significance: * = P<0.05 ** = P<0.01

Table 3
F_1 Summary of Body Weight Gains (Grams)
Pubertal Toxicity Study of Vinclozolin, Flutamide and Phenobarbital in Male Sprague Dawley Rats and Methoxychlor, Ethinyl Estradiol and Phenobarbital in Female Sprague Dawley Rats when Administered in Corn Oil by Oral Gavage

			Postnatal Days, From-To							
Group Sex		Base Weight	23	23-24	24-25	25-26	26-27	27-28	28-29	29-30	30-31
1M	Mean	67.41	7.09	6.91	6.62	5.59	7.75	7.65	6.58	9.06
	S.E.	1.00	0.25	0.29	0.16	0.22	0.37	0.35	0.30	0.43
	N	(15)	(15)	(15)	(15)	(15)	(15)	(15)	(15)	(15)
2M	Mean	67.32	8.29	6.29	*5.40	4.93	7.53	7.49	7.07	7.97
	S.E.	1.15	0.29	0.18	0.29	0.33	0.27	0.39	0.36	0.31
	N	(15)	(15)	(15)	(15)	(15)	(15)	(15)	(15)	(15)
3M	Mean	67.84	*8.62	7.21	*5.45	3.09	9.01	7.67	7.29	8.22
	S.E.	1.00	0.30	0.41	0.43	1.62	1.17	0.37	0.36	0.35
	N	(15)	(15)	(15)	(15)	(15)	(15)	(15)	(15)	(15)
4M	Mean	67.53	5.24	7.26	5.66	4.98	6.92	6.69	6.02	*7.38
	S.E.	1.12	1.29	0.59	0.29	0.24	0.25	0.42	0.54	0.47
	N	(15)	(14)	(14)	(14)	(14)	(14)	(14)	(14)	(14)
	P	0.987	(c)	0.279	0.023	(c)	(c)	0.239	0.123	0.033

Dose Level: Group 1 - 0 mg/kg/day Group 2 - 25 mg/kg/day Phenobarbital Group 3 - 50 mg/kg/day Phenobarbital
Group 4 - 100 mg/kg/day Phenobarbital
M = Male P = P-value from ANOVA c = Dunn's test used for pairwise comparisons
Statistical Significance: * = P<0.05 ** = P<0.01

Table 3 (continued)
F_1 Summary of Body Weight Gains (Grams)
Pubertal Toxicity Study of Vinclozolin, Flutamide and Phenobarbital in Male Sprague Dawley Rats and Methoxychlor, Ethinyl Estradiol and Phenobarbital in Female Sprague Dawley Rats when Administered in Corn Oil by Oral Gavage

Group Sex		Postnatal Days, From-To 31-32	32-33	33-34	34-35	35-36	36-37	37-38	38-39	39-40
1M	Mean	8.35	8.71	8.79	8.61	8.45	9.34	9.99	7.65	10.41
	S.E.	0.29	0.52	0.38	0.44	0.64	0.47	0.48	0.48	0.37
	N	(15)	(15)	(15)	(15)	(15)	(15)	(15)	(15)	(15)
2M	Mean	8.82	8.37	9.04	8.41	9.83	8.55	8.39	8.27	9.74
	S.E.	0.39	0.23	0.35	0.35	0.35	0.91	1.16	0.76	0.44
	N	(15)	(15)	(15)	(15)	(15)	(15)	(15)	(15)	(15)
3M	Mean	9.19	8.11	9.66	9.47	9.37	8.99	8.48	*9.63	9.35
	S.E.	0.34	0.60	0.49	0.54	0.46	0.53	0.55	0.41	0.43
	N	(15)	(15)	(15)	(15)	(15)	(15)	(15)	(15)	(15)
4M	Mean	8.00	8.23	**6.55	9.93	8.24	7.79	9.17	8.21	**7.90
	S.E.	0.49	0.52	0.46	0.50	0.45	0.55	0.38	0.43	0.49
	N	(14)	(14)	(14)	(14)	(14)	(14)	(14)	(14)	(14)
	P	0.147	0.839	0.000	0.078	0.080	0.371	0.362	0.072	0.002

Dose Level: Group 1 - 0 mg/kg/day Group 2 - 25 mg/kg/day Phenobarbital Group 3 - 50 mg/kg/day Phenobarbital
Group 4 - 100 mg/kg/day Phenobarbital
M = Male P = P-value from ANOVA
Statistical Significance: * = P<0.05 ** = P<0.01

Table 3 (continued)
F_1 Summary of Body Weight Gains (Grams)
Pubertal Toxicity Study of Vinclozolin, Flutamide and Phenobarbital in Male Sprague Dawley Rats and Methoxychlor, Ethinyl Estradiol and Phenobarbital in Female Sprague Dawley Rats when Administered in Corn Oil by Oral Gavage

Group Sex		Postnatal Days, From-To 40-41	41-42	42-43	43-44	44-45	45-46	46-47	47-48	48-49
1M	Mean	9.71	6.36	9.10	7.90	9.97	8.56	9.01	9.10	9.63
	S.E.	0.63	0.43	0.36	0.64	0.40	0.68	0.56	0.98	0.65
	N	(15)	(15)	(15)	(15)	(15)	(15)	(15)	(15)	(15)
2M	Mean	7.87	7.35	8.76	6.81	10.39	7.99	8.57	8.55	10.07
	S.E.	0.72	0.45	0.51	0.46	0.58	0.40	0.41	0.57	0.54
	N	(15)	(15)	(15)	(15)	(15)	(15)	(15)	(15)	(15)
3M	Mean	7.59	**8.87	8.55	7.29	10.34	8.29	9.01	8.39	9.57
	S.E.	0.72	0.59	0.69	0.40	0.60	0.55	0.49	0.46	0.74
	N	(15)	(15)	(15)	(15)	(15)	(15)	(15)	(15)	(15)
4M	Mean	8.34	7.26	8.77	6.48	9.93	**5.20	9.17	8.49	9.25
	S.E.	0.46	0.55	0.54	0.42	0.57	0.56	0.63	0.69	0.57
	N	(14)	(14)	(14)	(14)	(14)	(14)	(14)	(14)	(14)
	P	0.107	0.008	0.907	0.205	0.898	0.000	0.865	0.889	0.840

Dose Level: Group 1 - 0 mg/kg/day Group 2 - 25 mg/kg/day Phenobarbital Group 3 - 50 mg/kg/day Phenobarbital
Group 4 - 100 mg/kg/day Phenobarbital
M = Male P = P-value from ANOVA
Statistical Significance: * = P<0.05 ** = P<0.01

Table 3 (continued)
F_1 Summary of Body Weight Gains (Grams)
Pubertal Toxicity Study of Vinclozolin, Flutamide and Phenobarbital in Male Sprague Dawley Rats and Methoxychlor, Ethinyl Estradiol and Phenobarbital in Female Sprague Dawley Rats when Administered in Corn Oil by Oral Gavage

		Postnatal Days, From-To								
Group Sex		49-50	50-51	51-52	52-53	53-54	Absolute Gains 23-53	% Gains 23-53	Absolute Gains 23-54	% Gains 23-54
1M	Mean	8.73	7.38	9.90	10.67	6.84	250.73	356.93	263.69	411.09
	S.E.	1.04	0.87	0.56	0.64	1.36	8.06	9.014	4.75	9.768
	N	(15)	(15)	(15)	(15)	(7)	(8)	(8)	(7)	(7)
2M	Mean	7.78	6.95	10.00	9.39	7.74	244.95	350.73	252.54	393.24
	S.E.	0.66	0.87	0.49	0.52	1.00	5.97	6.485	7.26	14.732
	N	(15)	(15)	(15)	(15)	(7)	(8)	(8)	(7)	(7)
3M	Mean	8.77	*3.99	11.35	9.19	10.37	251.96	356.34	258.20	399.69
	S.E.	0.76	0.77	0.76	0.94	2.36	7.70	8.287	8.43	12.946
	N	(15)	(15)	(15)	(15)	(7)	(8)	(8)	(7)	(7)
4M	Mean	8.19	5.41	8.86	**6.69	9.41	234.51	329.69	**227.27	**357.46
	S.E.	0.92	0.73	1.00	0.97	0.88	8.58	11.265	3.96	7.057
	N	(14)	(14)	(14)	(14)	(7)	(7)	(7)	(7)	(7)
	P	0.819	0.018	0.126	0.008	0.365	0.390	0.138	0.002	0.018

Dose Level: Group 1 - 0 mg/kg/day Group 2 - 25 mg/kg/day Phenobarbital Group 3 - 50 mg/kg/day Phenobarbital
Group 4 - 100 mg/kg/day Phenobarbital
M = Male P = P-value from ANOVA
Statistical Significance: * = P<0.05 ** = P<0.01

Table 3 (continued)
F_1 Summary of Body Weight Gains (Grams)
Pubertal Toxicity Study of Vinclozolin, Flutamide and Phenobarbital in Male Sprague Dawley Rats and Methoxychlor, Ethinyl Estradiol and Phenobarbital in Female Sprague Dawley Rats when Administered in Corn Oil by Oral Gavage

			Postnatal Days, From-To							
Group Sex		Base Weight	23	23-24	24-25	25-26	26-27	27-28	28-29	29-30	30-31
1M	Mean	67.41	7.09	6.91	6.62	5.59	7.75	7.65	6.58	9.06
	S.E.	1.00	0.25	0.29	0.16	0.22	0.37	0.35	0.30	0.43
	N	(15)	(15)	(15)	(15)	(15)	(15)	(15)	(15)	(15)
5M	Mean	67.65	7.10	7.33	6.77	**7.19	*6.47	*8.93	6.72	9.88
	S.E.	0.81	0.26	0.16	0.34	0.38	0.38	0.32	0.38	0.51
	N	(15)	(15)	(15)	(15)	(15)	(15)	(15)	(15)	(15)
6M	Mean	68.08	7.17	7.03	7.27	*6.87	7.64	7.58	7.29	10.23
	S.E.	1.13	0.26	0.43	0.55	0.44	0.39	0.39	0.25	0.44
	N	(15)	(15)	(15)	(15)	(15)	(15)	(15)	(15)	(15)
7M	Mean	67.91	*6.14	7.15	5.97	6.66	7.28	7.59	5.27	10.15
	S.E.	1.02	0.28	0.31	0.36	0.32	0.34	0.38	0.83	0.81
	N	(15)	(15)	(15)	(15)	(15)	(15)	(15)	(15)	(15)
	P	0.967	0.020	0.797	0.121	0.012	0.072	0.024	0.039	0.460

Dose Level: Group 1 - 0 mg/kg/day Group 5 - 10 mg/kg/day Vinclozolin Group 6 - 30 mg/kg/day Vinclozolin
Group 7 - 100 mg/kg/day Vinclozolin
M = Male P = P-value from ANOVA
Statistical Significance: * = P<0.05 ** = P<0.01

Table 3 (continued)
F_1 Summary of Body Weight Gains (Grams)
Pubertal Toxicity Study of Vinclozolin, Flutamide and Phenobarbital in Male Sprague Dawley Rats and Methoxychlor, Ethinyl Estradiol and Phenobarbital in Female Sprague Dawley Rats when Administered in Corn Oil by Oral Gavage

Group Sex		Postnatal Days, From-To 31-32	32-33	33-34	34-35	35-36	36-37	37-38	38-39	39-40
1M	Mean	8.35	8.71	8.79	8.61	8.45	9.34	9.99	7.65	10.41
	S.E.	0.29	0.52	0.38	0.44	0.64	0.47	0.48	0.48	0.37
	N	(15)	(15)	(15)	(15)	(15)	(15)	(15)	(15)	(15)
5M	Mean	7.78	9.05	9.23	8.41	8.22	8.94	10.79	8.53	10.03
	S.E.	0.33	0.34	0.50	0.31	0.39	0.45	0.50	0.50	0.36
	N	(15)	(15)	(15)	(15)	(15)	(15)	(15)	(15)	(15)
6M	Mean	8.65	8.72	10.18	8.65	8.79	9.81	10.35	9.09	10.15
	S.E.	0.55	0.34	0.58	0.39	0.55	0.59	0.46	0.62	0.61
	N	(15)	(15)	(15)	(15)	(15)	(15)	(15)	(15)	(15)
7M	Mean	7.61	8.35	9.87	8.23	8.68	8.15	10.31	8.09	9.36
	S.E.	0.42	0.46	0.42	0.38	0.49	0.63	0.49	0.30	0.56
	N	(15)	(15)	(15)	(15)	(15)	(15)	(15)	(15)	(15)
	P	0.249	0.708	0.177	0.852	0.875	0.177	0.709	0.204	0.474

Dose Level: Group 1 - 0 mg/kg/day Group 5 - 10 mg/kg/day Vinclozolin Group 6 - 30 mg/kg/day Vinclozolin
Group 7 - 100 mg/kg/day Vinclozolin
M = Male P = P-value from ANOVA
Statistical Significance: * = P<0.05 ** = P<0.01

Table 3 (continued)
F_1 Summary of Body Weight Gains (Grams)
Pubertal Toxicity Study of Vinclozolin, Flutamide and Phenobarbital in Male Sprague Dawley Rats and Methoxychlor, Ethinyl Estradiol and Phenobarbital in Female Sprague Dawley Rats when Administered in Corn Oil by Oral Gavage

Group Sex		Postnatal Days, From-To 40-41	41-42	42-43	43-44	44-45	45-46	46-47	47-48	48-49
1M	Mean	9.71	6.36	9.10	7.90	9.97	8.56	9.01	9.10	9.63
	S.E.	0.63	0.43	0.36	0.64	0.40	0.68	0.56	0.98	0.65
	N	(15)	(15)	(15)	(15)	(15)	(15)	(15)	(15)	(15)
5M	Mean	8.34	*8.37	8.76	8.07	9.13	10.11	8.55	9.82	9.69
	S.E.	0.69	0.55	0.43	0.70	0.75	0.47	0.42	0.47	0.55
	N	(15)	(15)	(15)	(15)	(15)	(15)	(15)	(15)	(15)
6M	Mean	9.79	**8.63	9.63	6.59	10.23	10.31	9.04	9.53	11.21
	S.E.	0.56	0.60	0.36	0.48	0.46	0.51	0.53	0.57	0.48
	N	(15)	(15)	(15)	(15)	(15)	(15)	(15)	(15)	(15)
7M	Mean	8.13	8.11	9.32	6.76	9.05	9.03	8.17	8.72	10.23
	S.E.	0.42	0.49	0.64	0.52	0.64	0.73	0.57	0.55	0.45
	N	(15)	(15)	(15)	(15)	(15)	(15)	(15)	(15)	(15)
	P	0.091	0.014	0.600	0.181	0.383	0.133	0.595	0.670	0.149

Dose Level: Group 1 - 0 mg/kg/day Group 5 - 10 mg/kg/day Vinclozolin Group 6 - 30 mg/kg/day Vinclozolin
Group 7 - 100 mg/kg/day Vinclozolin
M = Male P = P-value from ANOVA
Statistical Significance: * = P<0.05 ** = P<0.01

Table 3 (continued)
F_1 Summary of Body Weight Gains (Grams)
Pubertal Toxicity Study of Vinclozolin, Flutamide and Phenobarbital in Male Sprague Dawley Rats and Methoxychlor, Ethinyl Estradiol and Phenobarbital in Female Sprague Dawley Rats when Administered in Corn Oil by Oral Gavage

		Postnatal Days, From-To								
Group Sex		49-50	50-51	51-52	52-53	53-54	Absolute Gains 23-53	% Gains 23-53	Absolute Gains 23-54	% Gains 23-54
1M	Mean	8.73	7.38	9.90	10.67	6.84	250.73	356.93	263.69	411.09
	S.E.	1.04	0.87	0.56	0.64	1.36	8.06	9.01	4.75	9.77
	N	(15)	(15)	(15)	(15)	(7)	(8)	(8)	(7)	(7)
5M	Mean	9.61	6.63	11.13	11.07	9.01	257.49	369.24	273.37	418.94
	S.E.	0.81	0.53	0.78	0.82	1.17	6.96	10.23	9.96	13.52
	N	(15)	(15)	(15)	(15)	(7)	(8)	(8)	(7)	(7)
6M	Mean	9.69	7.79	11.77	11.50	10.14	265.19	374.46	288.14	444.19
	S.E.	0.87	0.71	0.84	0.66	0.96	5.95	7.40	8.43	11.79
	N	(15)	(15)	(15)	(15)	(7)	(8)	(8)	(7)	(7)
7M	Mean	7.57	6.49	9.90	8.93	8.40	251.11	357.33	246.96	378.66
	S.E.	0.68	0.72	0.80	0.73	0.95	3.86	3.38	9.74	12.12
	N	(15)	(15)	(15)	(15)	(7)	(8)	(8)	(7)	(7)
	P	0.278	0.535	0.216	0.070	0.239	0.355	0.321	0.017	0.007

Dose Level: Group 1 - 0 mg/kg/day Group 5 - 10 mg/kg/day Vinclozolin Group 6 - 30 mg/kg/day Vinclozolin
Group 7 - 100 mg/kg/day Vinclozolin
M = Male P = P-value from ANOVA
Statistical Significance: * = P<0.05 ** = P<0.01

Table 3 (continued)
F_1 Summary of Body Weight Gains (Grams)
Pubertal Toxicity Study of Vinclozolin, Flutamide and Phenobarbital in Male Sprague Dawley Rats and Methoxychlor, Ethinyl Estradiol and Phenobarbital in Female Sprague Dawley Rats when Administered in Corn Oil by Oral Gavage

			Postnatal Days, From-To							
Group Sex		Base Weight	23	23-24	24-25	25-26	26-27	27-28	28-29	29-30	30-31
1M	Mean	67.41	7.09	6.91	6.62	5.59	7.75	7.65	6.58	9.06
	S.E.	0.995	0.249	0.293	0.158	0.216	0.372	0.351	0.298	0.43
	N	(15)	(15)	(15)	(15)	(15)	(15)	(15)	(15)	(15)
8M	Mean	66.93	6.90	6.69	5.99	5.90	6.52	7.68	6.12	9.01
	S.E.	0.85	0.23	0.177	0.299	0.315	0.29	0.408	0.245	0.299
	N	(15)	(15)	(15)	(15)	(15)	(15)	(15)	(15)	(15)
9M	Mean	67.06	6.43	6.87	*5.71	5.61	7.13	7.94	6.15	9.53
	S.E.	1.054	0.356	0.353	0.214	0.646	0.478	0.284	0.242	0.37
	N	(15)	(15)	(15)	(15)	(15)	(15)	(15)	(15)	(15)
	P	0.938	0.250	0.853	0.025	0.849	0.092	0.812	0.389	0.555

Dose Level: Group 1 - 0 mg/kg/day Group 8 - 25 mg/kg/day Flutamide Group 9 - 50 mg/kg/day Flutamide
M = Male P = P-value from ANOVA
Statistical Significance: * = P<0.05 ** = P<0.01

Table 3 (continued)
F_1 Summary of Body Weight Gains (Grams)
Pubertal Toxicity Study of Vinclozolin, Flutamide and Phenobarbital in Male Sprague Dawley Rats and Methoxychlor, Ethinyl Estradiol and Phenobarbital in Female Sprague Dawley Rats when Administered in Corn Oil by Oral Gavage

Group Sex		Postnatal Days, From-To 31-32	32-33	33-34	34-35	35-36	36-37	37-38	38-39	39-40
1M	Mean	8.35	8.71	8.79	8.61	8.45	9.34	9.99	7.65	10.41
	S.E.	0.288	0.516	0.379	0.436	0.636	0.466	0.482	0.477	0.372
	N	(15)	(15)	(15)	(15)	(15)	(15)	(15)	(15)	(15)
8M	Mean	8.22	7.60	8.82	8.48	7.65	9.19	8.88	9.09	*8.34
	S.E.	0.476	0.509	0.58	0.386	0.482	0.322	0.563	0.588	0.513
	N	(15)	(15)	(15)	(15)	(15)	(15)	(15)	(15)	(15)
9M	Mean	7.59	8.24	9.19	8.43	8.62	7.64	9.68	7.99	8.78
	S.E.	0.504	0.34	0.424	0.491	0.325	0.894	0.979	0.426	0.73
	N	(15)	(15)	(15)	(15)	(15)	(15)	(15)	(15)	(15)
	P	0.424	0.247	0.797	0.953	0.349	0.105	0.525	0.118	0.030

Dose Level: Group 1 - 0 mg/kg/day Group 8 - 25 mg/kg/day Flutamide Group 9 - 50 mg/kg/day Flutamide
M = Male P = P-value from ANOVA
Statistical Significance: * = P<0.05 ** = P<0.01

Table 3 (continued)
F_1 Summary of Body Weight Gains (Grams)
Pubertal Toxicity Study of Vinclozolin, Flutamide and Phenobarbital in Male Sprague Dawley Rats and Methoxychlor, Ethinyl Estradiol and Phenobarbital in Female Sprague Dawley Rats when Administered in Corn Oil by Oral Gavage

Group Sex		Postnatal Days, From-To 40-41	41-42	42-43	43-44	44-45	45-46	46-47	47-48	48-49
1M	Mean	9.71	6.36	9.10	7.90	9.97	8.56	9.01	9.10	9.63
	S.E.	0.63	0.431	0.36	0.637	0.401	0.678	0.556	0.977	0.651
	N	(15)	(15)	(15)	(15)	(15)	(15)	(15)	(15)	(15)
8M	Mean	**7.23	**8.84	9.11	7.85	9.13	8.49	7.87	8.08	10.00
	S.E.	0.573	0.385	0.476	0.633	0.556	0.458	0.5	0.58	0.592
	N	(15)	(15)	(15)	(15)	(15)	(15)	(15)	(15)	(15)
9M	Mean	**7.27	*8.23	8.37	7.23	8.51	8.19	8.51	7.05	9.57
	S.E.	0.495	0.565	0.357	0.579	0.518	0.448	0.532	0.56	0.713
	N	(15)	(15)	(15)	(15)	(15)	(15)	(15)	(15)	(15)
	P	0.004	0.001	0.335	0.697	0.125	0.879	0.322	0.154	0.882

Dose Level: Group 1 - 0 mg/kg/day Group 8 - 25 mg/kg/day Flutamide Group 9 - 50 mg/kg/day Flutamide
M = Male P = P-value from ANOVA
Statistical Significance: * = P<0.05 ** = P<0.01

Table 3 (continued)
F_1 Summary of Body Weight Gains (Grams)
Pubertal Toxicity Study of Vinclozolin, Flutamide and Phenobarbital in Male Sprague Dawley Rats and Methoxychlor, Ethinyl Estradiol and Phenobarbital in Female Sprague Dawley Rats when Administered in Corn Oil by Oral Gavage

		Postnatal Days, From-To								
Group Sex		49-50	50-51	51-52	52-53	53-54	Absolute Gains 23-53	% Gains 23-53	Absolute Gains 23-54	% Gains 23-54
1M	Mean	8.73	7.38	9.90	10.67	6.84	250.73	356.93	263.69	411.09
	S.E.	1.041	0.871	0.555	0.638	1.355	8.061	9.014	4.749	9.768
	N	(15)	(15)	(15)	(15)	(7)	(8)	(8)	(7)	(7)
8M	Mean	8.75	5.87	10.74	10.03	7.40	244.05	353.16	249.41	388.13
	S.E.	0.706	0.549	0.783	0.756	1.298	5.757	7.643	7.759	15.892
	N	(15)	(15)	(15)	(15)	(7)	(8)	(8)	(7)	(7
9M	Mean	8.86	**4.05	10.79	9.95	7.44	241.58	347.46	241.69	375.9
	S.E.	0.693	0.65	0.319	0.563	1.197	10.455	12.485	6.695	9.425
	N	(15)	(15)	(15)	(15)	(7)	(8)	(8)	(7)	(7)
	P	0.993	0.007	0.485	0.700	0.935	0.727	0.796	0.079	0.141

Dose Level: Group 1 - 0 mg/kg/day Group 8 - 25 mg/kg/day Flutamide Group 9 - 50 mg/kg/day Flutamide
M = Male P = P-value from ANOVA
Statistical Significance: * = P<0.05 ** = P<0.01

Table 3 (continued)
F_1 Summary of Body Weight Gains (Grams)
Pubertal Toxicity Study of Vinclozolin, Flutamide and Phenobarbital in Male Sprague Dawley Rats and Methoxychlor, Ethinyl Estradiol and Phenobarbital in Female Sprague Dawley Rats when Administered in Corn Oil by Oral Gavage

			Postnatal Days, From-To							
Group Sex		Base Weight 22	22-23	23-24	24-25	25-26	26-27	27-28	28-29	29-30
1F	Mean	58.87	5.39	6.01	5.30	5.37	5.69	6.26	6.61	5.46
	S.E.	0.755	0.249	0.172	0.209	0.335	0.246	0.387	0.435	0.229
	N	(15)	(15)	(15)	(15)	(15)	(15)	(15)	(15)	(15)
2F	Mean	58.17	*6.41	6.17	4.87	5.86	5.58	6.75	5.77	5.51
	S.E.	0.884	0.232	0.295	0.26	0.252	0.32	0.351	0.415	0.447
	N	(15)	(15)	(15)	(15)	(15)	(15)	(15)	(15)	(15)
3F	Mean	58.03	*6.20	5.95	5.41	5.58	5.21	6.73	**4.85	6.23
	S.E.	0.999	0.253	0.222	0.26	0.224	0.285	0.305	0.337	0.325
	N	(15)	(15)	(15)	(15)	(15)	(15)	(15)	(15)	(15)
	P	0.774	0.013	0.792	0.268	0.459	0.464	0.533	0.012	0.227

Dose Level: Group 1 - 0 mg/kg/day Group 2 - 0.0025 mg/kg/day Ethinyl Estradiol Group 3 - 0.005 mg/kg/day Ethinyl Estradiol
F = Female P = P-value from ANOVA
Statistical Significance: * = P<0.05 ** = P<0.01

Table 3 (continued)
F_1 Summary of Body Weight Gains (Grams)
Pubertal Toxicity Study of Vinclozolin, Flutamide and Phenobarbital in Male Sprague Dawley Rats and Methoxychlor, Ethinyl Estradiol and Phenobarbital in Female Sprague Dawley Rats when Administered in Corn Oil by Oral Gavage

Group Sex		Postnatal Days, From-To 30-31	31-32	32-33	33-34	34-35	35-36	36-37	37-38	38-39
1F	Mean	7.14	6.96	5.67	7.18	5.31	5.05	7.02	6.73	2.87
	S.E.	0.359	0.302	0.258	0.507	0.486	0.858	0.609	0.565	0.744
	N	(15)	(15)	(15)	(15)	(15)	(15)	(15)	(15)	(15)
2F	Mean	6.15	6.37	5.43	6.90	5.46	5.49	6.40	6.05	4.54
	S.E.	0.583	0.481	0.431	0.326	0.617	0.789	0.602	0.902	0.768
	N	(15)	(15)	(15)	(15)	(15)	(15)	(15)	(15)	(15)
3F	Mean	6.59	6.62	4.93	9.03	6.41	5.11	6.13	5.77	*5.25
	S.E.	0.454	0.346	1.027	0.841	0.317	0.732	0.61	0.72	0.635
	N	(15)	(15)	(15)	(15)	(15)	(15)	(15)	(15)	(15)
	P	0.342	0.553	0.719	0.032	0.234	0.912	0.574	0.649	0.067

Dose Level: Group 1 - 0 mg/kg/day Group 2 - 0.0025 mg/kg/day Ethinyl Estradiol Group 3 - 0.005 mg/kg/day Ethinyl Estradiol
F = Female P = P-value from ANOVA
Statistical Significance: * = P<0.05 ** = P<0.01

Table 3 (continued)
F_1 Summary of Body Weight Gains (Grams)
Pubertal Toxicity Study of Vinclozolin, Flutamide and Phenobarbital in Male Sprague Dawley Rats and Methoxychlor, Ethinyl Estradiol and Phenobarbital in Female Sprague Dawley Rats when Administered in Corn Oil by Oral Gavage

		Postnatal Days, From-To							
Group Sex		39-40	40-41	41-42	42-43	Absolute Gains 22-42	% Gains 22-42	Absolute Gains 22-43	% Gains 22-43
1F	Mean	4.94	2.57	3.43	2.73	111.83	183.24	112.70	200.29
	S.E.	0.623	0.859	1.037	1.365	2.657	4.76	2.981	6.463
	N	(15)	(15)	(15)	(7)	(8)	(8)	(7)	(7)
2F	Mean	3.83	3.77	3.52	1.94	112.98	186.65	110.33	199.14
	S.E.	0.894	0.886	0.519	1.271	2.839	3.212	3.322	6.94
	N	(15)	(15)	(15)	(7)	(8)	(8)	(7)	(7)
3F	Mean	3.66	3.05	4.81	3.63	117.89	192.66	112.16	205.96
	S.E.	0.817	0.841	1.14	1.02	4.184	5.491	3.504	6.35
	N	(15)	(15)	(15)	(7)	(8)	(8)	(7)	(7)
	P	0.463	0.612	0.517	0.631	0.402	0.357	0.867	0.740

Dose Level: Group 1 - 0 mg/kg/day Group 2 - 0.0025 mg/kg/day Ethinyl Estradiol Group 3 - 0.005 mg/kg/day Ethinyl Estradiol
F = Female P = P-value from ANOVA
Statistical Significance: * = P<0.05 ** = P<0.01

Table 3 (continued)
F_1 Summary of Body Weight Gains (Grams)
Pubertal Toxicity Study of Vinclozolin, Flutamide and Phenobarbital in Male Sprague Dawley Rats and Methoxychlor, Ethinyl Estradiol and Phenobarbital in Female Sprague Dawley Rats when Administered in Corn Oil by Oral Gavage

			Postnatal Days, From-To							
Group Sex		Base Weight 22	22-23	23-24	24-25	25-26	26-27	27-28	28-29	29-30
1F	Mean	58.87	5.39	6.01	5.30	5.37	5.69	6.26	6.61	5.46
	S.E.	0.76	0.25	0.17	0.21	0.34	0.25	0.39	0.44	0.23
	N	(15)	(15)	(15)	(15)	(15)	(15)	(15)	(15)	(15)
4F	Mean	58.97	*6.39	5.54	5.70	5.25	5.23	6.44	5.80	5.67
	S.E.	1.00	0.25	0.29	0.37	0.38	0.20	0.36	0.34	0.32
	N	(15)	(15)	(15)	(15)	(15)	(15)	(15)	(15)	(15)
5F	Mean	58.32	6.13	6.01	5.80	5.48	5.57	5.53	*5.07	**7.16
	S.E.	1.15	0.30	0.24	0.24	0.29	0.43	0.36	0.36	0.33
	N	(15)	(15)	(15)	(15)	(15)	(15)	(15)	(15)	(15)
6F	Mean	57.47	5.66	5.15	6.20	4.77	5.67	6.65	**4.89	6.17
	S.E.	1.00	0.23	0.34	0.31	0.28	0.31	0.19	0.30	0.36
	N	(15)	(15)	(15)	(15)	(15)	(15)	(15)	(15)	(15)
	P	0.695	0.039	0.075	0.188	0.433	0.701	0.110	0.005	0.002

Dose Level: Group 1 - 0 mg/kg/day Group 4 - 12.5 mg/kg/day Methoxychlor Group 5 - 25 mg/kg/day Methoxychlor
Group 6 - 50 mg/kg/day Methoxychlor
F = Female P = P-value from ANOVA
Statistical Significance: * = P<0.05 ** = P<0.01

Table 3 (continued)
F_1 Summary of Body Weight Gains (Grams)
Pubertal Toxicity Study of Vinclozolin, Flutamide and Phenobarbital in Male Sprague Dawley Rats and Methoxychlor, Ethinyl Estradiol and Phenobarbital in Female Sprague Dawley Rats when Administered in Corn Oil by Oral Gavage

Group Sex		Postnatal Days, From-To 30-31	31-32	32-33	33-34	34-35	35-36	36-37	37-38	38-39
1F	Mean	7.14	6.96	5.67	7.18	5.31	5.05	7.02	6.73	2.87
	S.E.	0.36	0.30	0.26	0.51	0.49	0.86	0.61	0.57	0.74
	N	(15)	(15)	(15)	(15)	(15)	(15)	(15)	(15)	(15)
4F	Mean	5.83	6.31	5.92	3.68	8.53	4.53	5.49	6.84	3.26
	S.E.	0.49	0.55	0.29	2.20	1.91	0.72	0.79	0.77	0.84
	N	(15)	(15)	(15)	(15)	(15)	(15)	(15)	(15)	(15)
5F	Mean	5.69	6.33	4.93	4.94	7.67	3.73	9.49	6.14	3.57
	S.E.	0.51	0.34	0.65	1.71	1.60	2.24	1.56	0.53	0.57
	N	(15)	(15)	(15)	(15)	(15)	(15)	(15)	(15)	(15)
6F	Mean	*5.32	5.69	5.42	6.91	5.57	4.82	6.17	5.47	3.47
	S.E.	0.37	0.32	0.47	0.50	0.37	0.56	0.68	0.54	0.64
	N	(15)	(15)	(15)	(15)	(15)	(15)	(15)	(15)	(15)
	P	0.028	(c)	0.441	0.271	0.220	0.896	0.033	0.369	0.902

Dose Level: Group 1 – 0 mg/kg/day Group 4 – 12.5 mg/kg/day Methoxychlor Group 5 – 25 mg/kg/day Methoxychlor
Group 6 – 50 mg/kg/day Methoxychlor
F = Female P = P-value from ANOVA c = Dunn's test used for pairwise comparisons
Statistical Significance: * = P<0.05 ** = P<0.01

Table 3 (continued)
F_1 Summary of Body Weight Gains (Grams)
Pubertal Toxicity Study of Vinclozolin, Flutamide and Phenobarbital in Male Sprague Dawley Rats and Methoxychlor, Ethinyl Estradiol and Phenobarbital in Female Sprague Dawley Rats when Administered in Corn Oil by Oral Gavage

		Postnatal Days, From-To							
Group Sex		39-40	40-41	41-42	42-43	Absolute Gains 22-42	% Gains 22-42	Absolute Gains 22-43	% Gains 22-43
1F	Mean	4.94	2.57	3.43	2.73	111.83	183.24	112.70	200.29
	S.E.	0.62	0.86	1.04	1.37	2.66	4.76	2.98	6.46
	N	(15)	(15)	(15)	(7)	(8)	(8)	(7)	(7)
4F	Mean	4.64	1.63	4.14	4.43	109.76	178.61	107.87	192.64
	S.E.	0.60	0.85	1.11	1.43	2.22	4.76	2.47	2.65
	N	(15)	(15)	(15)	(7)	(8)	(8)	(7)	(7)
5F	Mean	5.16	4.40	3.90	3.23	118.11	192.09	109.76	201.26
	S.E.	0.88	0.60	0.84	1.17	3.21	4.39	1.96	5.81
	N	(15)	(15)	(15)	(7)	(8)	(8)	(7)	(7)
6F	Mean	4.39	3.65	4.04	4.39	105.24	174.50	111.40	205.79
	S.E.	0.59	0.43	0.33	0.90	1.62	4.12	2.87	3.34
	N	(15)	(15)	(15)	(7)	(8)	(8)	(7)	(7)
	P	0.867	0.040	0.945	0.703	0.010	0.058	0.594	0.307

Dose Level: Group 1 - 0 mg/kg/day Group 4 - 12.5 mg/kg/day Methoxychlor Group 5 - 25 mg/kg/day Methoxychlor
Group 6 - 50 mg/kg/day Methoxychlor
F = Female P = P-value from ANOVA
Statistical Significance: * = P<0.05 ** = P<0.01

Table 3 (continued)
F_1 Summary of Body Weight Gains (Grams)
Pubertal Toxicity Study of Vinclozolin, Flutamide and Phenobarbital in Male Sprague Dawley Rats and Methoxychlor, Ethinyl Estradiol and Phenobarbital in Female Sprague Dawley Rats when Administered in Corn Oil by Oral Gavage

			Postnatal Days, From-To							
Group Sex		Base Weight	22	22-23	23-24	24-25	25-26	26-27	27-28	28-29	29-30
1F	Mean	58.87	5.39	6.01	5.30	5.37	5.69	6.26	6.61	5.46
	S.E.	0.76	0.25	0.17	0.21	0.34	0.25	0.39	0.44	0.23
	N	(15)	(15)	(15)	(15)	(15)	(15)	(15)	(15)	(15)
7F	Mean	56.70	**7.94	5.49	5.18	5.25	5.67	6.19	*5.02	*6.77
	S.E.	0.77	0.35	0.43	0.28	0.28	0.23	0.24	0.17	0.26
	N	(15)	(15)	(15)	(15)	(15)	(15)	(15)	(15)	(15)
8F	Mean	57.96	**8.05	5.82	5.01	4.69	6.11	5.81	5.42	6.57
	S.E.	1.07	0.41	0.30	0.26	0.24	0.29	0.37	0.24	0.23
	N	(15)	(15)	(15)	(15)	(15)	(15)	(15)	(15)	(15)
9F	Mean	58.14	6.21	4.91	5.15	**3.65	5.59	*4.95	6.55	5.05
	S.E.	0.97	0.75	0.44	0.49	0.26	0.53	0.28	0.59	0.52
	N	(15)	(15)	(15)	(15)	(15)	(15)	(15)	(15)	(15)
	P	0.399	0.000	(c)	(c)	0.000	0.709	0.022	(c)	0.001

Dose Level: Group 1 - 0 mg/kg/day Group 7 - 25 mg/kg/day Phenobarbital Group 8 - 50 mg/kg/day Phenobarbital
Group 9 - 100 mg/kg/day Phenobarbital
F = Female P = P-value from ANOVA c = Dunn's test used for pairwise comparisons
Statistical Significance: * = P<0.05 ** = P<0.01

Table 3 (continued)
F_1 Summary of Body Weight Gains (Grams)
Pubertal Toxicity Study of Vinclozolin, Flutamide and Phenobarbital in Male Sprague Dawley Rats and Methoxychlor, Ethinyl Estradiol and Phenobarbital in Female Sprague Dawley Rats when Administered in Corn Oil by Oral Gavage

Group Sex		Postnatal Days, From-To 30-31	31-32	32-33	33-34	34-35	35-36	36-37	37-38	38-39
1F	Mean	7.14	6.96	5.67	7.18	5.31	5.05	7.02	6.73	2.87
	S.E.	0.36	0.30	0.26	0.51	0.49	0.86	0.61	0.57	0.74
	N	(15)	(15)	(15)	(15)	(15)	(15)	(15)	(15)	(15)
7F	Mean	6.85	5.90	3.60	9.58	4.69	7.39	4.72	5.80	5.09
	S.E.	0.49	0.59	1.81	1.72	0.69	0.90	0.89	0.65	0.82
	N	(15)	(15)	(15)	(15)	(15)	(15)	(15)	(15)	(15)
8F	Mean	5.81	7.05	3.26	9.26	5.50	4.47	7.69	5.66	5.26
	S.E.	0.44	0.36	1.96	1.36	0.72	0.67	0.62	0.55	0.79
	N	(15)	(15)	(15)	(15)	(15)	(15)	(15)	(15)	(15)
9F	Mean	6.79	6.41	5.77	8.37	4.37	5.80	7.24	5.36	**6.53
	S.E.	0.62	0.40	0.42	0.33	0.43	0.52	0.69	0.76	0.75
	N	(15)	(15)	(15)	(15)	(15)	(15)	(15)	(15)	(15)
	P	0.245	0.204	0.417	0.450	0.505	0.047	0.022	0.468	0.014

Dose Level: Group 1 - 0 mg/kg/day Group 7 - 25 mg/kg/day Phenobarbital Group 8 - 50 mg/kg/day Phenobarbital
Group 9 - 100 mg/kg/day Phenobarbital
F = Female P = P-value from ANOVA
Statistical Significance: * = P<0.05 ** = P<0.01

Table 3 (continued)
F_1 Summary of Body Weight Gains (Grams)
Pubertal Toxicity Study of Vinclozolin, Flutamide and Phenobarbital in Male Sprague Dawley Rats and Methoxychlor, Ethinyl Estradiol and Phenobarbital in Female Sprague Dawley Rats when Administered in Corn Oil by Oral Gavage

		Postnatal Days, From-To							
Group Sex		39-40	40-41	41-42	42-43	Absolute Gains 22-42	% Gains 22-42	Absolute Gains 22-43	% Gains 22-43
1F	Mean	4.94	2.57	3.43	2.73	111.83	183.24	112.70	200.29
	S.E.	0.62	0.86	1.04	1.37	2.66	4.76	2.98	6.46
	N	(15)	(15)	(15)	(7)	(8)	(8)	(7)	(7)
7F	Mean	5.09	2.81	4.65	2.07	117.31	201.35	111.63	203.23
	S.E.	0.49	0.62	1.32	1.97	4.38	6.52	4.35	6.90
	N	(15)	(15)	(15)	(7)	(8)	(8)	(7)	(7)
8F	Mean	4.26	3.90	5.55	4.84	121.01	197.89	113.29	209.56
	S.E.	0.79	0.91	1.24	1.86	2.63	4.23	4.92	11.36
	N	(15)	(15)	(15)	(7)	(8)	(8)	(7)	(7)
9F	Mean	3.96	4.73	3.61	4.94	113.16	186.38	113.50	206.21
	S.E.	0.75	0.58	1.63	2.08	3.69	6.76	3.91	7.06
	N	(15)	(15)	(15)	(7)	(8)	(8)	(7)	(7)
	P	0.588	0.161	0.650	0.600	0.239	0.090	0.988	0.870

Dose Level: Group 1 - 0 mg/kg/day Group 7 - 25 mg/kg/day Phenobarbital Group 8 - 50 mg/kg/day Phenobarbital
Group 9 - 100 mg/kg/day Phenobarbital
F = Female P = P-value from ANOVA
Statistical Significance: * = P<0.05 ** = P<0.01

Table 4
F_1 Summary of Preputial Separation Data
Pubertal Toxicity Study of Vinclozolin, Flutamide and Phenobarbital in Male Sprague Dawley Rats and Methoxychlor, Ethinyl Estradiol and Phenobarbital in Female Sprague Dawley Rats when Administered in Corn Oil by Oral Gavage

Group Sex		Body Weight at Preputial Separation (grams)	Postnatal Day of Preputial Separation
1M	Mean	213.63	41.1
	S.E.	4.522	0.38
	N	(15)	(15)
2M	Mean	213.44	41.5
	S.E.	3.609	0.36
	N	(15)	(15)
3M	Mean	215.78	41.1
	S.E.	3.748	0.39
	N	(15)	(15)
4M	Mean	219.81	**43.6
	S.E.	4.903	0.54
	N	(14)	(14)
	P	0.697	0.000

Dose Level: Group 1 - 0 mg/kg/day Group 2 - 25 mg/kg/day Phenobarbital
Group 3 - 50 mg/kg/day Phenobarbital Group 4 - 100 mg/kg/day Phenobarbital
M = Male P = P-value from ANOVA
Statistical Significance: * = P<0.05 ** = P<0.01

Table 4 (continued)
F_1 Summary of Preputial Separation Data
Pubertal Toxicity Study of Vinclozolin, Flutamide and Phenobarbital in Male Sprague Dawley Rats and Methoxychlor, Ethinyl Estradiol and Phenobarbital in Female Sprague Dawley Rats when Administered in Corn Oil by Oral Gavage

Group Sex		Body Weight at Preputial Separation (grams)	Postnatal Day of Preputial Separation
1M	Mean	213.63	41.1
	S.E.	4.522	0.376
	N	(15)	(15)
5M	Mean	231.29	*42.6
	S.E.	5.12	0.434
	N	(15)	(15)
6M	Mean	**248.59	**43.9
	S.E.	4.902	0.336
	N	(15)	(15)
7M	Mean	**267.83	**47.7
	S.E.	6.046	0.319
	N	(15)	(15)
	P	0.000	0.000

Dose Level: Group 1 – 0 mg/kg/day Group 5 – 10 mg/kg/day Vinclozolin
Group 6 – 30 mg/kg/day Vinclozolin Group 7 – 100 mg/kg/day Vinclozolin
M = Male P = P-value from ANOVA
Statistical Significance: * = P<0.05 ** = P<0.01

Table 4 (continued)
F_1 Summary of Preputial Separation Data
Pubertal Toxicity Study of Vinclozolin, Flutamide and Phenobarbital in Male Sprague Dawley Rats and Methoxychlor, Ethinyl Estradiol and Phenobarbital in Female Sprague Dawley Rats when Administered in Corn Oil by Oral Gavage

Group Sex		Body Weight at Preputial Separation (grams)	Postnatal Day of Preputial Separation
1M	Mean	213.63	41.1
	S.E.	4.522	0.38
	N	(15)	(15)
8M	Mean	**318.57	**53.3
	S.E.	8.237	0.33
	N	(6)	(6)
9M	Mean	**311.00	**54.0
	S.E.	0	0.0
	N	(1)	(1)
	P	0.000	0.000

Dose Level: Group 1 – 0 mg/kg/day Group 8 – 25 mg/kg/day Flutamide
Group 9 – 50 mg/kg/day Flutamide
M = Male P = P-value from ANOVA
Statistical Significance: * = P<0.05 ** = P<0.01

Table 5
F_1 Summary of Vaginal Opening Data
Pubertal Toxicity Study of Vinclozolin, Flutamide and Phenobarbital in Male Sprague Dawley Rats and Methoxychlor, Ethinyl Estradiol and Phenobarbital in Female Sprague Dawley Rats when Administered in Corn Oil by Oral Gavage

Group Sex		Body Weight at Vaginal Opening (grams)	Postnatal Day of Vaginal Opening
1F	Mean	117.58	31.9
	S.E.	2.482	0.32
	N	(15)	(15)
2F	Mean	112.05	31.1
	S.E.	3.967	0.63
	N	(15)	(15)
3F	Mean	**95.20	**28.4
	S.E.	1.797	0.22
	N	(15)	(15)
	P	0.000	0.000

Dose Level: Group 1 - 0 mg/kg/day Group 2 - 0.0025 mg/kg/day Ethinyl Estradiol
Group 3 - 0.005 mg/kg/day Ethinyl Estradiol
F = Female P = P-value from ANOVA
Statistical Significance: * = P<0.05 ** = P<0.01

Table 5 (continued)
F_1 Summary of Vaginal Opening Data
Pubertal Toxicity Study of Vinclozolin, Flutamide and Phenobarbital in Male Sprague Dawley Rats and Methoxychlor, Ethinyl Estradiol and Phenobarbital in Female Sprague Dawley Rats when Administered in Corn Oil by Oral Gavage

Group Sex		Body Weight at Vaginal Opening (grams)	Postnatal Day of Vaginal Opening
1F	Mean	117.58	31.9
	S.E.	2.482	0.322
	N	(15)	(15)
4F	Mean	**92.41	**27.9
	S.E.	2.255	0.215
	N	(15)	(15)
5F	Mean	**87.48	**27.0
	S.E.	2.214	0.195
	N	(15)	(15)
6F	Mean	**82.02	**26.5
	S.E.	1.217	0.13
	N	(15)	(15)
	P	0	0

Dose Level: Group 1 – 0 mg/kg/day Group 4 – 12.5 mg/kg/day Methoxychlor
Group 5 – 25 mg/kg/day Methoxychlor Group 6 – 50 mg/kg/day Methoxychlor
F = Female P = P-value from ANOVA
Statistical Significance: * = P<0.05 ** = P<0.01

Table 5 (continued)
F_1 Summary of Vaginal Opening Data
Pubertal Toxicity Study of Vinclozolin, Flutamide and Phenobarbital in Male Sprague Dawley Rats
and Methoxychlor, Ethinyl Estradiol and Phenobarbital in Female Sprague Dawley Rats
when Administered in Corn Oil by Oral Gavage

Group Sex		Body Weight at Vaginal Opening (grams)	Postnatal Day of Vaginal Opening
1F	Mean	117.58	31.9
	S.E.	2.482	0.32
	N	(15)	(15)
7F	Mean	122.63	33.1
	S.E.	3.311	0.42
	N	(15)	(15)
8F	Mean	120.67	32.8
	S.E.	4.548	0.53
	N	(15)	(15)
9F	Mean	128.04	**34.5
	S.E.	5.107	0.67
	N	(15)	(15)
	P	0.315	0.006

Dose Level: Group 1 - 0 mg/kg/day Group 7 - 25 mg/kg/day Phenobarbital
Group 8 - 50 mg/kg/day Phenobarbital Group 9 - 100 mg/kg/day Phenobarbital
F = Female P = P-value from ANOVA
Statistical Significance: * = P<0.05 ** = P<0.01

Table 6
F_1 Summary of Estrous Cyclicity Data
Pubertal Toxicity Study of Vinclozolin, Flutamide and Phenobarbital in Male Sprague Dawley Rats and Methoxychlor, Ethinyl Estradiol and Phenobarbital in Female Sprague Dawley Rats when Administered in Corn Oil by Oral Gavage

Group Sex		Cycle Length (days)	Number of Cycles	Age at First Estrus (PND)
1F	Mean	4.9	1.7	33.4
	S.E.	0.2	0.13	0.631
	N	(15)	(15)	(15)
2F	Mean	5.1	1.9	32.5
	S.E.	0.26	0.18	0.646
	N	(13)[a]	(13)[a]	(15)
3F	Mean	5.1	1.9	*28.7
	S.E.	0.17	0.12	0.153
	N	(15)	(15)	(15)

Dose Level: Group 1 – 0 mg/kg/day Group 2 – 0.0025 mg/kg/day Ethinyl Estradiol
Group 3 – 0.005 mg/kg/day Ethinyl Estradiol
Statistical Significance: * = P<0.05 ** = P<0.01
a = Estrous cycle longer than twelve days, unclear, or not cycling in 2 of 15 animals
F = Female

Table 6 (continued)
F_1 Summary of Estrous Cyclicity Data
Pubertal Toxicity Study of Vinclozolin, Flutamide and Phenobarbital in Male Sprague Dawley Rats and Methoxychlor, Ethinyl Estradiol and Phenobarbital in Female Sprague Dawley Rats when Administered in Corn Oil by Oral Gavage

Group Sex		Cycle Length (days)	Number of Cycles	Age at First Estrus (PND)
1F	Mean	4.9	1.7	33.4
	S.E.	0.2	0.13	0.631
	N	(15)	(15)	(15)
4F	Mean	5.2	*2.3	*30.9
	S.E.	0.14	0.12	0.376
	N	(15)	(15)	(15)
5F	Mean	**6.2	1.7	*30.7
	S.E.	0.24	0.18	0.715
	N	(15)	(15)	(15)
6F	Mean	**5.9	1.9	*28.6
	S.E.	0.18	0.13	0.6
	N	(14)[a]	(14)[a]	(15)

Dose Level: Group 1 – 0 mg/kg/day Group 4 – 12.5 mg/kg/day Methoxychlor
Group 5 – 25 mg/kg/day Methoxychlor Group 6 – 50 mg/kg/day Methoxychlor
Statistical Significance: * = P<0.05 ** = P<0.01
a = Estrous cycle longer than twelve days, unclear, or not cycling in 1 of 15 animals
F = Female

Table 6 (continued)
F_1 Summary of Estrous Cyclicity Data
Pubertal Toxicity Study of Vinclozolin, Flutamide and Phenobarbital in Male Sprague Dawley Rats and Methoxychlor, Ethinyl Estradiol and Phenobarbital in Female Sprague Dawley Rats when Administered in Corn Oil by Oral Gavage

Group Sex		Cycle Length (days)	Number of Cycles	Age at First Estrus (PND)
1F	Mean	4.9	1.7	33.4
	S.E.	0.2	0.13	0.631
	N	(15)	(15)	(15)
7F	Mean	4.9	1.6	35.6
	S.E.	0.16	0.16	0.735
	N	(15)	(15)	(15)
8F	Mean	5.0	1.6	34.0
	S.E.	0.21	0.13	0.617
	N	(14)[a]	(14)[a]	(15)
9F	Mean	5.3	1.5	34.5
	S.E.	0.21	0.16	0.689
	N	(11)[b]	(11)[b]	(15)

Dose Level: Group 1 – 0 mg/kg/day Group 7 – 25 mg/kg/day Phenobarbital
Group 8 – 50 mg/kg/day Phenobarbital Group 9 – 100 mg/kg/day Phenobarbital
Statistical Significance: * = P<0.05 ** = P<0.01
a = Estrous cycle longer than twelve days, unclear, or not cycling in 1 of 15 animals
b = Estrous cycle longer than twelve days, unclear, or not cycling in 4 of 15 animals
F = Female

Table 6 (continued)
F_1 Summary of Estrous Cyclicity Data
Pubertal Toxicity Study of Vinclozolin, Flutamide and Phenobarbital in Male Sprague Dawley Rats and Methoxychlor, Ethinyl Estradiol and Phenobarbital in Female Sprague Dawley Rats when Administered in Corn Oil by Oral Gavage

Group	Estrous Stage (%)							Number of	Number of Females
Sex	P	E	M	D	N	EE	ED	Cycling Females	with Regular Cycle
1F	11.5	25.9	14.4	47.7	0.6	7.0	13.0	15/15(100%)	12/15(80%)
2F	13.0	26.5	13.5	47.0	0.0	0.0	27.0	14/15(93%)	10/15(67%)
3F	12.0	39.1	14.2	34.7	0.0	80.0	20.0	15/15(100%)	**3/15(20%)

Dose Level: Group 1 - 0 mg/kg/day Group 2 - 0.0025 mg/kg/day Ethinyl Estradiol Group 3 - 0.005 mg/kg/day Ethinyl Estradiol
Each dose group is compared to the control group using the Chi-Square test
Statistical Significance: * = P<0.05 ** = P<0.01
P = Proestrus E = Estrus M = Metestrus D = Diestrus EE = Extended Estrous ED = Extended Diestrus
N = Not Clear, Poor Quality, or Insufficient Number of Cells
F = Female

Table 6 (continued)
F_1 Summary of Estrous Cyclicity Data
Pubertal Toxicity Study of Vinclozolin, Flutamide and Phenobarbital in Male Sprague Dawley Rats and Methoxychlor, Ethinyl Estradiol and Phenobarbital in Female Sprague Dawley Rats when Administered in Corn Oil by Oral Gavage

Group Sex	Estrous Stage (%) P	E	M	D	N	EE	ED	Number of Cycling Females	Number of Females with Regular Cycle
1F	11.5	25.9	14.4	47.7	0.6	7.0	13.0	15/15(100%)	12/15(80%)
4F	15.4	21.8	15.0	47.4	0.4	0.0	20.0	15/15(100%)	12/15(80%)
5F	11.7	19.0	13.0	56.3	0.0	0.0	67.0	15/15(100%)	**4/15(27%)
6F	13.4	28.3	18.9	39.4	0.0	40.0	33.0	15/15(100%)	**3/15(20%)

Dose Level: Group 1 – 0 mg/kg/day Group 4 – 12.5 mg/kg/day Methoxychlor Group 5 – 25 mg/kg/day Methoxychlor
Group 6 – 50 mg/kg/day Methoxychlor
Each dose group is compared to the control group using the Chi-Square test
Statistical Significance: * = P<0.05 ** = P<0.01
P = Proestrus E = Estrus M = Metestrus D = Diestrus EE = Extended Estrous ED = Extended Diestrus
N = Not Clear, Poor Quality, or Insufficient Number of Cells
F = Female

Table 6 (continued)
F_1 Summary of Estrous Cyclicity Data
Pubertal Toxicity Study of Vinclozolin, Flutamide and Phenobarbital in Male Sprague Dawley Rats and Methoxychlor, Ethinyl Estradiol and Phenobarbital in Female Sprague Dawley Rats when Administered in Corn Oil by Oral Gavage

Group	Estrous Stage (%)							Number of	Number of Females
Sex	P	E	M	D	N	EE	ED	Cycling Females	with Regular Cycle
1F	11.5	25.9	14.4	47.7	0.6	7.0	13.0	15/15(100%)	12/15(80%)
7F	14.1	24.4	17.3	44.2	0.0	0.0	20.0	15/15(100%)	12/15(80%)
8F	8.8	26.9	16.3	48.1	0.0	0.0	20.0	14/15(93%)	12/15(80%)
9F	8.9	27.4	14.8	48.9	0.0	7.0	20.0	*11/15(73%)	10/15(67%)

Dose Level: Group 1 – 0 mg/kg/day Group 7 – 25 mg/kg/day Phenobarbital Group 8 – 50 mg/kg/day Phenobarbital
Group 9 – 100 mg/kg/day Phenobarbital
Each dose group is compared to the control group using the Chi-Square test
Statistical Significance: * = P<0.05 ** = P<0.01
P = Proestrus E = Estrus M = Metestrus D = Diestrus EE = Extended Estrous ED = Extended Diestrus
N = Not Clear, Poor Quality, or Insufficient Number of Cells
F = Female

Table 7
F_1 Gross Pathology Summary
Pubertal Toxicity Study of Vinclozolin, Flutamide and Phenobarbital in Male Sprague Dawley Rats and Methoxychlor, Ethinyl Estradiol and Phenobarbital in Female Sprague Dawley Rats when Administered in Corn Oil by Oral Gavage

	MALES								
Group:	1	2	3	4	5	6	7	8	9
Number of Animals on Study :	15	15	15	15	15	15	15	15	15
Number of Animals Completed:	(15)	(15)	(15)	(15)	(15)	(15)	(15)	(15)	(15)
adrenal glands;									
Submitted	(15)	(15)	(15)	(15)	(15)	(15)	(15)	(15)	(15)
No Visible Lesions	15	15	15	15	15	15	15	15	15
brain;									
Submitted	(0)	(0)	(0)	(1)	(0)	(0)	(0)	(0)	(0)
No Visible Lesions	0	0	0	1	0	0	0	0	0
coagulating glands;									
Submitted	(0)	(0)	(0)	(1)	(0)	(0)	(0)	(0)	(0)
No Visible Lesions	0	0	0	1	0	0	0	0	0
dorsolateral prostate;									
Submitted	(0)	(0)	(0)	(1)	(0)	(0)	(0)	(0)	(1)
No Visible Lesions	0	0	0	2	0	0	0	0	0
reduction in size	0	0	0	0	1	0	1	10	12
epididymides;									
Submitted	(15)	(15)	(15)	(15)	(15)	(15)	(15)	(15)	(15)
No Visible Lesions	14	15	15	13	15	15	14	10	8
enlargement; bilateral	0	0	0	1	0	0	0	0	0
reduction in size; bilateral	1	0	0	1	0	0	1	5	6
reduction in size; right	0	0	0	0	0	0	0	0	1

Only positive findings are reported.
Dose Level: Group 1 - 0 mg/kg/day; Group 2 - 25 mg/kg/day Phenobarbital; Group 3 - 50 mg/kg/day Phenobarbital
Group 4 - 100 mg/kg/day Phenobarbital; Group 5 - 10 mg/kg/day Vinclozolin; Group 6 - 30 mg/kg/day Vinclozolin
Group 7 - 100 mg/kg/day Vinclozolin; Group 8 - 25 mg/kg/day Flutamide; Group 9 - 50 mg/kg/day Flutamide

Table 7 (continued)
F_1 Gross Pathology Summary
Pubertal Toxicity Study of Vinclozolin, Flutamide and Phenobarbital in Male Sprague Dawley Rats and Methoxychlor, Ethinyl Estradiol and Phenobarbital in Female Sprague Dawley Rats when Administered in Corn Oil by Oral Gavage

	MALES								
Group:	1	2	3	4	5	6	7	8	9
Number of Animals on Study :	15	15	15	15	15	15	15	15	15
Number of Animals Completed:	(15)	(15)	(15)	(15)	(15)	(15)	(15)	(15)	(15)
esophagus;									
Submitted	(0)	(0)	(0)	(0)	(0)	(0)	(0)	(0)	(0)
No Visible Lesions	0	0	0	1	0	0	0	0	0
kidneys;									
Submitted	(15)	(15)	(15)	(15)	(15)	(15)	(15)	(15)	(15)
No Visible Lesions	14	12	11	12	12	11	9	15	13
discoloration; dark; left; focal	0	1	0	0	0	0	0	0	0
discoloration; dark; left; single	0	0	1	0	0	0	0	0	0
discoloration; dark; right; single	0	0	0	0	0	0	1	0	0
dilation; bilateral	0	0	1	1	0	0	0	0	0
dilation; left	0	0	0	0	0	0	1	0	0
dilation; right	1	2	2	2	3	4	5	0	2
liver;									
Submitted	(15)	(15)	(15)	(15)	(15)	(15)	(15)	(15)	(15)
No Visible Lesions	15	14	15	15	15	15	14	15	15
discoloration; pale	0	0	0	0	0	0	1	0	0
nodule(s); white	0	1	0	0	0	0	0	0	0
pituitary;									
Submitted	(15)	(15)	(15)	(15)	(14)[a]	(15)	(15)	(15)	(15)
No Visible Lesions	15	15	15	15	15	15	15	15	15

Only positive findings are reported.
a = Pituitary examined but not collected for animal 9086
Dose Level: Group 1 - 0 mg/kg/day; Group 2 - 25 mg/kg/day Phenobarbital; Group 3 - 50 mg/kg/day Phenobarbital
Group 4 - 100 mg/kg/day Phenobarbital; Group 5 - 10 mg/kg/day Vinclozolin; Group 6 - 30 mg/kg/day Vinclozolin
Group 7 - 100 mg/kg/day Vinclozolin; Group 8 - 25 mg/kg/day Flutamide; Group 9 - 50 mg/kg/day Flutamide

Table 7 (continued)
F_1 Gross Pathology Summary
Pubertal Toxicity Study of Vinclozolin, Flutamide and Phenobarbital in Male Sprague Dawley Rats and Methoxychlor, Ethinyl Estradiol and Phenobarbital in Female Sprague Dawley Rats when Administered in Corn Oil by Oral Gavage

	MALES								
Group:	1	2	3	4	5	6	7	8	9
Number of Animals on Study :	15	15	15	15	15	15	15	15	15
Number of Animals Completed:	(15)	(15)	(15)	(15)	(15)	(15)	(15)	(15)	(15)
skin;									
Submitted	(0)	(0)	(0)	(1)	(0)	(1)	(0)	(0)	(0)
No Visible Lesions	0	0	0	0	0	0	0	0	0
abrasion(s); multiple	0	0	0	1	0	0	0	0	0
abrasion(s); red; single	0	0	0	0	0	1	0	0	0
spleen;									
Submitted	(2)	(0)	(0)	(1)	(1)	(1)	(0)	(0)	(0)
No Visible Lesions	0	0	0	1	0	0	0	0	0
enlargement	2	0	0	0	1	0	0	0	0
nodule(s); red	0	0	0	0	0	1	0	0	0
nodule(s); white	1	0	0	0	0	0	0	0	0
rough	1	0	0	0	0	0	0	0	0
stomach;									
Submitted	(0)	(0)	(0)	(1)	(0)	(0)	(0)	(0)	(0)
No Visible Lesions	0	0	0	1	0	0	0	0	0

Only positive findings are reported.
Dose Level: Group 1 – 0 mg/kg/day; Group 2 – 25 mg/kg/day Phenobarbital; Group 3 – 50 mg/kg/day Phenobarbital
Group 4 – 100 mg/kg/day Phenobarbital; Group 5 – 10 mg/kg/day Vinclozolin; Group 6 – 30 mg/kg/day Vinclozolin
Group 7 – 100 mg/kg/day Vinclozolin; Group 8 – 25 mg/kg/day Flutamide; Group 9 – 50 mg/kg/day Flutamide

Table 7 (continued)
F_1 Gross Pathology Summary
Pubertal Toxicity Study of Vinclozolin, Flutamide and Phenobarbital in Male Sprague Dawley Rats and Methoxychlor, Ethinyl Estradiol and Phenobarbital in Female Sprague Dawley Rats when Administered in Corn Oil by Oral Gavage

	MALES								
Group:	1	2	3	4	5	6	7	8	9
Number of Animals on Study :	15	15	15	15	15	15	15	15	15
Number of Animals Completed:	(15)	(15)	(15)	(15)	(15)	(15)	(15)	(15)	(15)
testes;									
Submitted	(15)	(15)	(15)	(15)	(15)	(15)	(15)	(15)	(15)
No Visible Lesions	14	15	15	13	15	15	15	15	12
enlargement; bilateral	0	0	0	0	0	0	0	0	2
enlargement; left	0	0	0	0	0	0	0	0	1
reduction in size; bilateral	1	0	0	2	0	0	0	0	0
thymus;									
Submitted	(2)	(1)	(1)	(2)	(0)	(0)	(0)	(0)	(0)
No Visible Lesions	0	0	0	1	0	0	0	0	0
discoloration; dark; bilateral	0	1	0	0	0	0	0	0	0
discoloration; dark; left	0	0	1	0	0	0	0	0	0
discoloration; red; bilateral	1	0	0	0	0	0	0	0	0
discoloration; red; bilateral; diffuse	1	0	0	0	0	0	0	0	0
discoloration; left	0	0	0	1	0	0	0	0	0
thyroid glands;									
Submitted	(15)	(15)	(15)	(15)	(15)	(15)	(15)	(15)	(15)
No Visible Lesions	15	15	14	15	15	15	15	15	15
discoloration; bilateral	0	0	1	0	0	0	0	0	0

Only positive findings are reported.
Dose Level: Group 1 – 0 mg/kg/day; Group 2 – 25 mg/kg/day Phenobarbital; Group 3 – 50 mg/kg/day Phenobarbital
Group 4 – 100 mg/kg/day Phenobarbital; Group 5 – 10 mg/kg/day Vinclozolin; Group 6 – 30 mg/kg/day Vinclozolin
Group 7 – 100 mg/kg/day Vinclozolin; Group 8 – 25 mg/kg/day Flutamide; Group 9 – 50 mg/kg/day Flutamide

Table 7 (continued)
F_1 Gross Pathology Summary
Pubertal Toxicity Study of Vinclozolin, Flutamide and Phenobarbital in Male Sprague Dawley Rats and Methoxychlor, Ethinyl Estradiol and Phenobarbital in Female Sprague Dawley Rats when Administered in Corn Oil by Oral Gavage

	MALES								
Group:	1	2	3	4	5	6	7	8	9
Number of Animals on Study :	15	15	15	15	15	15	15	15	15
Number of Animals Completed:	(15)	(15)	(15)	(15)	(15)	(15)	(15)	(15)	(15)
urinary bladder;									
Submitted	(0)	(0)	(0)	(1)	(0)	(0)	(0)	(0)	(0)
No Visible Lesions	0	0	0	0	0	0	0	0	0
distension	0	0	0	1	0	0	0	0	0
levator ani plus bulbocavernosus;									
Submitted	(0)	(0)	(0)	(0)	(0)	(0)	(0)	(0)	(0)
No Visible Lesions	0	0	0	0	0	0	0	0	0
reduction in size	0	0	0	0	0	0	0	0	1
reduction in size; bilateral	0	0	0	0	0	0	0	0	3
ventral prostate;									
Submitted	(0)	(0)	(0)	(1)	(0)	(0)	(0)	(0)	(0)
No Visible Lesions	0	0	0	1	0	0	0	0	0
reduction in size	0	0	0	0	1	0	1	10	12
seminal vesicles and coagulating glands with fluid;									
Submitted	(0)	(0)	(0)	(0)	(0)	(0)	(0)	(0)	(0)
No Visible Lesions	1	1	0	0	0	1	0	0	0
reduction in size; bilateral	0	0	1	1	0	0	1	14	15
reduction in size; left	0	0	0	0	1	0	0	0	0
reduction in size; right	0	0	0	0	0	0	1	0	0

Only positive findings are reported.
Dose Level: Group 1 - 0 mg/kg/day; Group 2 - 25 mg/kg/day Phenobarbital; Group 3 - 50 mg/kg/day Phenobarbital
Group 4 - 100 mg/kg/day Phenobarbital; Group 5 - 10 mg/kg/day Vinclozolin; Group 6 - 30 mg/kg/day Vinclozolin
Group 7 - 100 mg/kg/day Vinclozolin; Group 8 - 25 mg/kg/day Flutamide; Group 9 - 50 mg/kg/day Flutamide

Table 7 (continued)
F_1 Gross Pathology Summary
Pubertal Toxicity Study of Vinclozolin, Flutamide and Phenobarbital in Male Sprague Dawley Rats and Methoxychlor, Ethinyl Estradiol and Phenobarbital in Female Sprague Dawley Rats when Administered in Corn Oil by Oral Gavage

	FEMALES								
Group:	1	2	3	4	5	6	7	8	9
Number of Animals on Study :	15	15	15	15	15	15	15	15	15
Number of Animals Completed:	(15)	(15)	(15)	(15)	(15)	(15)	(15)	(15)	(15)
adrenal glands;									
Submitted	(15)	(15)	(15)	(15)	(15)	(15)	(15)	(15)	(15)
No Visible Lesions	15	15	15	15	14	15	14	15	15
discoloration; pale; bilateral	0	0	0	0	1	0	0	0	0
enlargement; right	0	0	0	0	0	0	1	0	0
kidneys;									
Submitted	(15)	(15)	(15)	(15)	(15)	(15)	(15)	(15)	(15)
No Visible Lesions	13	14	13	11	14	14	11	11	12
discoloration; dark; bilateral; multifocal	1	0	0	2	0	0	1	1	0
discoloration; dark; left; single	0	0	0	0	0	0	1	0	0
discoloration; white; bilateral; multifocal	0	0	0	0	0	0	0	1	0
enlargement; bilateral	0	0	0	0	0	0	0	0	1
cyst(s); clear; bilateral; focal	0	0	0	1	0	0	0	0	0
cyst(s); clear; right; single	0	0	0	0	0	0	1	0	0
dilation; bilateral	0	0	0	0	0	0	0	0	1
dilation; left	0	0	0	0	0	0	1	0	0
dilation; right	1	1	2	2	1	1	1	2	2
liver;									
Submitted	(15)	(15)	(15)	(15)	(15)	(15)	(15)	(15)	(15)
No Visible Lesions	14	15	15	15	15	15	15	15	15
discoloration; pale	1	0	0	0	0	0	0	0	0

Only positive findings are reported.
Dose Level: Group 1 - 0 mg/kg/day; Group 2 - 0.0025 mg/kg/day Ethinyl Estradiol; Group 3 - 0.005 mg/kg/day Ethinyl Estradiol
Group 4 - 12.5 mg/kg/day Methoxychlor; Group 5 - 25 mg/kg/day Methoxychlor; Group 6 - 50 mg/kg/day Methoxychlor
Group 7 - 25 mg/kg/day Phenobarbital; Group 8 - 50 mg/kg/day Phenobarbital; Group 9 - 100 mg/kg/day Phenobarbital

Table 7 (continued)
F_1 Gross Pathology Summary
Pubertal Toxicity Study of Vinclozolin, Flutamide and Phenobarbital in Male Sprague Dawley Rats and Methoxychlor, Ethinyl Estradiol and Phenobarbital in Female Sprague Dawley Rats when Administered in Corn Oil by Oral Gavage

	FEMALES								
Group:	1	2	3	4	5	6	7	8	9
Number of Animals on Study :	15	15	15	15	15	15	15	15	15
Number of Animals Completed:	(15)	(15)	(15)	(15)	(15)	(15)	(15)	(15)	(15)
ovaries;									
Submitted	(15)	(15)	(15)	(15)	(15)	(15)	(15)	(15)	(15)
No Visible Lesions	15	14	15	15	15	14	14	14	14
discoloration; red; bilateral; multifocal	0	0	0	0	0	0	1	0	1
discoloration; red; left; focal	0	0	0	0	0	1	0	0	0
discoloration; red; right; single	0	1	0	0	0	0	0	0	0
cyst(s); clear; right; single	0	0	0	0	0	0	0	1	0
pituitary;									
Submitted	(15)	(15)	(15)	(15)	(15)	(15)	(15)	(15)	(14)[a]
No Visible Lesions	15	15	15	15	15	15	15	15	15
spleen;									
Submitted	(0)	(0)	(0)	(0)	(2)	(0)	(0)	(1)	(0)
enlargement	0	0	0	0	2	0	0	1	0
tail;									
Submitted	(0)	(0)	(1)	(0)	(0)	(0)	(1)	(0)	(0)
irregularly shaped	0	0	1	0	0	0	0	0	0
abrasion(s); single	0	0	0	0	0	0	1	0	0
thyroid glands;									
Submitted	(15)	(15)	(15)	(15)	(15)	(15)	(15)	(15)	(15)
No Visible Lesions	15	15	15	15	15	15	15	15	15

Only positive findings are reported.
a = Pituitary examined but not collected for animal 9291.
Dose Level: Group 1 - 0 mg/kg/day; Group 2 - 0.0025 mg/kg/day Ethinyl Estradiol; Group 3 - 0.005 mg/kg/day Ethinyl Estradiol
Group 4 - 12.5 mg/kg/day Methoxychlor; Group 5 - 25 mg/kg/day Methoxychlor; Group 6 - 50 mg/kg/day Methoxychlor
Group 7 - 25 mg/kg/day Phenobarbital; Group 8 - 50 mg/kg/day Phenobarbital; Group 9 - 100 mg/kg/day Phenobarbital

Table 7 (continued)
F_1 Gross Pathology Summary
Pubertal Toxicity Study of Vinclozolin, Flutamide and Phenobarbital in Male Sprague Dawley Rats and Methoxychlor, Ethinyl Estradiol and Phenobarbital in Female Sprague Dawley Rats when Administered in Corn Oil by Oral Gavage

	FEMALES								
Group:	1	2	3	4	5	6	7	8	9
Number of Animals on Study :	15	15	15	15	15	15	15	15	15
Number of Animals Completed:	(15)	(15)	(15)	(15)	(15)	(15)	(15)	(15)	(15)
ureter(s);									
Submitted	(0)	(0)	(0)	(0)	(0)	(0)	(1)	(0)	(0)
dilation; right	0	0	0	0	0	0	1	0	0
urinary bladder;									
Submitted	(0)	(0)	(0)	(0)	(0)	(0)	(0)	(1)	(1)
thickening	0	0	0	0	0	0	0	0	1
calculus(i); white; multiple	0	0	0	0	0	0	0	1	1
uterus and cervix without fluid;									
Submitted	(15)	(15)	(15)	(15)	(15)	(15)	(15)	(15)	(15)
No Visible Lesions	11	13	14	13	14	12	12	12	12
distension; bilateral	4	1	1	2	1	3	3	3	3
distension; left	0	1	0	0	0	0	0	0	0

Only positive findings are reported.
Dose Level: Group 1 - 0 mg/kg/day; Group 2 - 0.0025 mg/kg/day Ethinyl Estradiol; Group 3 - 0.005 mg/kg/day Ethinyl Estradiol
Group 4 - 12.5 mg/kg/day Methoxychlor; Group 5 - 25 mg/kg/day Methoxychlor; Group 6 - 50 mg/kg/day Methoxychlor
Group 7 - 25 mg/kg/day Phenobarbital; Group 8 - 50 mg/kg/day Phenobarbital; Group 9 - 100 mg/kg/day Phenobarbital

Table 8
F_1 Summary of Organ Weights (Grams)
Pubertal Toxicity Study of Vinclozolin, Flutamide and Phenobarbital in Male Sprague Dawley Rats and Methoxychlor, Ethinyl Estradiol and Phenobarbital in Female Sprague Dawley Rats When Administered in Corn Oil by Oral Gavage

Group Sex		Terminal Body weight	Adrenal Glands	Dorsolateral Prostate	Ventral Prostate	Epididymides	Kidneys	Levator Ani plus Bulbocavernosus
1M	Mean	324.18	0.0493	0.2657	0.2384	0.5231	3.1139	0.5986
	S.E.	5.027	0.0021	0.0119	0.0135	0.0168	0.0480	0.0356
	N	(15)	(15)	(15)	(15)	(15)	(15)	(15)
2M	Mean	315.81	0.0532	0.2230	0.1998	0.5264	2.9043	0.6348
	S.E.	4.780	0.0022	0.0141	0.0136	0.0237	0.0611	0.0433
	N	(15)	(15)	(15)	(15)	(15)	(15)	(15)
3M	Mean	322.71	0.0479	0.2523	0.2106	0.5380	3.0668	0.5847
	S.E.	5.789	0.0030	0.0210	0.0134	0.0209	0.0655	0.0439
	N	(15)	(15)	(15)	(15)	(15)	(15)	(15)
4M	Mean	**298.27	0.0526	0.2119	**0.1792	0.5086	**2.8119	0.5850
	S.E.	5.153	0.0029	0.0135	0.0114	0.0256	0.0840	0.0280
	N	(14)	(14)	(13)	(14)	(14)	(14)	(14)
	P	0.004	0.400	0.069	0.021	0.825	0.006	0.770

Dose Level: Group 1 – 0 mg/kg/day Group 2 – 25 mg/kg/day Phenobarbital Group 3 – 50 mg/kg/day Phenobarbital
Group 4 – 100 mg/kg/day Phenobarbital
M = Male P = P-value from ANOVA
Statistical Significance: * = P<0.05 ** = P<0.01

Table 8 (continued)
F_1 Summary of Organ Weights (Grams)
Pubertal Toxicity Study of Vinclozolin, Flutamide and Phenobarbital in Male Sprague Dawley Rats and Methoxychlor, Ethinyl Estradiol and Phenobarbital in Female Sprague Dawley Rats When Administered in Corn Oil by Oral Gavage

Group Sex		Liver	Pituitary	Right Epididymis	Seminal Vesicles With Fluid	Seminal Vesicles Without Fluid	Testes	Thyroid with Parathyroids
1M	Mean	16.6198	0.0104	0.2519	0.6980	0.4109	2.7035	0.0216
	S.E.	0.4143	0.0004	0.0075	0.0452	0.0258	0.0410	0.0013
	N	(15)	(15)	(15)	(15)	(15)	(15)	(15)
2M	Mean	18.1356	0.0096	0.2588	0.7762	0.4530	2.7446	0.0204
	S.E.	0.4970	0.0005	0.0141	0.0351	0.0233	0.0342	0.0009
	N	(15)	(15)	(15)	(15)	(15)	(15)	(15)
3M	Mean	**19.5922	0.0100	0.2627	0.6863	0.3927	2.8150	0.0219
	S.E.	0.5854	0.0004	0.0137	0.0598	0.0272	0.0601	0.0008
	N	(15)	(15)	(15)	(15)	(15)	(15)	(15)
4M	Mean	**19.8170	*0.0087	0.2552	0.5857	0.3634	2.6539	0.0232
	S.E.	0.6773	0.0003	0.0155	0.0383	0.0291	0.0720	0.0013
	N	(14)	(14)	(14)	(14)	(14)	(14)	(14)
	P	0.000	0.029	0.942	0.046	0.124	0.196	0.354

Dose Level: Group 1 – 0 mg/kg/day Group 2 – 25 mg/kg/day Phenobarbital Group 3 – 50 mg/kg/day Phenobarbital
Group 4 – 100 mg/kg/day Phenobarbital
M = Male P = P-value from ANOVA
Statistical Significance: * = P<0.05 ** = P<0.01

Table 8 (continued)
F_1 Summary of Organ Weights (Grams)
Pubertal Toxicity Study of Vinclozolin, Flutamide and Phenobarbital in Male Sprague Dawley Rats and Methoxychlor, Ethinyl Estradiol and Phenobarbital in Female Sprague Dawley Rats When Administered in Corn Oil by Oral Gavage

Group Sex		Terminal Body weight	Adrenal Glands	Dorsolateral Prostate	Ventral Prostate	Epididymides	Kidneys	Levator Ani plus Bulbocavernosus
1M	Mean	324.18	0.0493	0.2658	0.2384	0.5231	3.1139	0.5986
	S.E.	5.027	0.00214	0.01186	0.01351	0.01680	0.04801	0.03563
	N	(15)	(15)	(15)	(15)	(15)	(15)	(15)
5M	Mean	332.56	0.0531	0.2493	0.2036	0.5833	3.0251	0.6207
	S.E.	6.112	0.00253	0.01429	0.01673	0.02018	0.06936	0.03269
	N	(15)	(15)	(15)	(15)	(15)	(15)	(15)
6M	Mean	*343.99	0.0518	0.2464	0.2102	0.5164	3.1449	0.6201
	S.E.	5.750	0.00230	0.00857	0.01338	0.02374	0.05584	0.02222
	N	(15)	(15)	(15)	(15)	(15)	(15)	(15)
7M	Mean	317.08	0.0563	**0.1890	*0.1834	0.4585	2.9573	**0.4590
	S.E.	5.448	0.00198	0.00720	0.01404	0.01594	0.04210	0.03720
	N	(15)	(15)	(15)	(15)	(15)	(15)	(15)
	P	0.009	0.180	0.000	0.071	0.000	0.075	0.002

Dose Level: Group 1 – 0 mg/kg/day Group 5 – 10 mg/kg/day Vinclozolin Group 6 – 30 mg/kg/day Vinclozolin
Group 7 – 100 mg/kg/day Vinclozolin
M = Male P = P-value from ANOVA
Statistical Significance: * = P<0.05 ** = P<0.01

Table 8 (continued)
F_1 Summary of Organ Weights (Grams)
Pubertal Toxicity Study of Vinclozolin, Flutamide and Phenobarbital in Male Sprague Dawley Rats and Methoxychlor, Ethinyl Estradiol and Phenobarbital in Female Sprague Dawley Rats When Administered in Corn Oil by Oral Gavage

Group Sex		Liver	Pituitary	Right Epididymis	Seminal Vesicles With Fluid	Seminal Vesicles Without Fluid	Testes	Thyroid with Parathyroids
1M	Mean	16.6198	0.0104	0.2519	0.6981	0.4109	2.7035	0.0217
	S.E.	0.41427	0.00037	0.00747	0.04515	0.02580	0.04104	0.00126
	N	(15)	(15)	(15)	(15)	(15)	(15)	(15)
5M	Mean	17.0859	0.0102	0.2863	0.6724	0.4097	2.8175	0.0192
	S.E.	0.52393	0.00041	0.01106	0.04042	0.02005	0.03143	0.00083
	N	(15)	(15)	(15)	(15)	(15)	(15)	(15)
6M	Mean	18.0730	0.0102	0.2552	0.6258	0.3742	**3.0094	0.0219
	S.E.	0.45738	0.00029	0.01248	0.02929	0.01272	0.03707	0.00085
	N	(15)	(15)	(15)	(15)	(15)	(15)	(15)
7M	Mean	16.3842	0.0095	0.2240	**0.4564	**0.2980	**2.9717	0.0210
	S.E.	0.53887	0.00041	0.01034	0.02698	0.01474	0.04893	0.00085
	N	(15)	(15)	(15)	(15)	(15)	(15)	(15)
	P	0.081	0.319	0.001	0.000	0.000	0.000	0.208

Dose Level: Group 1 – 0 mg/kg/day Group 5 – 10 mg/kg/day Vinclozolin Group 6 – 30 mg/kg/day Vinclozolin
Group 7 – 100 mg/kg/day Vinclozolin
M = Male P = P-value from ANOVA
Statistical Significance: * = P<0.05 ** = P<0.01

Table 8 (continued)
F_1 Summary of Organ Weights (Grams)
Pubertal Toxicity Study of Vinclozolin, Flutamide and Phenobarbital in Male Sprague Dawley Rats and Methoxychlor, Ethinyl Estradiol and Phenobarbital in Female Sprague Dawley Rats When Administered in Corn Oil by Oral Gavage

Group Sex		Terminal Body weight	Adrenal Glands	Dorsolateral Prostate	Ventral Prostate	Epididymides	Kidneys	Levator Ani plus Bulbocavernosus
1M	Mean	324.18	0.0493	0.2657	0.2384	0.5231	3.1139	0.5986
	S.E.	5.027	0.00214	0.01186	0.01351	0.0168	0.04801	0.03563
	N	(15)	(15)	(15)	(15)	(15)	(15)	(15)
8M	Mean	313.49	0.0518	**0.1238	**0.1062	**0.3443	**2.7121	**0.3878
	S.E.	4.523	0.00156	0.00738	0.00667	0.01614	0.05221	0.01825
	N	(15)	(15)	(15)	(15)	(15)	(15)	(15)
9M	Mean	308.69	**0.0592	**0.1019	**0.0835	**0.3504	**2.7644	**0.3849
	S.E.	6.675	0.00229	0.00708	0.00951	0.01632	0.07413	0.02385
	N	(15)	(15)	(15)	(15)	(15)	(15)	(15)
	P	0.137	0.004	0.000	0.000	0.000	0.000	0.000

Dose Level: Group 1 – 0 mg/kg/day Group 8 – 25 mg/kg/day Flutamide Group 9 – 50 mg/kg/day Flutamide
M = Male P = P-value from ANOVA
Statistical Significance: * = P<0.05 ** = P<0.01

Table 8 (continued)
F_1 Summary of Organ Weights (Grams)
Pubertal Toxicity Study of Vinclozolin, Flutamide and Phenobarbital in Male Sprague Dawley Rats and Methoxychlor, Ethinyl Estradiol and Phenobarbital in Female Sprague Dawley Rats When Administered in Corn Oil by Oral Gavage

Group Sex		Liver	Pituitary	Right Epididymis	Seminal Vesicles With Fluid	Seminal Vesicles Without Fluid	Testes	Thyroid with Parathyroids
1M	Mean	16.6198	0.0104	0.2519	0.6980	0.4109	2.7027	0.0216
	S.E.	0.41427	0.00037	0.00747	0.04515	0.0258	0.04104	0.00126
	N	(15)	(15)	(15)	(15)	(15)	(15)	(15)
8M	Mean	16.7339	0.0110	**0.1688	*0.1740	**0.1449	*2.9993	0.0212
	S.E.	0.49512	0.00054	0.00969	0.01763	0.01421	0.05388	0.00108
	N	(15)	(15)	(15)	(15)	(15)	(15)	(15)
9M	Mean	17.3366	0.0110	**0.1648	*0.1367	**0.1165	*3.4953	0.0204
	S.E.	0.45703	0.0005	0.0102	0.01108	0.00942	0.13064	0.00116
	N	(15)	(15)	(15)	(15)	(15)	(15)	(15)
	P	0.497	0.584	0.000	(c)	0.000	(c)	0.741

Dose Level: Group 1 – 0 mg/kg/day Group 8 – 25 mg/kg/day Flutamide Group 9 – 50 mg/kg/day Flutamide
M = Male P = P-value from ANOVA
Statistical Significance: * = P<0.05 ** = P<0.01
c = Dunn's Test used for pair-wise comparisons

Table 8 (continued)
F_1 Summary of Organ Weights (Grams)
Pubertal Toxicity Study of Vinclozolin, Flutamide and Phenobarbital in Male Sprague Dawley Rats and Methoxychlor, Ethinyl Estradiol and Phenobarbital in Female Sprague Dawley Rats When Administered in Corn Oil by Oral Gavage

Group Sex		Terminal Body weight	Adrenal Glands	Kidneys	Liver	Ovaries	Pituitary	Thyroid with Parathyroids	Uterus, Cervix With Fluid	Uterus, Cervix Without Fluid
1F	Mean	171.10	0.0414	1.7290	8.6670	0.0930	0.0086	0.0165	0.3553	0.4194
	S.E.	1.953	0.00175	0.02895	0.14691	0.00416	0.00053	0.00105	0.02763	0.04917
	N	(15)	(15)	(15)	(15)	(15)	(15)	(15)	(15)	(15)
2F	Mean	169.91	**0.0501	1.7157	8.4112	0.0944	0.0082	0.0169	0.3871	0.4357
	S.E.	2.541	0.00206	0.03515	0.16290	0.00426	0.00056	0.00076	0.02365	0.03722
	N	(15)	(15)	(15)	(15)	(15)	(15)	(15)	(15)	(15)
3F	Mean	173.24	**0.0487	1.6992	8.7559	0.0987	0.0093	0.0168	0.3603	0.3972
	S.E.	3.358	0.00127	0.02981	0.15814	0.00458	0.00045	0.00107	0.02009	0.03386
	N	(15)	(15)	(15)	(15)	(15)	(15)	(15)	(15)	(15)
	P	0.676	0.002	0.798	0.280	0.627	0.277	0.959	0.605	0.799

Dose Level: Group 1 – 0 mg/kg/day Group 2 – 0.0025 mg/kg/day Ethinyl Estradiol Group 3 – 0.005 mg/kg/day Ethinyl Estradiol
F = Female P = P-value from ANOVA
Statistical Significance: * = P<0.05 ** = P<0.01

Table 8 (continued)
F_1 Summary of Organ Weights (Grams)
Pubertal Toxicity Study of Vinclozolin, Flutamide and Phenobarbital in Male Sprague Dawley Rats and Methoxychlor, Ethinyl Estradiol and Phenobarbital in Female Sprague Dawley Rats When Administered in Corn Oil by Oral Gavage

Group Sex		Terminal Body weight	Adrenal Glands	Kidneys	Liver	Ovaries	Pituitary	Thyroid with Parathyroids	Uterus, Cervix With Fluid	Uterus, Cervix Without Fluid
1F	Mean	171.10	0.0414	1.7290	8.6670	0.0930	0.0086	0.0165	0.3553	0.4194
	S.E.	1.953	0.00175	0.02895	0.14691	0.00416	0.00053	0.00105	0.02763	0.04917
	N	(15)	(15)	(15)	(15)	(15)	(15)	(15)	(15)	(15)
4F	Mean	167.85	*0.0487	1.6687	8.1228	0.0953	0.0079	0.0173	0.3472	0.3778
	S.E.	2.126	0.00133	0.02910	0.21855	0.00434	0.00047	0.00063	0.02277	0.03733
	N	(15)	(15)	(15)	(15)	(15)	(15)	(15)	(15)	(15)
5F	Mean	172.53	0.0463	1.6664	8.3961	0.0850	0.0077	0.0185	0.3310	0.3580
	S.E.	3.009	0.00220	0.03303	0.24842	0.00397	0.00032	0.00087	0.01827	0.02300
	N	(15)	(15)	(15)	(15)	(15)	(15)	(15)	(15)	(15)
6F	Mean	165.59	0.0448	1.6662	*7.8981	*0.0789	*0.0069	0.0169	0.3316	0.4373
	S.E.	1.761	0.00225	0.03244	0.16802	0.00337	0.00054	0.00093	0.02396	0.05597
	N	(15)	(15)	(15)	(15)	(15)	(15)	(15)	(15)	(15)
	P	0.136	0.066	0.398	0.049	0.019	0.102	0.415	0.852	0.547

Dose Level: Group 1 – 0 mg/kg/day Group 4 – 12.5 mg/kg/day Methoxychlor Group 5 – 25 mg/kg/day Methoxychlor
Group 6 – 50 mg/kg/day Methoxychlor
F = Female P = P-value from ANOVA
Statistical Significance: * = P<0.05 ** = P<0.01

Table 8 (continued)
F_1 Summary of Organ Weights (Grams)
Pubertal Toxicity Study of Vinclozolin, Flutamide and Phenobarbital in Male Sprague Dawley Rats and Methoxychlor, Ethinyl Estradiol and Phenobarbital in Female Sprague Dawley Rats When Administered in Corn Oil by Oral Gavage

Group Sex		Terminal Body Weight	Adrenal Glands	Kidneys	Liver	Ovaries	Pituitary	Thyroid with Parathyroids	Uterus, Cervix With Fluid	Uterus, Cervix Without Fluid
1F	Mean	171.10	0.0414	1.7290	8.6670	0.0930	0.0086	0.0165	0.3553	0.4194
	S.E.	1.953	0.00175	0.02895	0.14691	0.00416	0.00053	0.00105	0.02763	0.04917
	N	(15)	(15)	(15)	(15)	(15)	(15)	(15)	(15)	(15)
7F	Mean	171.36	**0.0520	1.9541	*9.7852	0.0977	0.0089	0.0177	0.3515	0.4070
	S.E.	3.543	0.00252	0.20915	0.31274	0.00401	0.00095	0.00099	0.01817	0.03914
	N	(15)	(15)	(15)	(15)	(15)	(15)	(15)	(15)	(15)
8F	Mean	175.37	**0.0526	1.7419	**10.7576	0.1007	0.0079	0.0181	0.3475	0.3932
	S.E.	3.151	0.00156	0.07392	0.37280	0.00339	0.00052	0.00115	0.02081	0.03734
	N	(15)	(15)	(15)	(15)	(15)	(15)	(15)	(15)	(15)
9F	Mean	171.46	**0.0518	1.7403	**11.3215	0.0942	0.0083	*0.0120	0.3519	0.4087
	S.E.	2.753	0.00197	0.06703	0.33043	0.00430	0.00036	0.00069	0.02302	0.03618
	N	(15)	(15)	(15)	(15)	(15)	(14)	(15)	(15)	(15)
	P	0.691	0.000	0.464	0.000	0.516	0.717	0.100	0.996	0.976

Dose Level: Group 1 – 0 mg/kg/day Group 7 – 25 mg/kg/day Phenobarbital Group 8 – 50 mg/kg/day Phenobarbital
Group 9 – 100 mg/kg/day Phenobarbital
F = Female P = P-value from ANOVA
Statistical Significance: * = P<0.05 ** = P<0.01

Appendix 1
Bulk Chemical Analysis
Pubertal Toxicity Study of Vinclozolin, Flutamide and Phenobarbital in Male Sprague Dawley Rats and Methoxychlor, Ethinyl Estradiol and Phenobarbital in Female Sprague Dawley Rats when Administered in Corn Oil by Oral Gavage

50-06-6

COPY

Bulk Chemical Reanalysis for Phenobarbital Free Acid

NIEHS Contract No. N01-ES-05457
ETP Task No. CHEM07191
MRI Project No. 110100
MRI Task No. 590

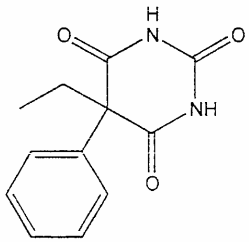
Midwest Research Institute
425 Volker Boulevard
Kansas City, Missouri 64110

January 23, 2003

7244-600
Rocca
Wolfe
Holley

Bulk Chemical Reanalysis for Phenobarbital Free Acid

CAS No.: 50-06-6 MRI Task No.: 590 ETP Task No.: CHEM07191 Program Supported: RTX Analysis Dates: 12/9/2002 to 12/11/2002 Interim Result Dates: 12/16/2002 (e-mail)	Lot No.: QI0645 MRI Assigned Batch No.: 01 Amount Received: 1 x 5 g Sample Receipt Date: 12/5/02 Storage conditions (@ Analytical Lab): Frozen Supplier: Therimmune Research Sample ID: 1909A Study No.: 7244-600

Structure	Mol. Wt.	Mol. Formula
	232.2	$C_{12}H_{12}N_2O_3$

Quality Assurance Statement

Bulk Chemical Reanalysis for Phenobarbital Free Acid

ETP Task No. CHEM07191
MRI Project No. 110100
MRI Task No. 590

This study was inspected by the Quality Assurance Unit of MRI (QAU) and the findings reported to the Study Director and Management as follows:

Phase inspected	Date inspected	Date reported
Protocol audit	12/11/02	12/11/02
In-life audit; GC analysis	12/11/02	12/11/02
Data audit	1/17/03	1/17/03
Report audit	1/17/03	1/17/03

The raw data and report will be stored in the MRI Archives.

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

Facility/equipment	Inspection date	Management submitted date
PCA laboratory complex	9/13/02	9/13/02
FT-IR facility	11/18/02	11/18/02
GC facility	11/18/02	11/19/02

MIDWEST RESEARCH INSTITUTE

Rosemary Moran

Rosemary Moran
Quality Assurance Officer

Approved:

James Dworak

James E. Dworak, Ph.D.
Manager, Quality Assurance

January 23, 2003

MRI-ETP\TASK 590.DOC

Good Laboratory Practice Compliance Statement

Bulk Chemical Reanalysis for Phenobarbital Free Acid

ETP Task No. CHEM07191
MRI Project No. 110100
MRI Task No. 590

This study was conducted in compliance with the Good Laboratory Practice regulations of the U.S. Food and Drug Administration (21 *CFR* 58).

Jason McClintock
Study Director

1/23/2003
Date

Executive Summary

The purpose of this task was to perform a bulk chemical reanalysis for phenobarbital free acid (Lot No. QI0645) received from Therimmune Research. The identity of the test article was confirmed as Phenobarbital-free acid by infrared spectroscopy. A major peak comparison assay was performed using gas chromatography to compare the purity of the test article with that of a frozen reference sample. The relative percent purity of the test article was determined to be 99.4 ± 0.9(s)%.

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Bulk Chemical Reanalysis for Phenobarbital Free Acid

1. Introduction

The purpose of this task was to perform a bulk chemical reanalysis of a sample of phenobarbital free acid (Lot No. QI0645) received from Therimmune Research. The identity of the test article was verified by infrared spectroscopy. Gas chromatography (GC) was used to perform a major peak comparison assay of a frozen reference sample (maintained at MRI) and the bulk chemical test article. For GC, a HP-5 column was used with flame ionization detection to analyze solutions of the test article in methanol. This task was initiated on December 5, 2002.

2. Chemical Information

Test Article:	Phenobarbital free acid
Lot No.:	QI0645
MRI Assigned Batch No.:	01
Supplier:	Therimmune Research (Study No. 7244-600)
Molecular Formula:	$C_{12}H_{12}N_2O_3$
Molecular Weight:	232.2
CAS No.:	50-06-6

3. Analysis Methods

3.1 Infrared Spectroscopy

3.1.1 Sample Preparation

The bulk chemical test article was incorporated into a potassium bromide pellet at a concentration of approximately 2.0%.

3.1.2 Instrument

Perkin Elmer Series 1605 FT-IR with the following parameters.

Scan Range:	400 cm^{-1} to 4000 cm^{-1}
Number of Scans:	Background: 32 Sample: 32
Resolution:	2.00 cm^{-1}
Apodization:	Strong

3.1.3 Analysis

The spectrum of the sample was obtained from 400 to 4000 cm^{-1} and corrected for the background spectrum of air. A polystyrene film was also scanned to verify instrument calibration.

3.1.4 Results

The FT-IR spectrum of the sample is contained in Figure 1, attached. The sample spectrum was consistent with a previously obtained spectrum[1] for phenobarbital free acid.

3.2 Gas Chromatography

3.2.1 Solution Preparation

3.2.1.1 Internal Standard Stock Solution

The internal standard stock solution was prepared by weighing approximately 126 mg of caffeine and transferring into a 50-mL volumetric flask. The contents of the flask were brought to volume with methanol, sonicated for about 10 minutes, cooled to ambient temperature, and mixed by inversion.

3.2.1.2 Internal Standard Blank Solution

A 1-mL portion of the internal standard stock solution was volumetrically pipetted into a 10-mL volumetric flask. The contents of the flask were diluted to volume with methanol and mixed by inversion.

3.2.1.3 Sample and Standard Solutions

Triplicate test article and frozen reference solutions were prepared for the purity assay by accurately weighing approximately 123 to 129 mg of the test article or frozen reference sample into individual 50-mL volumetric flasks. The contents of the flasks were then diluted to volume with methanol and mixed by inversion (target concentration, ~ 2.5 mg/mL).

A 1-mL portion of each of the solutions was transferred by volumetric pipette to individual 10-mL volumetric flasks. A 1-mL portion of internal standard stock solution was added to each flask by volumetric pipette. The contents of the flasks were diluted to volume with methanol and mixed by inversion (target concentration, ~ 0.25 mg/mL).

---

[1] MRI Final Report, "*Bulk Chemical Limited Analysis of Phenobarbital Free Acid,*" NIEHS Contract No. N01-ES-05457, ETP Task No. CHEM06834, MRI Project No. 110100, Task No. 495, July 19, 2002.

3.2.2 Instrument

Instrument:	Agilent 6890 gas chromatograph with Agilent 7683 autosampler
Injection Mode:	Splitless, purge on at 0.75 min
Injection Volume:	1 µL
Column:	HP-5, 30 m x 0.53 mm ID, 1.5-µm thickness
Temperature Program:	220°C, isothermal
Detector:	Flame ionization
Range:	0
Temperatures:	
Inlet:	250°C
Detector:	275°C
Carrier Gas:	Helium
Flow Rate:	9.7 mL/min
Makeup Gas:	Nitrogen
Flow Rate:	22.3 mL/min
Air Flow Rate:	320 mL/min
Hydrogen Flow Rate:	31.2 mL/min
Electronic Integration:	Turbochrom Version 6.1.1
Retention Times:	Caffeine, ~ 3.2 min
Phenobarbital,	~ 4.4 min

3.2.3 Analysis

Portions of each sample, frozen reference, internal standard blank, and solvent blank solution were transferred to individual autosampler vials. Each solution was injected and analyzed using the system and parameters described in Section 3.2.2.

3.2.4 Results

3.2.4.1 System Suitability

The analytical system described in Section 3.2.2 was evaluated for system precision, theoretical plates, and tailing factor, according to USP guidelines.[2] System precision was calculated using the average response ratio obtained from six injections of a bulk chemical solution. Theoretical plates, tailing factor, and resolution were evaluated for a single injection of the same solution.

[2] *The United States Pharmacopeia*, USP 24, Physical Tests/<621> Chromatography—System Suitability, ©1999, pp. 1923-1924.

Table 1. System Suitability

Test	Phenobarbital free acid		Caffeine (internal standard)	
	Acceptable range	Assay	Acceptable range	Assay
System Precision	< 2	1.3	–	–
Retention Time (min)	~ 7.6	4.4	~ 5.4	3.2
Tailing Factor	< 2	2.3	< 2	1.2
Theoretical Plates	> 20,000	12,340	> 25,000	14,263
Resolution	> 5	9.0	–	–

Several of the system suitability criteria were not met due to the use of a different, but equivalent, column. This did not adversely affect the results for the analysis of the test article.

3.2.4.2 Purity Assay

The relative purity (%) was calculated for each solution of the bulk chemical test article using the average relative weight ratio for the frozen reference solutions and the relative weight ratio for the bulk chemical test article solution. The average relative purity and standard deviation (n = 3) were calculated. Results are presented in Table 2, and representative chromatograms are shown in Figure 2.

Table 2. Relative Purity of Bulk Chemical

Test article sample	Relative purity (%)
1	99.0
2	98.8
3	100.4
Average	99.4 ± 0.9(s)%

4. Conclusions

The test article was identified by infrared spectroscopy as phenobarbital free acid. A major peak comparison analysis by gas chromatography indicated a relative purity of 99.4 ± 0.9(s)% for the bulk chemical.

5. Contributors

Matt Armstrong performed the analyses for this study.

MIDWEST RESEARCH INSTITUTE

Jason McClintock
Study Director

Linda Siemann
Group Leader
Bulk Chemical Analysis

Approved:

for Roger K. Harris, Ph.D.
Principal Investigator
Environmental Toxicology Program

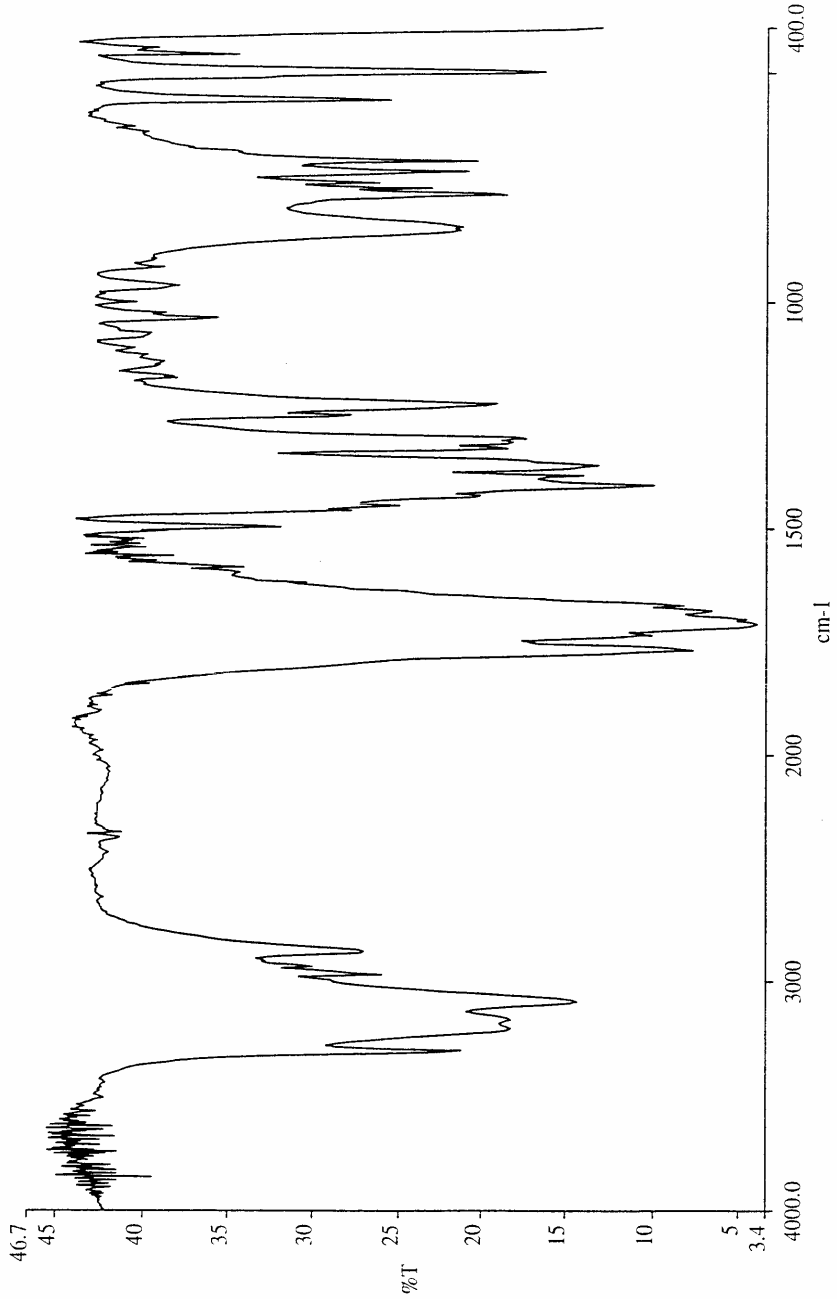

Figure 1. Fourier Transform Infrared Spectrum of Phenobarbital Free Acid Bulk Chemical (Lot No. QI0645)

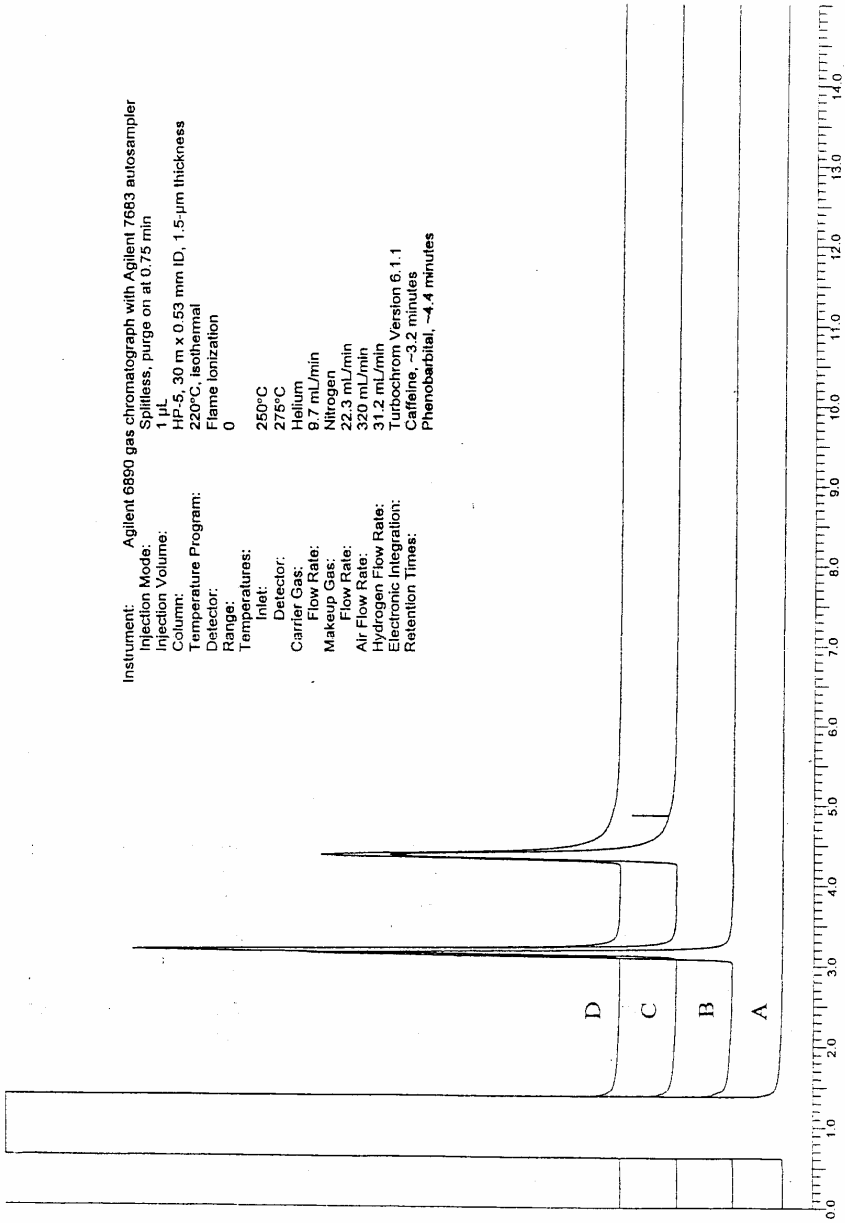

Figure 2. Typical Chromatograms for GC Analysis of Phenobarbital Free Acid (Lot No. QI0645)
(A) Blank (B) IS Blank (C) Frozen Reference (D) Bulk Chemical

MRI-ETPTASK-590.DOC

7

BATTELLE-BCR

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Chemistry Support Services for the NTP
NIH Contract No.: N01-ES-05456
Battelle Project No.: G004110-BPZ
NTP ChemTask No.: CHEM07214
CAS No.: 50471-44-8

COPY

BULK CHEMICAL REANALYSIS REPORT

VINCLOZOLIN

8-039-BCR-69

January 31, 2003

Prepared By:

Wendy M. Black
Study Director

Approved By:

Steven W. Graves, B.S.
Principal Investigator

Submitted to:

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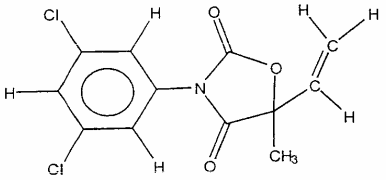
4401 Commons Building, Suite 100
Research Triangle Park, NC 27709

7244-600
Rocca
Wolfe
Holley

BULK CHEMICAL REANALYSIS REPORT

VINCLOZOLIN

CAS No.: 50471-44-8	Lot No.: 102996 (Battelle Organic Synthesis Group)
Battelle Chemical ID Code: 039	Samples/Amount Received: Bulk Chemical
Battelle Task No.: 8-039-BCR-69	Sample Receipt Date: 12/05/02
NTP Task No.: CHEM07214	Submitter: Gary Holley
Program Supported: RDGT	Study Lab: Therimmune
Analysis Date: 1/9/03	Receipt Condition/Appearance: On Dry Ice/Good
Interim Results Date: 1/14/03	Shipping Container: Amber Glass Vial
	Storage Conditions (@ Battelle): Frozen (≤-20°C)

STRUCTURE	Mol. Wt.	Mol. Formula
	286.1 g/mol	$C_{12}H_9NO_3Cl_2$

EXECUTIVE SUMMARY

The purpose of this study was to compare the purity of a sample of the bulk chemical currently in use to the same lot of a frozen reference standard maintained at ≤-20°C. The sample was analyzed by gas chromatography (GC) with flame ionization detection and determined to have a purity of 100.1% relative to the frozen reference, which was statistically identical to the frozen reference. This indicates the purity of the sample is acceptable for continued use.

QUALITY ASSURANCE STATEMENT

BULK CHEMICAL REANALYSIS REPORT

VINCLOZOLIN

NTP ChemTask No.: CHEM07214
Battelle Project No.: G004110-BPZ
Battelle Task No.: 8-039-BCR-69

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

Critical Phase Inspected	Date Inspected	Date Reported to Study Director and Management
Audit study file	1/30/03	1/30/03
Audit analytical report	1/30/03	1/30/03

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

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

Facility/Equipment	Date Inspected	Date of Report to Management
Chemistry Technical Center inspection	4/23/00	4/24/00
	12/8-12/12/00	12/13/00
	10/17/01	10/19/01
	9/6/02	9/6/02

1-31-03
Quality Assurance Unit Date
Battelle

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Figure 2. Control Chart 4

1 INTRODUCTION

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

2 TEST ARTICLE

Vinclozolin, Lot No. 102996, was received from Battelle's Organic Synthesis Group on October 30, 1996. The frozen reference standard was removed from this lot shortly after receipt and has been stored at ≤-20°C since that time. The bulk chemical reanalysis sample was received from Therimmune on December 5, 2002 and stored at ≤-20°C until being transferred on January 9, 2003 to the analytical chemistry laboratory for analysis.

3 ANALYSIS METHOD

The test article was analyzed according to the "Standard Operating Procedure (SOP) for the Bulk Chemical Reanalysis of Vinclozolin." This section describes the method, results, and conclusions.

3.1 Preparation of Internal Standard Solution

Internal standard (IS) solution was prepared by accurately weighing 400 ± 40 mg of isodrin into a 100-mL volumetric flask and dissolving in and diluting to volume with ethyl acetate.

3.2 Preparation of Frozen Reference Standards

Triplicate stock solutions were prepared by transferring 50 ± 5 mg of frozen reference standard into individual 100-mL volumetric flasks. The samples were dissolved in and diluted to volume with ethyl acetate and mixed well. One (1) mL of each of these solutions and 1 mL of internal standard solution were pipetted into individual 10-mL volumetric flasks. The flasks were diluted to volume with ethyl acetate and mixed.

3.3 Preparation of Bulk Chemical Samples

Triplicate stock solutions were prepared by transferring 50 ± 5 mg of bulk chemical sample into individual 100-mL volumetric flasks. The samples were dissolved in and diluted to volume with ethyl acetate and mixed well. One (1) mL of each of these solutions and 1 mL of internal standard solution were pipetted into individual 10-mL volumetric flasks. The flasks were diluted to volume with ethyl acetate and mixed.

3.4 Preparation of Blanks

The blank containing internal standard was prepared by diluting 1 mL of internal standard solution to 10 mL with ethyl acetate. The blank was ethyl acetate.

3.5 Analysis

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

Table 1 – GC System

GC	Agilent Model 6890 (Palo Alto, CA)
Column	RTX-5, 30 m x 0.25 mm (ID), 0.25 µm film thickness (Restek, Bellefonte, PA)
Carrier Gas and Flow Rate	Helium at 2 mL/minute
Hydrogen Flow Rate	30 mL/minute
Air Flow Rate	380 mL/minute
Detector	Flame Ionization (FID)
Column Temperature	120°C with hold 1 minute, increase at 15°C per minute to 315°C, hold 3 minutes
Detector Temperature	225°C
Injector Temperature	200°C
Injection Volume/Mode	1 µL/Splitless
Run Time	17 minutes
Retention Times	
Vinclozolin	~7.5 minutes
IS	~8.4 minutes

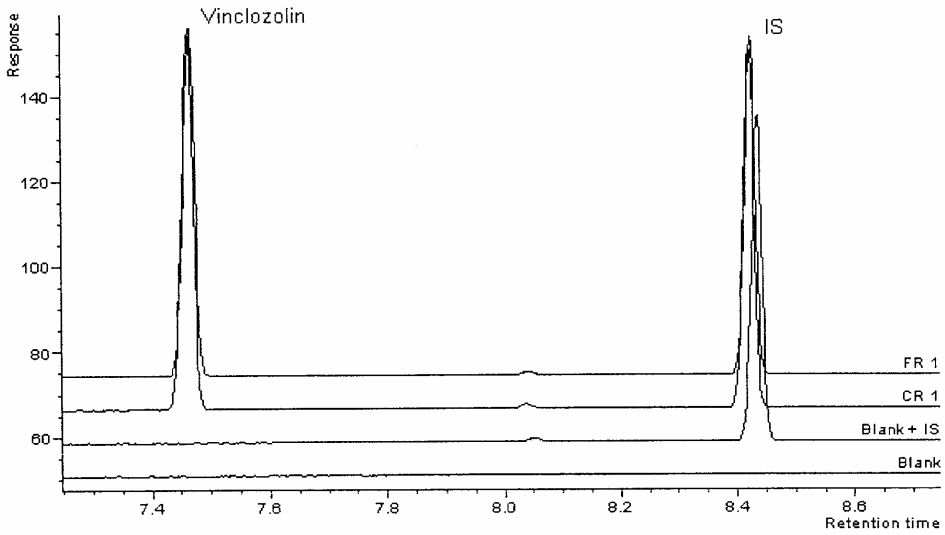

Figure 1 – Representative Overlaid Chromatograms of Frozen Reference Standard, Bulk Chemical Sample, Blank with Internal Standard, and Blank

4 RESULTS

The integration of the vinclozolin and internal standard peaks by the chromatography data system was examined and manually modified, if necessary. The relative response factor for each reference and bulk chemical sample was calculated using the peak area ratio (vinclozolin /internal standard) and the individual weight of each sample. The relative response factor for each bulk chemical samples was compared to the average relative response factor for the frozen reference standard. The average relative purity of the bulk chemical when compared to the frozen reference was 100.1%. The results are shown in Table 2.

Table 2 – Results for Bulk Chemical Reanalysis

Sample ID	Relative % Purity	Avg Relative % Purity ± s
A	100.0	
B	99.7	100.1 ± 0.5
C	100.6	

The average percent purity of the test article was compared to the average value of the frozen reference using the following formula for the uncertainty value U, where t is the student t variable at the chosen significance level (95% in this case) and the degrees of freedom, s_p is the pooled standard deviation of the method and n is the number of replicates of the means under comparison. The calculated value of U (significant difference), 1.0% for this analysis,

$$U = ts_p\sqrt{(n_a + n_b) \div (n_a \times n_b)}$$

indicated that the average value of the test article was equivalent to the purity of the frozen reference.

5 CONCLUSIONS

The bulk chemical is 100.1% pure relative to the frozen reference standard, which is statistically equivalent to the frozen reference. The purity of the bulk chemical is acceptable for continued use. A chemical reanalysis control chart for demonstration of purity over time is included in Figure 2.

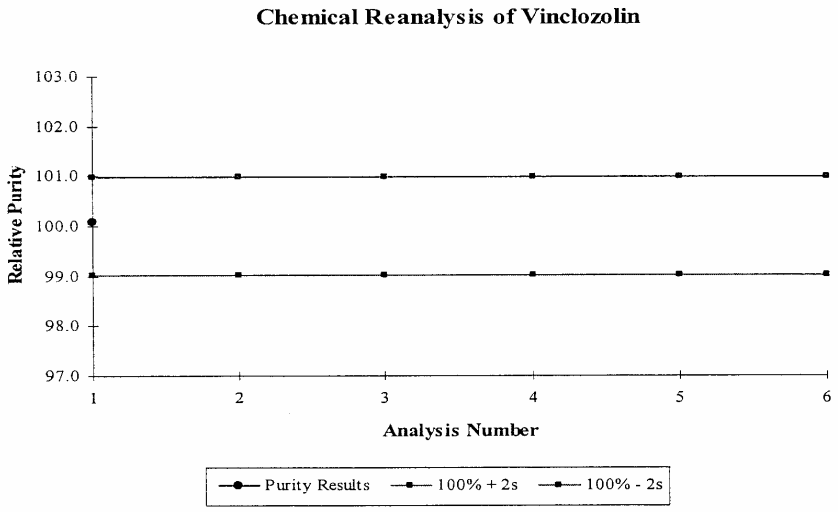

Figure 2 – Control Chart

6 ACKNOWLEDGMENTS

John Kelly performed the analytical work. Wendy Black wrote the report. Padmini Fernando reviewed the analysis raw data for completeness and accuracy.

BATTELLE-BCR

Chemistry Support Services for the NTP
NIH Contract No.: N01-ES-05456
Battelle Project No.: G004110-BPY
NTP ChemTask No.: CHEM07216
CAS No.: 13311-84-7

COPY

BULK CHEMICAL REANALYSIS REPORT

FLUTAMIDE

8-139-BCR-68

January 31, 2003

Prepared By:

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Study Director

Approved By:

Steven W. Graves, B.S.
Principal Investigator

Submitted to:

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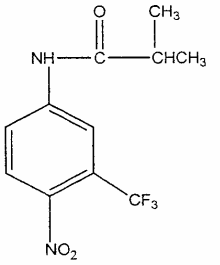
7249-600
Rocca
Wolfe
Holley

BULK CHEMICAL REANALYSIS REPORT

FLUTAMIDE

CAS No.: 13311-84-7	Lot No.: 109H0952 (Sigma)
Battelle Chemical ID Code: 139	Samples/Amount Received: One 5 g chemical reanalysis sample
Battelle Task No.: 8-139-BCR-68	Sample Receipt Date: 12/05/02
NTP Task No.: CHEM07216	Submitter: G. Holley
Program Supported: RDGT	Study Lab: Therimmune
Analysis Date: 1/08/03	Receipt Condition/Appearance: Good
Interim Results Date: 1/10/03	Shipping Container: Amber glass vial
	Storage Conditions (@ Battelle): Frozen ≤-20°C

STRUCTURE	Mol. Wt.	Mol. Formula
	276.2 g/mol	$C_{11}H_{11}F_3N_2O_3$

EXECUTIVE SUMMARY

The purpose of this study was to compare the purity of a sample of the bulk chemical currently in use to the same lot of a reference standard maintained at ≤-20°C. The sample was analyzed by high performance liquid chromatography (HPLC) with UV detection and determined to have a purity of 99.3% relative to the reference.

QUALITY ASSURANCE STATEMENT

BULK CHEMICAL REANALYSIS REPORT

FLUTAMIDE

NTP ChemTask No.: CHEM07216
Battelle Project No.: G004110-BPY
Battelle Task No.: 8-139-BCR-68

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

Critical Phase Inspected	Date Inspected	Date Reported to Study Director and Management
Audit study file	1/30/03	1/30/03
Audit analytical report	1/30/03	1/30/03

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

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

Facility/Equipment	Date Inspected	Date of Report to Management
Chemistry Technical Center inspection	4/23/00	4/24/00
	12/8-12/12/00	12/13/00
	10/17/01	10/19/01
	9/6/02	9/6/02

1-31-03
Quality Assurance Unit Date
Battelle

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Figure 1. Representative Overlaid Chromatograms of Frozen Reference Standard, Bulk Chemical Sample, Blank with Internal Standard, and Blank 3
Figure 2. Control Chart 4

1 INTRODUCTION

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

2 TEST ARTICLE

Flutamide, Lot No. 109H0952, was received at Battelle from Sigma on January 23, 2001. The frozen reference standard was removed from this lot shortly after receipt and has been stored at ≤-20°C since that time. The bulk chemical reanalysis sample was received from Therimmune on December 5, 2002 and stored at ≤-20°C until being transferred on January 8, 2003 to the analytical chemistry laboratory for analysis.

3 ANALYSIS METHOD

The test article was analyzed according to the "Standard Operating Procedure (SOP) for the Bulk Chemical Reanalysis of Flutamide." This section describes the method, results, and conclusions.

3.1 Preparation of Internal Standard Solution

Internal standard (IS) solution was prepared by pipetting 200 µL of acetophenone into a 100-mL volumetric flask and dissolving in and diluting to volume with HPLC mobile phase (see Table 1 for composition).

3.2 Preparation of Frozen Reference Samples

Triplicate stock solutions were prepared by transferring 100 ± 10 mg of accurately weighed frozen reference sample into individual 50-mL volumetric flasks. The samples were dissolved in and diluted to volume with acetone and mixed well. One (1) mL of each of these solutions and 1 mL of internal standard solution were pipetted into individual 50-mL volumetric flasks. The flasks were diluted to volume with HPLC mobile phase and mixed.

3.3 Preparation of Chemical Reanalysis Samples

Triplicate stock solutions were prepared by transferring 100 ± 10 mg of accurately weighed chemical reanalysis sample into individual 50-mL volumetric flasks. The samples were dissolved in and diluted to volume with acetone and mixed well. One (1) mL of each of these solutions and 1 mL of internal standard solution were pipetted into individual 50-mL volumetric flasks. The flasks were diluted to volume with HPLC mobile phase and mixed.

3.4 Preparation of Blanks

The blank containing internal standard was prepared by diluting 1 mL of internal standard solution to 50 mL with HPLC mobile phase. The blank was HPLC mobile phase.

3.5 Analysis

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

Table 1 – HPLC System

HPLC System	Agilent (Plo Alto, CA)
Analytical Column	Phenomenex Ultremex 5 µ, C-18, 250 X 4.6 mm ID (Torrance, CA)
Guard Column	Phenomenex, 5 µ
Mobile Phase	65:35 (v:v) Acetonitrile:Milli-Q Water*
Flow Rate	0.7 mL/minute
Injection Volume	10 µL
UV Detection Wavelength	240 nm
Run Time	20 minutes
Retention Time	
IS	~5.7 minutes
Flutamide	~7.5 minutes

*Milli-Q water has a resistivity of ≥ 18MΩ-cm.

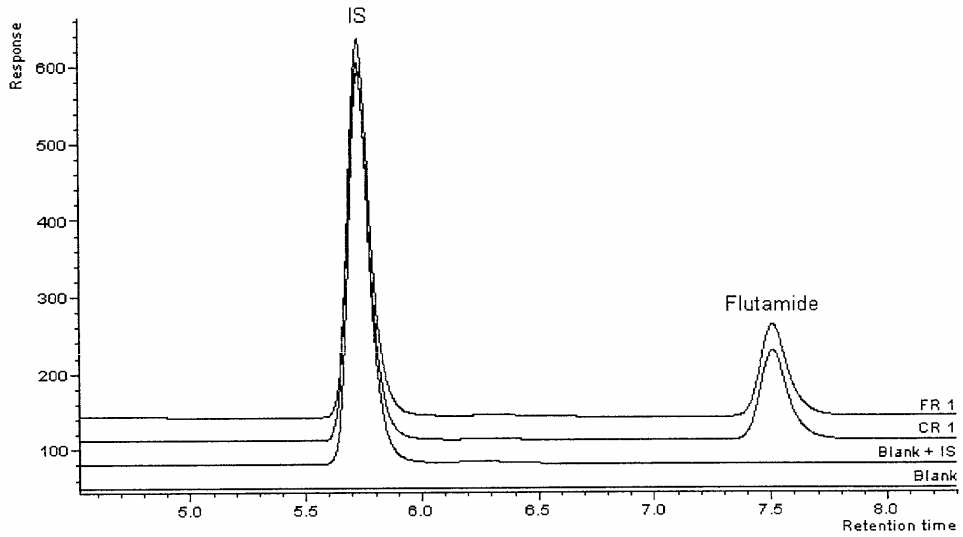


Figure 1 – Representative Overlaid Chromatograms of Frozen Reference Standard, Bulk Chemical Sample, Blank with Internal Standard, and Blank

4 RESULTS

The integration of the flutamide and internal standard peaks by the chromatography data system was examined and manually modified, if necessary to achieve consistency. The relative response factor for each frozen reference and chemical reanalysis sample was calculated using the peak area ratio (flutamide/internal standard) and the individual weight of each sample. The relative response factor for each bulk chemical sample was compared to the average relative response factor for the reference standard. The average relative purity of the bulk chemical when compared to the reference was 99.3%. No impurity peaks were present in the chemical reanalysis sample that were not in the frozen reference sample also. The results are shown in Table 2.

Table 2 – Results for Bulk Chemical Reanalysis

Sample ID	Relative % Purity	Avg Relative % Purity ± s
A	99.1	
B	99.3	99.3 ± 0.1
C	99.3	

The average percent purity of the test article was compared to the average value of the frozen reference using the following formula for the uncertainty value U, where t is the student t variable at the chosen significance level (95% in this case) and the degrees of freedom, s_p is the pooled standard deviation of the method and n is the number of replicates of the means under comparison. The calculated value of U (significant difference) was 0.6% for this analysis.

$$U = ts_p\sqrt{(n_a + n_b) \div (n_a \times n_b)}$$

5 CONCLUSIONS

The chemical reanalysis sample is 99.3% pure relative to the frozen reference sample, which was statistically different from the purity of the frozen reference. This difference is more than likely due to the excellent precision of the method, which resulted in a small value for U, rather than any real difference in purity. A chemical reanalysis control chart showing the purity over time is included in Figure 2.

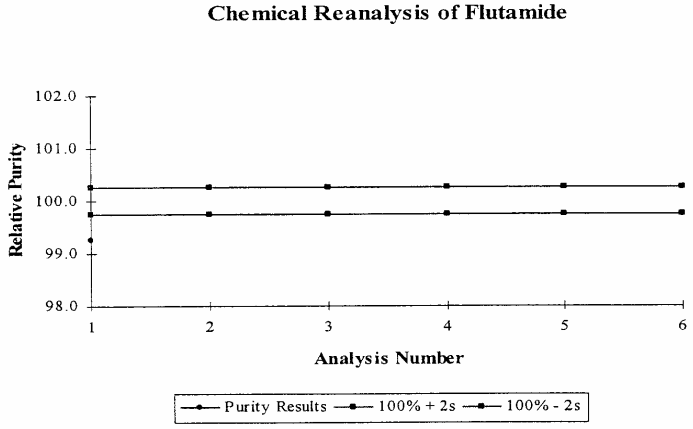


Figure 2 – Control Chart

6 ACKNOWLEDGMENTS

John Kelly performed the analytical work. Wendy M. Black wrote the report. Padmini Fernando reviewed the analysis raw data for completeness and accuracy.

COPY

Bulk Chemical Reanalysis for Ethinyl estradiol

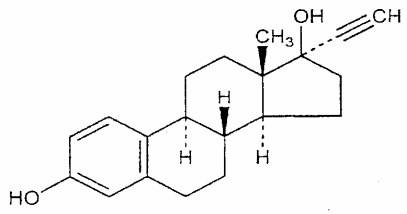
NIEHS Contract No. N01-ES-05457
ETP Task No. CHEM07193
MRI Project No. 110100
MRI Task No. 592

Midwest Research Institute
425 Volker Boulevard
Kansas City, Missouri 64110

January 31, 2003

7244-600
Rocca
Wolfe
Holley

Bulk Chemical Reanalysis for Ethinyl estradiol

CAS No. 57-63-6 MRI Task No.: 592 ETP Task No. CHEM07193 Program Supported: RACB Analysis Dates: 12/17/2002 Interim Result Dates: 1/3/2003 (e-mail)	Lot No.: 45H0716 MRI Assigned Batch No.: 01 Amount Received: 1 x 5 g Sample Receipt Date: 12/5/02 Storage Conditions (@ Analytical Lab): frozen Supplier: Therimmune Research Study No.: 7244-600 I.D. No. 1318B
Structure  	Mol. Wt. 296.44 Mol. Formula $C_{20}H_{24}O_2$

Quality Assurance Statement

Bulk Chemical Reanalysis for Ethinyl estradiol

ETP Task No. CHEM07193
MRI Project No. 110100
MRI Task No. 592

This study was inspected by the Quality Assurance Unit of MRI (QAU) and the findings reported to the Study Director and Management as follows:

Phase inspected	Date inspected	Date reported
Protocol audit	12/17/02	12/18/02
In-life audit; HPLC analysis	12/17/03	12/18/02
Data audit	1/30/03	1/30/03
Report audit	1/30/03	1/30/03
Protocol Amendment No. 1	1/30/03	1/30/03

The raw data and report will be stored in the MRI Archives.

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

Facility/equipment	Inspection date	Management submitted date
PCA laboratory complex	9/13/02	9/16/02
FT-IR facility	11/18/02	11/19/02
HPLC facility	11/18/02	11/19/02

MIDWEST RESEARCH INSTITUTE

Esther McClellan
Senior Quality Assurance Auditor

Approved:

James E. Dworak, Ph.D.
Manager, Quality Assurance

January 31, 2003

Executive Summary

The purpose of this task was to perform a bulk chemical reanalysis for ethinyl estradiol (Lot No. 45H0716, received from Therimmune Research). The identity of the test article was confirmed as ethinyl estradiol by infrared spectroscopy. A major peak comparison assay was performed using high performance liquid chromatography to compare the purity of the test article with that of a frozen reference sample maintained at MRI. The relative percent purity of the test article was determined to be 98.8 ± 0.2(s)%.

Good Laboratory Practice Compliance Statement

Bulk Chemical Reanalysis for Ethinyl estradiol

ETP Task No. CHEM07193
MRI Project No. 110100
MRI Task No. 592

This study was conducted in compliance with the Good Laboratory Practice regulations of the U.S. Food and Drug Administration (21 *CFR* 58).

Jason McClintock
Study Director

1/31/03
Date

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Bulk Chemical Reanalysis for Ethinyl estradiol

1. Introduction

The purpose of this task was to perform a bulk chemical reanalysis for a sample of ethinyl estradiol (Lot No. 45H0716, received from Therimmune Research). The identity of the test article was verified by infrared spectroscopy. High performance liquid chromatography (HPLC) was used to perform a major peak comparison assay of a frozen reference sample maintained at MRI and the bulk chemical test article. For HPLC, an Alltima C18 column was used, with a 70:30 methanol: water mobile phase and ultraviolet (UV) detection. This task was initiated on December 5, 2002.

2. Chemical Information

Test Article:	Ethinyl estradiol
Lot No.:	45H0716
Supplier:	Therimmune Research
Study No.:	7244-600
I.D. No.:	1318B
Molecular Formula:	$C_{20}H_{24}O_2$
Molecular Weight	296.44
CAS No.:	57-63-6

3. Analysis Methods

3.1 Infrared Spectroscopy

3.1.1 Sample Preparation

The bulk chemical test article was incorporated into a potassium bromide pellet at a concentration of approximately 0.7% (w/w).

3.1.2 Instrument

Perkin Elmer Model 1605 FT-IR with the following parameters.

Scan Range:	400 cm^{-1} to 4000 cm^{-1}
Number of Scans:	Background: 32 Sample: 32
Resolution:	16.00 cm^{-1}
Apodization:	Strong

3.1.3 Analysis

The spectrum of the sample was obtained from 400 to 4000 cm^{-1} and corrected for the background spectrum of air. A polystyrene film was also scanned to verify instrument calibration.

3.1.4 Results

The FT-IR spectrum of the sample is contained in Figure 1, attached. The sample spectrum was consistent with a previously obtained spectrum[1] for ethinyl estradiol.

3.2 High Performance Liquid Chromatography

3.2.1 Mobile Phase Preparation

The mobile phase was prepared combining 300 mL of ASTM reagent grade water and 700 mL of methanol. The solution was mixed, filtered, and degassed with vacuum sonication before use.

3.2.2 Solution Preparation

3.2.2.1 Internal Standard Stock Solution

Approximately 500 mg of propiophenone was weighed and transferred to an amber 100-mL volumetric flask. The contents of the flask were then diluted to volume with methanol and mixed thoroughly.

3.2.2.2 Internal Standard Blank Solution

A 2-mL portion of the internal standard stock solution was volumetrically pipetted into an amber 25-mL volumetric flask. The contents of the flask were diluted to volume with methanol and mixed thoroughly.

3.2.2.3 Sample and Standard Solutions

Triplicate bulk chemical test article and frozen reference solutions were prepared for the purity assay by accurately weighing approximately 100 mg of the test article or frozen reference sample into individual 100-mL amber volumetric flasks. An 8-mL portion of the internal standard stock solution was added to each flask using an 8-mL volumetric

[1] MRI Final Report. "*Bulk Chemical Reanalysis for Ethinyl Estrodiol,*" NIEHS Contract No. N01-ES-05457, ETP Task No. CHEM06067, MRI Project No. 110100, Task No. 256, May 24, 2001.

pipette. The contents of the flasks were then diluted to volume with methanol and mixed by inversion.

3.2.3 HPLC Instrument

Instrument:	LCS-36
Controller:	Waters 680
Autosampler:	Waters 717 plus, Model 717P
Pump:	Waters 510, Model M510
Detector:	Spectra Physics UV1000
Detection:	Ultraviolet, 280 nm wavelength
Column:	Alltima C18, 250 mm x 3.2 mm ID, 5 µm
Solvent System:	70% Methanol: 30% Water
Solvent Program:	Isocratic
Flow Rate:	0.65 mL/min
Injection Volume:	10 µL
Integration:	Turbochrom, Version 6.1.1
Retention Times:	Propiophenone (internal standard), ~ 5.8 min Ethinyl estradiol, ~ 10.0 min

3.2.4 Analysis

Portions of each sample, reference, and blank solution were transferred into individual autosampler vials. Each solution was injected and analyzed using the system and parameters described in Section 3.2.3.

3.2.5 Results

3.2.5.1 System Suitability

The analytical system described in Section 3.2.3 was evaluated for system precision, theoretical plates, and tailing factor, according to USP guidelines.[2] System precision was calculated using the average major peak area obtained from six injections of a single solution. Theoretical plates and tailing factor were evaluated for a single injection of the same solution.

[2] *The United States Pharmacopeia*, USP 24, Physical Tests / <621> Chromatography—System Suitability, ©1999, pp. 1923-1924.

Table 1. System Suitability

Test	Propiophenone		Ethinyl estradiol	
	Acceptable range[3]	Assay	Acceptable range[3]	Assay
System Precision	–	–	< 1	0.1
Retention Time (min)	~ 5.3	~ 5.8[a]	~ 8.8	~ 10.0[a]
Tailing Factor	< 1.5	1.2	< 1.5	1.2
Theoretical Plates	> 3500	6058	> 2900	6070
Resolution	–	–	> 6.0	10.4

[a] Increase in retention times did not adversely affect reported results.

3.2.5.2 Purity Assay

The relative purity (%) was calculated for each solution of the bulk chemical test article using the average relative weight ratio for the frozen reference solutions and the relative weight ratio for the bulk chemical test article solution. The average relative purity and standard deviation (n = 3) were calculated. Results are presented in Table 2, and representative chromatograms are shown in Figure 2.

Table 2. Relative Purity of Bulk Chemical

Test article sample	Relative purity (%)
1	98.89
2	98.97
3	98.67
Average	98.8 ± 0.2(s)%

4. Conclusions

The test article was identified by infrared spectroscopy as ethinyl estradiol. A major peak comparison analysis by high-pressure liquid chromatography indicated a relative purity of 98.8 ± 0.2(s)% for the bulk chemical.

[3] MRI ETP Standard Operating Procedure, Code ETP-1013, "Bulk Chemical Reanalysis of Ethinyl Estradiol."

5. Contributors

Andrew Hodges performed the analyses for this study.

MIDWEST RESEARCH INSTITUTE

Jason McClintock
Study Director

Linda Siemann
Group Leader
Bulk Chemical Analysis

Approved:

for Roger K. Harris, Ph.D.
Principal Investigator
Environmental Toxicology Program

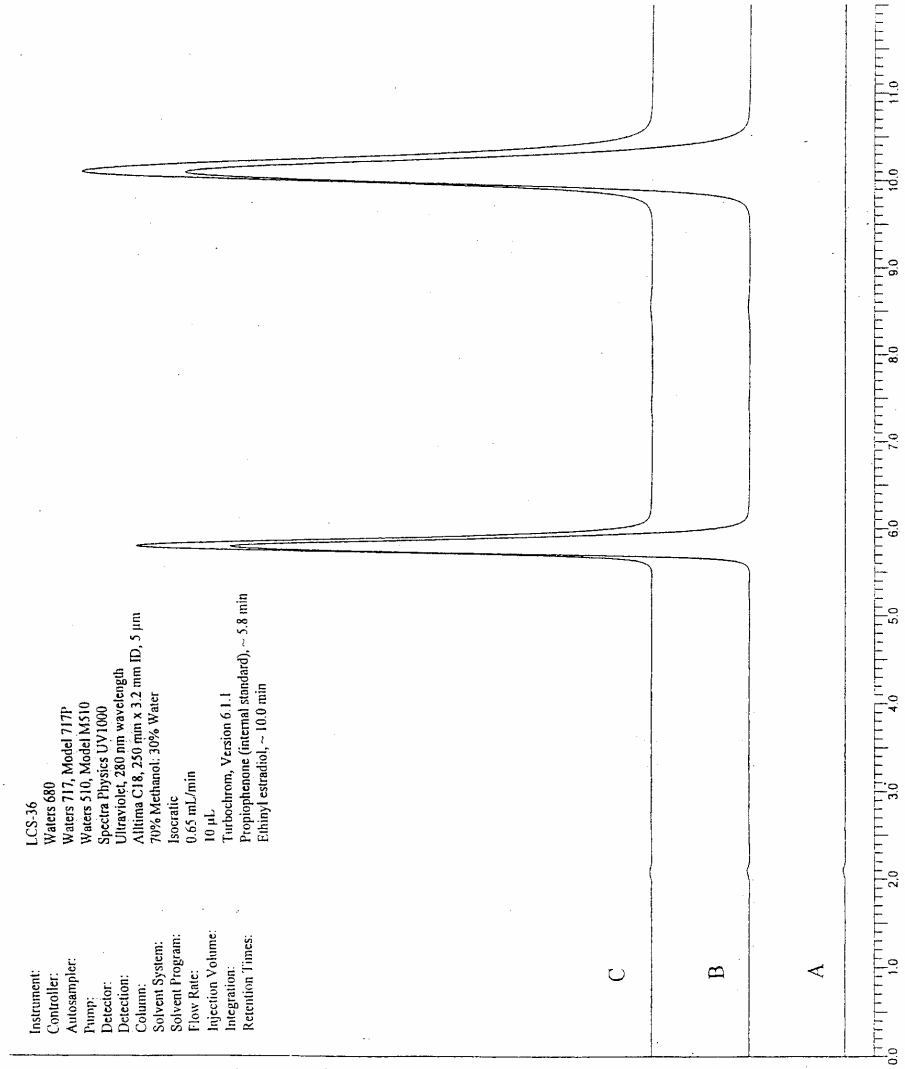


Figure 2. Typical Chromatograms for HPLC Analysis of Ethinyl Estradiol (Lot No. 45H0716)
(A) Blank (B) Frozen Reference (C) Bulk Chemical

MRI-ETINTASK 592.DOC

7

RTI INTERNATIONAL

NTP Chemistry Support Services

3040 Cornwallis Road ▪ PO Box 12194 ▪ Research Triangle Park, NC 27709-2194 ▪ USA
Telephone 919-541-6581 or 919-541-5975 ▪ Fax 919-541-7208 ▪ www.rti.org

COPY

Chemistry Support Services for the ETP
NIH Contract No. N01-ES-05455
RTI Project 07939.004.003.104
ChemTask No. CHEM07238

METHOXYCHLOR (95%)

BULK CHEMICAL REANALYSIS REPORT

March 5, 2003

Prepared by:

Gwen McNeill
Analytical Chemist

Approved by:

James Blake
Task Leader

Charles Sparacino, Ph.D.
Principal Investigator

Submitted to:

Mr. Bradley J. Collins
National Institute of Environmental Health Sciences
P.O. Box 12233
111 T. W. Alexander Drive
Research Triangle Park, NC 27709-2233

7244-600
Rocca
Nehrebecky
Patel

METHOXYCHLOR (95%)

CAS No.: 72-43-5

RTI Chemical ID Code: J93

ChemTask No.: CHEM07238

Program Supported: RTX

Analysis Dates: Jan 2-16, 2003

Interim Results Date: Jan 16, 2003

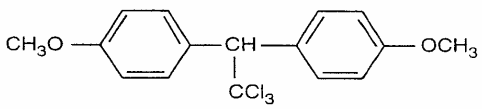
Storage Conditions:
Bulk: Refrigerated
Reference: Freezer

Lot No.: 124F0575 (Sigma)

Samples/Amounts Received:
Bulk: 5 g (10011-44-11)
Reference: 5 g (8404-72-05)

Sample Receipt Date:
Dec 12, 2002 (Bulk aliquot)
Jun 27, 1996 (Reference)

STRUCTURE



MOL. WT.

345.65

FORMULA

$C_{16}H_{15}Cl_3O_2$

EXECUTIVE SUMMARY

In support of the Reproductive Toxicology Program, an aliquot of Methoxychlor (95%) was submitted for bulk chemical reanalysis. As a precaution, the bulk aliquot of neat chemical was stored in a freezer by the RTI analytical laboratory prior to analysis. The minimum recommended storage condition for the material is however refrigerated. Chemical purity of the bulk sample was determined relative to a reference standard of the same lot/batch number which had been stored at RTI under freezer conditions. Analytical results obtained by a GC/FID chromatographic method indicated that the sample had a percent relative purity of 101% when compared to the frozen reference standard. Identity was confirmed by IR, which showed no additional impurities.

RTI INTERNATIONAL

Quality Assurance Statement

Chemical Name: Methoxychlor (95%)

Task Type: Bulk Chemical Reanalysis

RTI Task Number: 07939.004.003.104

Chem Task Number: CHEM07238

This task was audited by the Chemistry and Life Sciences Quality Assurance Unit and the results of the inspections and audits were reported to the task leader and management as identified below. To the best of our knowledge, the reported results accurately describe the study methods and procedures used, and the reported results accurately reflect the raw data.

Inspections and Audits	Inspection and Audit Date(s)	Date Inspection/Audit Report Sent to Task Leader and Management
Standard Preparation	1/9/03	3/5/03
Data and Report Audit	2/26,28/03	2/28/03

Jennifer Jones
Quality Assurance Specialist

3/5/03
Date

Approval:

Doris J. Smith
Quality Assurance Manager

3-5-2003
Date

turning knowledge into practice

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METHOXYCHLOR (95%)

1.0 INTRODUCTION

The objective of this work was to determine the stability of the study chemical during the current RTX studies being conducted at RTI labs. To accomplish this objective, a bulk chemical re-analysis (SOP CSC-1025.6) was performed. The identity of the chemical was assessed and its purity determined.

2.0 CHEMICAL ANALYSIS

A sample of methoxychlor (95%) was received on December 12, 2002 for bulk chemical reanalysis (RTI Log No. 10011-44-11). The aliquot was stored frozen until analyzed.

3.0 CONFIRMATION OF IDENTITY - INFRARED SPECTROMETRY (IR)

Procedure: SOP ESE-430.5
SOP CSC-1581.10
AM CSC-3802.0

Notebook No.: 10353 *p.:* 124

3.1 IR Parameters

System	Nicolet Magna-560 FTIR (4000 - 400 cm^{-1})
Software	Nicolet Omnic E.S.P., Ver. 5.1
Method	NaCl, Neat Melt

3.2 Results

Frequency (1/cm)	Assignment
2957	C-H Stretch
1609	C=C Stretch
1511	CCl_3 Stretch
1254	C-O Stretch
1035	C-C Stretch
751	CCl_3 Bend

1

The observed spectrum was consistent with the structure of methoxychlor. A visual comparison of the spectra showed the bulk chemical to be consistent with the frozen reference. Figures 1 and 2 show the bulk and RTI reference spectra. The observed differences in the measured intensities was a result of variance in sample amounts and plate thickness.

4.0 PURITY DETERMINATION - GAS CHROMATOGRAPHY

Procedure: SOP CSC-1321.13
AM CSC-3802.0

Notebook No.: *10353* *pp*: 125-133

This section describes the chromatographic method used to measure the composition of the sample and the data used to estimate sample purity. The following changes in the current analytical method were implemented during this effort to improve the chromatographic results.

1) Injection volume changed from 2 µL to 1 µL.
2) Injection solvent changed from methanol to hexane.
3) Column length changed from 15 m to 30 m.

4.1 Preparation of Internal Standard (IS) Solution

A stock solution of internal standard was prepared by weighing approximately 50 mg of pyrene into a 100-mL volumetric flask. The internal standard was diluted to volume with hexane and mixed by inversion. This constituted a 0.5 mg/mL.

4.2 Bulk Sample and Frozen Reference Standard Solution Preparation

Triplicate solutions of the reference standard and bulk samples were prepared by transferring approximately 10 mg of compound to individual 10-mL volumetric flasks and diluting to volume with the internal standard solution. All solutions were mixed by inversion and sonication.

4.3 Analysis

GC/FID Parameters

System	HP 5890 Series II Gas Chromatograph
Software	Agilent Chemstation/Chemstore; Version: A/08.03
Column	Supelco Equity 5 (30 m × 0.32 mm ID) with 0.25 µm film
Column Temperature	235°C
Carrier-Flow Rate	Helium - 1.0 mL/min
Injector Temp. - Type	250°C - Split (~20/1)
Injection Volume	1 µL
Injection Solvent	Hexane
Detector	FID at 325°C
Est. Retention Times	~9.3 min (IS) ~18.3 min (Methoxychlor-95%)

4.4 Results

Typical chromatograms are shown in Figure 3. Calculations based on a major peak comparison technique gave the results shown in the following table.

Chemical	RTI Log No.	RRF[a]	Mean RRF (%RSD)	% Relative Purity[b]
Bulk Replicate #1	10011-44-11	1.005		
Bulk Replicate #2	10011-44-11	0.9563	0.9810 (2.5)	101
Bulk Replicate #3	10011-44-11	0.9817		
Reference Replicate #1	8404-72-05	1.002		
Reference Replicate #2	8404-72-05	0.9450	0.9696 (3.0)	--
Reference Replicate #3	8404-72-05	0.9619		

[a]Relative Response Factor; normalized to sample concentration.
[b]% Relative Purity = (Mean RRF, bulk/Mean RRF, ref.) x 100.

5.0 ACKNOWLEDGMENT

Personnel contributing to the performance of this task: Brenda Fletcher and Gwen McNeill.

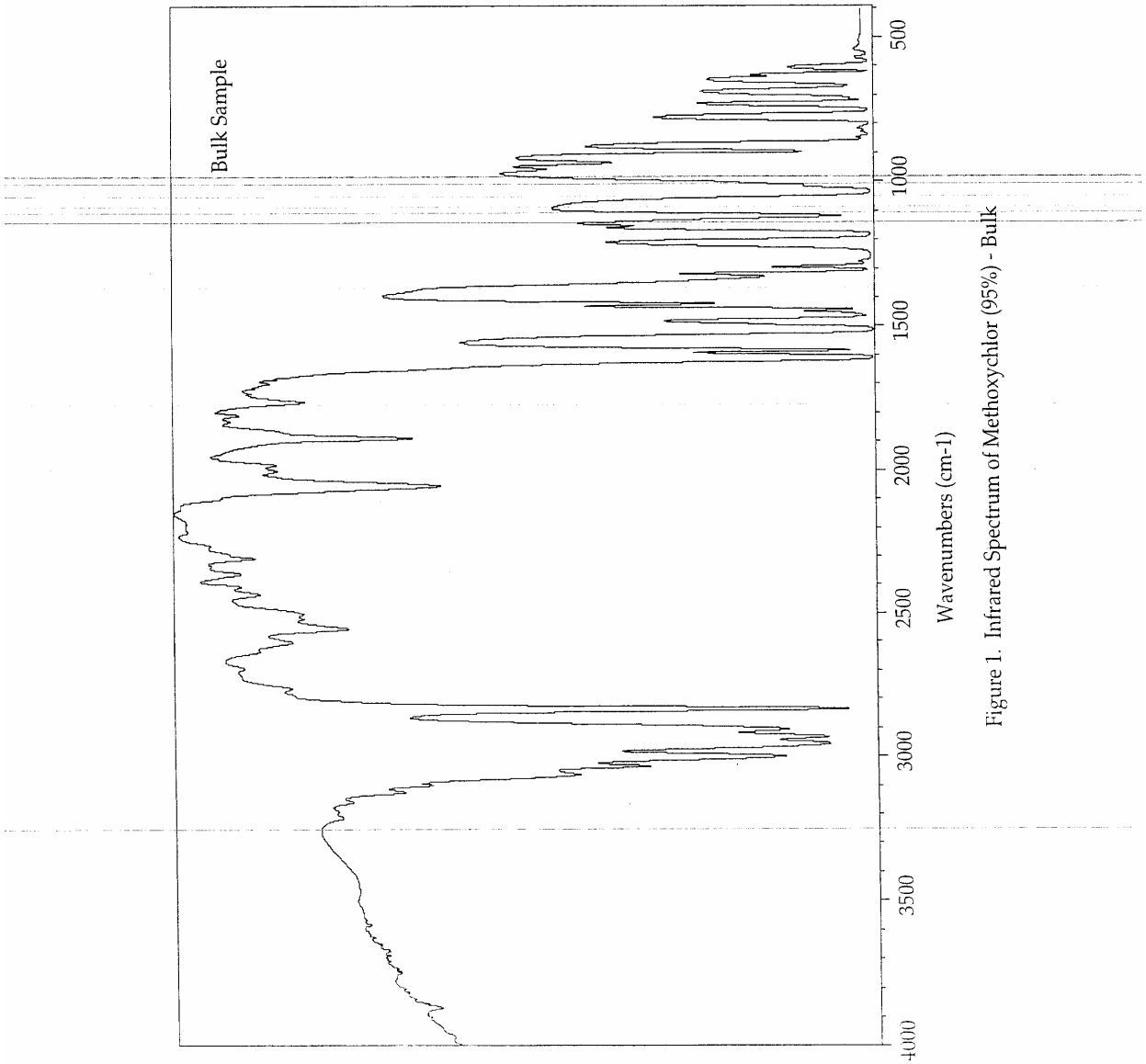


Figure 1. Infrared Spectrum of Methoxychlor (95%) - Bulk

4

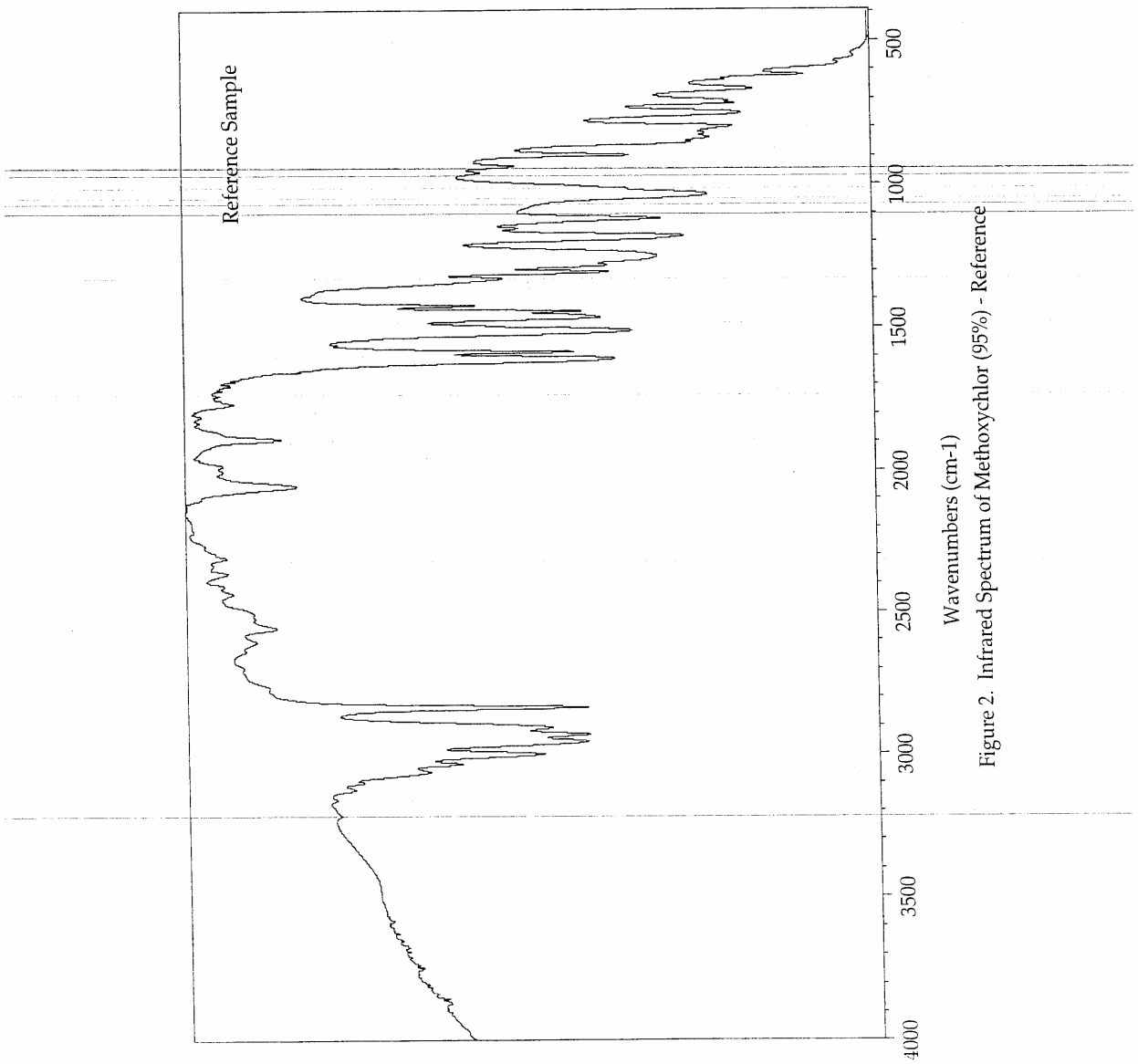


Figure 2. Infrared Spectrum of Methoxychlor (95%) - Reference

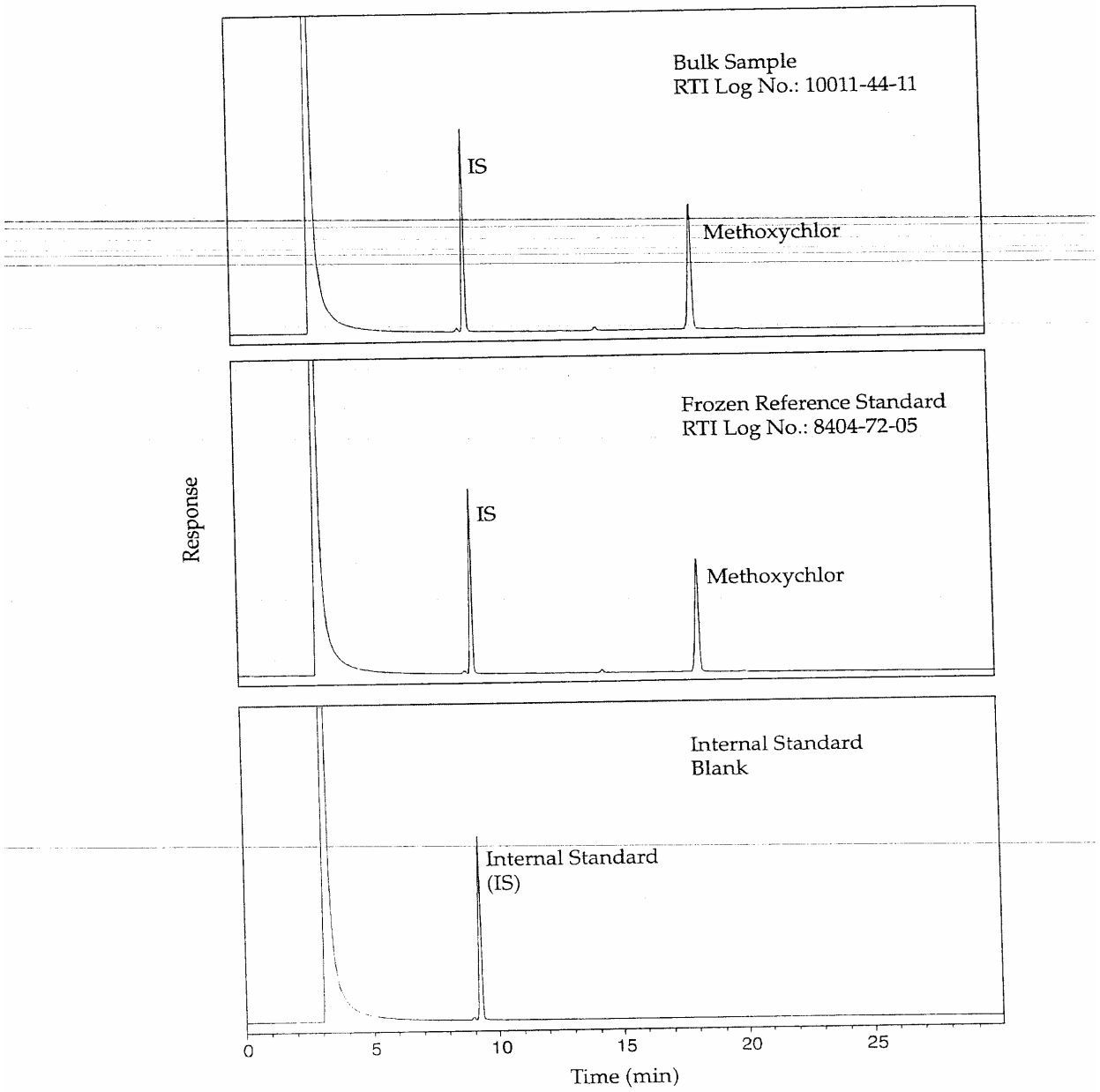


Figure 3. Gas Chromatograms of Methoxychlor (95%) and a Blank

6

APPENDIX

Analytical Method (AM CSC-3802.0)

Bulk Chemical Reanalysis of Methoxychlor

ANALYTICAL METHOD	RESEARCH TRIANGLE INSTITUTE 3040 CORNWALLIS ROAD RESEARCH TRIANGLE PARK, NC 27709-2194	AM CSC-3802.0 Page 1 of 5

TITLE: BULK CHEMICAL REANALYSIS OF METHOXYCHLOR

AUTHOR: Signed Rekha Banker
Analytical Chemist

Date 4/11/01

APPROVED BY: Signed
Principal Investigator

Date 4-11-01

ANALYTICAL METHOD	RESEARCH TRIANGLE INSTITUTE 3040 CORNWALLIS ROAD RESEARCH TRIANGLE PARK, NC 27709-2194	AM CSC-3802.0 Page 2 of 5

1.0 METHOD SUMMARY - START OF STUDY PURITY ANALYSIS

To fulfill a start of study BCR request, confirmation of identity and determination of purity for the bulk chemical as compared to a frozen reference standard stored at RTI is conducted using IR spectroscopy and gas chromatography (GC).

2.0 SAMPLE RECEIPT, STORAGE, AND HANDLING

The reference aliquot is maintained at the RTI MHF in a freezer (~ -20°C) until an analysis request is made. Upon study initiation, an aliquot of the bulk material may be supplied by the testing laboratory or by the RTI MHF. It is stored at room temperature (~ 20 to 25°C) at RTI until analyzed.

NOTE: All analytical transfers from the bulk aliquots should be conducted in a glove box and under yellow lights.

3.0 REAGENTS

Refer to Table 1 for all required chemicals. Use this table to document the lot number and expiration date for each reagent, making changes and/or additions where necessary. Any critical requirements for reagents are noted.

TABLE 1. REAGENTS

Reagent	RTI Log No.	Supplier	Lot Number	Exp. Date
Methoxychlor (Bulk Aliquot)				
Methoxychlor (Frozen Reference)				
Pyrene (IS)				
Methanol (Pesticide Grade)	NA			

4.0 EQUIPMENT

Refer to Table 2 for a list of the major equipment required. Equivalent pieces of equipment are acceptable. Use this table to document the specific information for each piece of equipment. Check the maintenance and calibration records for each piece of equipment (where applicable) to be sure that they are current.

ANALYTICAL METHOD	RESEARCH TRIANGLE INSTITUTE 3040 CORNWALLIS ROAD RESEARCH TRIANGLE PARK, NC 27709-2194	AM CSC-3802.0 Page 3 of 5

TABLE 2. EQUIPMENT

Equipment	Manufacturer/Model	Serial/ID Number/Location
glove box	NA	
analytical balance		
refrigerator		
freezer		
IR Spectrometer		
GC		

5.0 LABWARE PREPARATION

Be sure all labware is properly cleaned per normal procedures prior to use. No special procedures are required.

6.0 PROCEDURE FOR IDENTIFICATION CONFIRMATION

6.1 General Instructions

Check or calibrate equipment according to relevant SOPs.

Make reference to the relevant SOPs in an RTI notebook. It is also important to record scientific observations and deviations from the SOP in the notebook.

6.2 Confirmation of Identity by Infrared Spectrometry

The basis of this analysis is visual comparison of the infrared spectrum obtained for the bulk chemical to the spectrum of the reference standard supplied by RTI.

Obtain an infrared spectrum of the bulk and reference sample by preparing a neat melt on NaCl plates. Place a small amount of material on a NaCl plate (bottom) located on a hot plate. Warm the hot plate slowly to melt the study material and then quickly cover the melt with another pre-warmed NaCl plate (top) and seal.

NOTE: Due to handling logistics, preparation of the neat melt is conducted in a NTP certified hood while the analyst is fitted with a respirator. While working in a glove box, transfer from each bulk aliquot only a sufficient amount of neat material (<1 mg) to separate pre-cleaned conical vials and cap. Remove the vials from the glove box and place them in the hood adjacent to the hot plate. Continue as stated above.

Obtain a spectrum from 4000 to 600 cm^{-1} using a suitable infrared spectrometer. Adjust the instrument setting to obtain a baseline of about 80% transmission. Compare the spectrum

obtained for the bulk chemical to that of the reference sample. Identity is confirmed if the spectra are comparable.

7.0 PROCEDURE FOR PURITY DETERMINATION

7.1 General Instructions

Check or calibrate equipment according to relevant SOPs.

Make reference to the relevant SOPs in an RTI notebook. It is also important to record scientific observations and deviations from the SOP in the notebook.

7.2 Confirmation of Purity by Capillary Gas Chromatography

7.2.1 Preparation of Internal Standard Solution

Prepare a stock solution of internal standard by weighing ~50 mg of pyrene into a 100-mL volumetric flask and diluting to volume with methanol. Mix thoroughly. This will constitute a 0.5 mg/mL solution.

7.2.2 Preparation of Methoxychlor Reference Standard and Bulk Sample Solutions

1. Handle the neat material in a glove box under yellow lights.

2. Prepare triplicate primary solutions of the reference standard and bulk sample by transferring approximately 10 mg of compound to individual 10-mL volumetric flasks and diluting to volume with internal standard. Mix by inversion.

TABLE 3. RECORD OF GC ALIQUOT TRANSFERS

Reference Aliquot #	Target Mass (mg)	Actual Mass (mg)

Bulk Aliquot #	Target Mass (mg)	Actual mass (mg)

REVIEW AND REVISION LOG	RESEARCH TRIANGLE INSTITUTE POST OFFICE BOX 12194 RESEARCH TRIANGLE PARK, NC 27709-2194	AM CSC-3802 Page 1 of 1

BULK CHEMICAL REANALYSIS OF METHOXYCHLOR

REVIEW/REVISION LOG

Rev. #	Date	Description
0	04/11/01	Original version.

COPY
2/20/01

ANALYTICAL METHOD

METHOD NO. 002 PAGE 1 OF 6 EFFECTIVE DATE: APRIL 13, 2000
APPROVAL BY: _____

TITLE: Determination of Peroxide in Fats And Oils

DEVELOPED BY: The American Oil Chemists' Society (Official Method CD 8-53, 1986); adapted for use by TherImmune Research Corporation

1.0 SCOPE

This method determines all substances, in terms of milli-equivalents (meq) of peroxide per kilogram (kg) of sample (fats or oils), which oxidize potassium iodine under conditions of the test. These are generally assumed to be peroxide or other similar products of fat oxidation.

2.0 PRINCIPLE

Aliquots of the fats/oils are diluted with glacial acetic acid (HOAc) and chloroform ($CHCl_3$) mixture (3:2 ratio). The diluted samples are titrated potentiometrically with 0.005 N $Na_2S_2O_3$.

3.0 EQUIPMENT

3.1 Balance: Metteler AE 250 analytical balance, or equivalent.

3.2 Buret: Brinkmann 25 mL digital, or equivalent.

3.3 Electrode: Fisher Platinum/Ag/AgCl combination, or equivalent.

3.4 pH/mV meter: Corning Ion Analyzer 255, or equivalent.

3.5 General laboratory equipment and glassware.

4.0 REAGENTS

4.1 Chloroform ($CHCl_3$): Burdick & Jackson, ACS reagent, or equivalent.

METHOD NO. 002 PAGE 2 OF 6

4.2 Water (H_2O): Burdick & Jackson HPLC grade, or equivalent. Boil and cool to room temperature before using.

4.3 Glacial acetic acid (HOAc): Burdick & Jackson USP grade, or equivalent.

4.4 Potassium Iodine (KI): Sigma analytical reagent, or equivalent.

4.5 Sodium Thiosulfate ($Na_2S_2O_3$) Solution, 0.1000 N: Aldrich Volumetric Standard, or equivalent.

4.6 Diluent: combine 300 mL of HOAc with 200 mL of $CHCl_3$. Mix well.

4.7 Saturated KI solution: add KI to 10 mL previously boiled and cooled water until the solution become saturated, as indicated by the presence of undissolved crystals.

4.8 Titrant (suggested concentration): dilute 5.0mL of 0.1000 N $Na_2S_2O_3$ to 100 mL previously boiled and cooled water to make 0.005 N $Na_2S_2O_3$.

5.0 PROCEDURE

5.1 Weigh 5.00 ±0.05 g of sample into each of three previously tared 100 or 150 mL beakers.

5.2 Add 30 mL of diluent to one of the beakers and stir with a magnetic stir bar until dissolved.

5.3 Add 0.5 mL of saturated KI solution, stir thoroughly with the magnetic stir bar, and allow to stand for exactly one minute.

5.4 Add 30 mL previously boiled and cooled water, place the electrode in the mixture and stir magnetically. Continue to stir throughout the titration.

5.5 Take an initial reading in mV. Titrate with 0.005 N $Na_2S_2O_3$ in increments of 0.25 mL. Record the relative mV reading with each increment.

5.6 Continue the titration until the crossover occurs. (See table 2)

5.7 Repeat the step 5.2 to 5.6 for the other two beakers.

5.8 Repeat the step 5.2 to 5.6 with an empty beaker. This will serve as reagent blank. The titrant used for reagent blank should not be more than 1.0 mL of $Na_2S_2O_3$ before crossover occurs. (See table 1)

6.0 SAMPLE CALCULATIONS

6.1 The meq of each sample is calculated using following equation

$$Meq = A + B \times [C / (C + D)]$$

Where: A = titrant (mL) added to obtain the crossover
B = increment (mL) used for titration
C = Δ^2 mL/Δ^2 mV before crossover
D = Δ^2 mL/Δ^2 mV after crossover

6.2 The peroxide value of each sample is calculated using the following equation:

$$\text{Peroxide Value} = [(A - B)/C] \times D \times 1000$$

Where: A = meq of sample
B = meq of reagent blank
C = weight of sample in gram
D = normality of titrant
1000 is a conversion factor to convert kg to gram

6.3 When the normality of the titrant is 0.005, the sample weight is 5.00 g, and the meq of reagent blank is 0.00, the sample meq equals to sample peroxide value in meq/kg.

7.0 NOTES

7.1 This method is highly empirical and any variation in procedure may cause variation in results.

7.2 The quantities of reagents maybe adjusted to reflect large or small quantities of samples.

7.3 The concentration of titrant may be increased if the concentration of peroxide is found to be unusually high.

METHOD NO. 002 PAGE 4 OF 6

8.0 LIST OF TABLES

Table 1. Titration, Reagent Blank

Table 2 Titration, Fat or Oil sample

METHOD NO. 002 PAGE 5 OF 6

Table 1
Titration, Reagent Blank

Titrant (mL)	Relative (mV)	$\frac{\Delta mV}{\Delta mL}$	$\frac{\Delta^2 mV}{\Delta^2 mL}$
0.00	250		
		-53	
0.25	197		+43
		-10	
0.50	187		+5
		-5	
0.75	182		+2
		-3	
1.00	179		+1
		-2	
1.25	177		0
		-2	
1.50	175		

Shows immediate crossover. No negative $\Delta^2 mV/\Delta^2 mL$ values.
Meq=0

METHOD NO. 002 PAGE 6 OF 6

Table 2
Titration, Fat or Oil sample

Titrant (mL)	Relative (mV)	$\frac{\Delta mV}{\Delta mL}$	$\frac{\Delta^2 mV}{\Delta^2 mL}$
0.00	250		
		-9	
0.25	241		-23
		-32	
0.50	209		-16
		-48	
0.75	161		+39
		-9	
1.00	152		+5
		-4	
1.25	148		+2
		-2	
1.50	146		

Crossover occurs at 0.50 to 0.75 mL (changes from negative to positive).

Meq = 0.50 + 0.25 (16/(16+39)) = 0.573

Peroxide Value = 0.573 meq/kg

Project No.:	7244-600	Page No.:	1
Interval:	Corn Oil	SD:	Rocca
Date:	2/24/2003	Analyst:	AAA
Date Received:	1/3/2003	Name:	Corn Oil
Date Processed:	2/24/2003	Log No.:	705
Date Analyzed:	2/24/2003	Batch Size:	NA
Storage:	Refrigerated	Sample Size:	50 mL

1. Preparation of Reagents:

Diluent: 150mL of glacial acetic acid combined with 100mL of chloroform.
Saturated KI solution: KI added to 10mL of previously boiled and cooled water until the solution becomes saturated.
Titrant: 5.0mL of 0.1000N $Na_2S_2O_3$ was diluted to 100mL with previously boiled and cooled water to make 0.005N $Na_2S_2O_3$.
Water: 400 mL boiled and cooled to room temperature

2. Preparation of Samples:

	Sample No. 1	Sample No. 2	Sample No. 3
Weight of Corn Oil (1) (g)	5.00	5.00	5.00
Volume of diluent added	30mL	30mL	30mL
Volume of saturated KI solution added	0.5mL	0.5mL	0.5mL
Volume of water added	30mL	30mL	30mL

3. Preparation of Reagent Blank:

Volume of diluent added	30mL
Volume of saturated KI solution added	0.5mL
Volume of water added	30mL

4. Reagents Used:

	Manufacturer	Lot No.	Grade
Glacial acetic acid	Mallinckrodt	8817T18A06	A.C.S.
Chloroform	J. T. Baker	N29B29	A.C.S.
KI	EM Science	126465/M276922	A.C.S.
Water	Fisher	026810	HPLC
Sodium Thiosulfate	Aldrich	08930KA	A.C.S.

(1) Added. ~~AAA~~ 2/25/03.

Prepared By: AAA Date: 2/24/03

Reviewed By: Li Date: 2/27/2003

Project No.: 7244-600
Interval: Corn Oil
Date: 2/24/2003

Page No.: 2
SD: Rocca
Analyst: AAA

Sample No. 1

Titrant (mL)	Relative mV
0.00	287.2
1.00	281.8
1.50	277.6
1.75	274
2.00	268.3
2.25	261.8
2.50	245
2.75	207.1
3.00	203.0
3.25	197.1

Sample No. 2

Titrant (mL)	Relative mV
0.00	288.6
1.00	283.5
1.25	281.3
1.50	279.1
1.75	276.5
2.00	272.5
2.25	267.2
2.50	256.5
2.75	210.6
3.00	200.1
3.25	195.2

Sample No. 3

Titrant (mL)	Relative mV
0.00	288.3
1.00	281.9
1.25	278.6
1.50	274.9
1.75	271.5
2.00	265.1
2.25	230.3
2.50	214.6
2.75	210.5
3.00	208.1
3.25	204.7
3.50	201.8

Reagent Blank

Titrant (mL)	Relative mV
0.00	244.9
0.25	210.1
0.50	195.9
0.75	189.7
1.00	184.6
1.25	181.1
1.50	177.8

Prepared By: Aadeyos Date: 2/25/03

Reviewed By: Li Jin Date: 2/27/2003

Project No.: 7244-600
Interval: Corn Oil
Date: 2/24/2003

Page No.: 3
SD: Rocca
Analyst: AAA

Sample No. 1 Titrant (mL)	Relative mV	ΔMV / ΔML	Δ²MV / Δ²ML
1.75	274.0		
		-5.7	
2.00	268.3		-0.8
		-6.5	
2.25	261.8		-10.3
		-16.8	
2.50	245.0		-21.1
		-37.9	
2.75	207.1		33.8
		-4.1	
3.00	203.0		-1.8
		-5.9	
3.25	197.1		

Crossover occurs at 2.50 to 2.75 mL

Meq = 2.62

Sample No. 2 Titrant (mL)	Relative mV	ΔMV / ΔML	Δ²MV / Δ²ML
1.75	276.5		
		-4.0	
2.00	272.5		-1.3
		-5.3	
2.25	267.2		-5.4
		-10.7	
2.50	256.5		-35.2
		-45.9	
2.75	210.6		35.4
		-10.5	
3.00	200.1		5.6
		-4.9	
3.25	195.2		

Crossover occurs at 2.50 to 2.75 mL

Meq = 2.62

Sample No. 3 Titrant (mL)	Relative mV	ΔMV / ΔML	Δ²MV / Δ²ML
1.75	271.5		
		-6.4	
2.00	265.1		-28.4
		-34.8	
2.25	230.3		19.1
		-15.7	
2.50	214.6		11.6
		-4.1	
2.75	210.5		1.7
		-2.4	
3.00	208.1		-1.0
		-3.4	
3.25	204.7		

Crossover occurs at 2.00 to 2.25 mL

Meq = 2.12

Reagent Blank Titrant (mL)	Relative mV	ΔMV / ΔML	Δ²MV / Δ²ML
0.00	244.9		
		-34.8	
0.25	210.1		20.6
		-14.2	
0.50	195.9		8.0
		-6.2	
0.75	189.7		1.1
		-5.1	
1.00	184.6		1.6
		-3.5	
1.25	181.1		0.2
		-3.3	
1.50	177.8		

Shows immediate crossover, No negative Δ^2MV/Δ^2ML values.

Meq = 0

Averaged Peroxide Value = **2.45**

Prepared By: Aaadeyys Date: 2/25/03

Reviewed By: Li Jin Date: ~~2/27/2003~~ 2/27/2003 (EE) Li Jin 2/27/2003

Appendix 2
Formulation Analysis Reports
Pubertal Toxicity Study of Vinclozolin, Flutamide and Phenobarbital in Male Sprague Dawley Rats and Methoxychlor, Ethinyl Estradiol and Phenobarbital in Female Sprague Dawley Rats when Administered in Corn Oil by Oral Gavage

COPY

Formulation Analysis of Phenobarbital Free Acid in Corn Oil—Formulation Date: December 3, 2002

NIEHS Contract No. N01-ES-05457
ETP Task No. CHEM07192
MRI Project No. 110100
MRI Task No. 591

Midwest Research Institute
425 Volker Boulevard
Kansas City, Missouri 64110

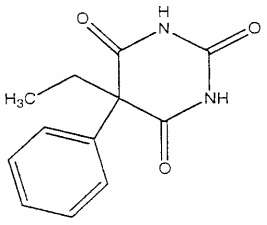
January 24, 2003

7244-600
Rorrer
Wolfe
Holley

Formulation Analysis (FA)

Chemical Information: Phenobarbital Free Acid

CAS No.: 50-06-6 MRI Task No.: 591 ETP Task No.: CHEM07192 Program Supported: RTX Analysis Dates: December 9 and 11, 2002 Interim Report Date: December 12, 2002	Lot No.: QI0645 (Spectrum Chemical Co., 99.4% Per BCLA at MRI) Samples Received: dose verification samples at 0, 10, 20, and 40 mg/mL phenobarbital free acid in corn oil; homogeneity samples at 10 and 40 mg/mL (top, middle, and bottom at each concentration) phenobarbital free acid in corn oil; and neat phenobarbital free acid Sample Receipt Date: December 5, 2002 (formulations and neat chemical) Submitter: TherImmune Research Corporation Study Lab: TherImmune Research Corporation Formulation Date: December 3, 2002 Receipt Condition: Intact, Dry ice (neat chemical) Intact, Ice Packs (formulations) Storage Conditions (@ MRI): Formulation samples, refrigerated (~ 5°C) and neat test article, frozen (~ –20°C, protected from heat)

Structure	Molecular Weight	Molecular Formula
	232.2	$C_{12}H_{12}N_2O_3$

Quality Assurance Statement

Formulation Analysis of Phenobarbital Free Acid in Corn Oil—Formulation
Date: December 3, 2002

ETP Task No. CHEM07192
MRI Project No. 110100
MRI Task No. 591

This study was inspected by the Quality Assurance Unit of MRI (QAU) and the findings reported to the Study Director and Management as follows:

Phase inspected	Date inspected	Date reported
Protocol audit	12/9/02	12/12/02
Protocol Amendment No. 1 audit	1/21/03	1/22/03
Protocol Amendment No. 2 audit	1/21/03	1/22/03
In-life audit; formulation analysis	12/9/02	12/12/02
Data audit	1/21/03	1/22/03
Report audit	1/21/03	1/22/03

The raw data and report will be stored in the MRI Archives.

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

Facility/equipment	Date inspected	Date reported
GC facility	11/18/02	11/19/02
PCA laboratory complex	9/13/02	9/16/02

MIDWEST RESEARCH INSTITUTE
Rosemary Moran
Rosemary Moran
Quality Assurance Officer

Approved:
James Dworak
James Dworak, Ph.D.
Manager, Quality Assurance

January 24, 2003

Good Laboratory Practice Compliance Statement

Formulation Analysis of Phenobarbital Free Acid in Corn Oil—Formulation
Date: December 3, 2002

ETP Task No. CHEM07192
MRI Project No. 110100
MRI Task No. 591

This formulation analysis study (MRI Task No. 591) of phenobarbital free acid in corn oil was conducted in compliance with the Good Laboratory Practice Regulations of the U.S. Food and Drug Administration (21 *CFR* Part 58).

Amir Kazerooni
Study Director

1/24/03
Date

Executive Summary

The purpose of this study was to perform a formulation analysis of phenobarbital free acid in corn oil received from TherImmune Research Corporation. Samples included dose verification samples (one vial each) at concentration of 0 (control) 10, 20, 40 mg/mL, and homogeneity samples (top, middle, and bottom) for formulations prepared at target concentration 10 and 40 mg/mL phenobarbital free acid in corn oil. The concentrations of the dose formulations were determined with TherImmune-supplied test article as an analytical standard.

The average concentrations of the dose verification samples were determined to be 0, 10.02, 19.24, and 42.09 mg/mL phenobarbital free acid in corn oil. The average percentages of the target concentrations were 100.2%, 96.2%, and 105.3% for the 10, 20, and 40 mg/mL formulations, respectively. All of the samples were determined to be within 5.3% of the respective target concentrations.

The 10 mg/mL and 40 mg/mL phenobarbital free acid in corn oil were evaluated for homogeneity, and the results indicated that the both formulations were homogeneous with % RSDs of 1.8 and 3.7, and recoveries of 101.8% and 100.3%, respectively. The mean (n = 6) concentration of the 10 mg/mL and 40 mg/mL formulations were 10.17 mg/mL and 40.13 mg/mL, respectively.

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Figure 1. Representative Chromatograms of Phenobarbital Free Acid in Corn Oil; 361.944 µg/mL Spiked Vehicle Standard, 40 mg/mL Sample (~ 435 µg/mL), IS Blank, and Undosed Vehicle Blank 10

Formulation Analysis of Phenobarbital in Corn Oil— Formulation Date: December 3, 2002

1. Introduction

The purpose of this study was to perform a formulation analysis of phenobarbital free acid in corn oil received from TherImmune Research Corporation. Samples included dose verification samples (one vial each) at concentration of 0 (control) 10, 20, 40 mg/mL, and homogeneity samples (top, middle and bottom) for formulations prepared at target concentration 10 and 40 mg/mL phenobarbital free acid in corn oil. The concentrations of the dose formulations were determined with TherImmune-supplied test article as an analytical standard. This study was initiated on December 5, 2002.

The formulation analysis was based on the validated method as described in the MRI report for MRI Task No. 496, "Dose Formulation Development of Phenobarbital in Corn Oil," MRI Project No. 110100, NIEHS Contract No. N01-ES-05457, ETP Task No. CHEM06833, issued on December 3, 2002.

2. Chemical and Sample Information

Test Article:	Phenobarbital Free Acid
Lot No.:	QI0645
Supplier:	Spectrum Chemical Company
Molecular Formula:	$C_{12}H_{12}N_2O_3$
Molecular Weight:	232.2
CAS No.:	50-06-6

The formulations submitted for formulation analysis from TherImmune Research Corporation were received at MRI on December 5, 2002, in amber glass injection vials and are identified in Table 1 using TherImmune Research Corporation designations. Neat phenobarbital free acid was also received from TherImmune Research Corporation on December 5, 2002. The formulation samples were stored under refrigerated (~ 5°C) conditions and neat test article was stored under frozen (~ –20°C) conditions prior to analysis.

Table 1. Formulations From TherImmune Research Corporation—Formulation Date: December 3, 2002

Study No.	Concentration (mg/mL)	Volume (~ mL)
Dose verification samples		
7244-600	0	35
7244-600	10	35
7244-600	20	35
7244-600	40	35
Homogeneity samples	Concentration (mg/mL)-Location	
7244-600	10-Top	35
7244-600	10-Middle	35
7244-600	10-Bottom	35
7244-600	40-Top	35
7244-600	40-Middle	35
7244-600	40-Bottom	35

3. Materials and Equipment

Corn oil, Spectrum Chemicals
Caffeine (IS), Aldrich
Acetone, Burdick and Jackson, High Purity
Vortex Mixer, VWR Scientific
Ultra Sonic Bath, Branson Model 5510
Agilent 6890 Gas Chromatograph with Flame Ionization Detector (FID) and Turbochrom data system, version 6.1.1

4. Formulation Analysis

A formulation analysis was performed to determine the concentration of phenobarbital free acid in corn oil formulations from TherImmune Research Corporation. The concentrations of the formulations were determined using neat phenobarbital free acid standard supplied by TherImmune Research Corporation. All formulations were analyzed in duplicate, and quantitation was performed using a spiked vehicle standard curve.

4.1 Determination of Corn Oil Density

The density of corn oil was determined by transferring triplicate aliquots of corn oil into appropriate individual volumetric flasks and obtaining the weight of each aliquot.

4.2 Preparation of Standard Solutions

4.2.1 Internal Standard Solution

An internal standard (IS) solution was prepared by accurately weighing ~ 600 mg of caffeine into a 200-mL volumetric flask. An aliquot of acetone was added to the volumetric flask and the contents of the flask were mixed by vortexing for ~ 2 min and mixed by sonication for ~ 2 min, diluted to volume with acetone, and mixed by inversion.

4.2.2 Stock Solutions

Four stock solutions of phenobarbital free acid were prepared by accurately weighing and quantitatively transferring 201.080 mg (Stock A), 100.655 mg (Stock B), 198.614 mg (Stock C), and 104.035 mg (Stock D) of phenobarbital free acid into respective 100-mL volumetric flasks. The contents of each flask were diluted to volume with acetone and mixed by inversion. Expected concentrations: Stock A, 2.01080 mg/mL; Stock B, 1.00655 mg/mL; Stock C, 1.98614 mg/mL; and Stock D, 1.04035 mg/mL.
(NOTE: Stocks C and D were prepared to ensure that the calibration curve was within the validated range.)

4.2.3 Spiked Vehicle Standards

NOTE: Due to lack of availability of recently purchased corn oil, expired corn oil was used for the preparation of the spiked vehicle standards. However, analysis of this corn oil (Dose vehicle analysis, MRI Task No. 595)[1] determined that the corn oil was below (2.44 meq/Kg) the ETP criteria for maximum peroxide content (< 3.0 meq/Kg). Inspection of the chromatography indicated that the corn oil contributed no interferences to the analysis, and the use of this corn oil did not affect the integrity of the study.

The spiked vehicle standards for phenobarbital free acid were prepared by transferring aliquots of stock solutions into individual 50-mL volumetric flasks containing ~ 1-g aliquot of corn oil (vehicle) and 5 mL of IS solution, diluting to volume with acetone, and mixing by inversion (see Table 2). The final analytical concentration of the spiked vehicle standard curve covered a range of 20.8070 to 556.119 µg/mL.

[1] MRI Report, "Dose Vehicle Analysis of Corn Oil," NIEHS Contract No. N01-ES-05457, ETP Task No. CHEM07197, MRI Project No. 110100, MRI Task No. 595, to be submitted.

Table 2. Preparation of Spiked Vehicle Standards

Spiked vehicle standard	Stock aliquot A, B, C, or D (mL)	Corn oil aliquot (~ g)[a]	IS (mL)	Final volume (mL)	Expected concentration in corn oil (mg/mL)	Expected analytical concentration (µg/mL)
B_{11}	D, 1	1	5	50	0.96481	20.8070
A_{21}	A, 2	1	5	50	3.7296	80.4320
B_{31}	B, 10	1	5	50	9.3346	201.310
A_{41}	A, 9	1	5	50	16.783	361.944
B_{51}	B, 23	1	5	50	21.470	463.013
A_{61}	C, 14	1	5	50	25.787	556.119

[a] Density of corn oil: 0.92739 g/mL.

4.2.4 Blank

4.2.4.1 IS Blank

An internal standard blank was prepared by volumetrically transferring 5 mL of IS solution into a 50-mL volumetric flask, diluting to volume with acetone, and mixing by inversion.

4.2.4.2 Reagent Blank (D0)

A reagent blank (D0) was prepared by transferring an aliquot of acetone into an autosampler vial for analysis.

4.2.4.3 Undosed Vehicle Blanks (C01 and C02)

An undosed vehicle blank (C01) was prepared by accurately weighing ~ 1 g of corn oil into a 50-mL volumetric flask, diluting the contents to volume with acetone, and mixing by inversion.

A second undosed vehicle blank (C02) was prepared with IS by accurately weighing ~ 1 g of corn oil into a 50-mL volumetric flask containing 5 mL of IS solution. The contents of the flask were diluted to volume with acetone and mixed by inversion.

4.3 Determination of Formulation Density

The density of each formulation was determined by transferring triplicate aliquots of formulation into individual 10-mL volumetric flasks and obtaining the weight of each aliquot.

4.4 Preparation of Formulation Samples

Each formulation was prepared in duplicate by transferring ~ 1.0-g aliquot of formulation into individual volumetric flasks containing IS solution (see Table 3). The contents of each flask were diluted to volume with acetone and mixed by inversion.

Table 3. Preparation of Formulation Samples

Expected formulation concentration (mg/mL)	Formulation aliquot (~ g)	IS (mL)	Final dilution (mL)	Expected analytical concentration (~ µg/mL)
0 (Control)	1.0	5	50	0.0
10	1.0	5	50	217
20	1.0	5	50	435
40	1.0	10	100	435

4.5 Instrumental System and Parameters

Aliquots of the spiked vehicle standards, blanks, and formulation were transferred to autosampler vials and analyzed using the instrumentation outlined in Table 4. Representative chromatograms for the formulation analysis of phenobarbital free acid in corn oil are presented in Figure 1.

Table 4. GC Conditions

Instrument:	Agilent 6890 Gas Chromatograph
Injector:	Agilent 7683 Autosampler
Injection Temperature:	250°C
Injector Mode:	Spitless, purge on at 0.75 min
Injector Volume:	1 µL
Detector:	Flame Ionization Detector (FID)
Detector Temperature:	275°C
Carrier Gas, Flow Rate:	Helium, ~ 10 mL/min
Purge Gas, Flow Rate:	Nitrogen, ~ 30 mL/min
Air, Flow Rate:	300 mL/min
Hydrogen, Flow Rate:	30 mL/min
Range:	5
Column:	DB-5 (J&W Scientific) 30 m x 0.53 mm ID, 1.5-µm film
Data System:	PE Nelson Turbochrom, version 6.1.1
Oven Program:	Initial temp: 185°C, 5-min hold, ramp at 10°C/min to 250°C, 5 min hold
Retention Time:	Caffeine (IS): ~ 7.4 min
	Phenobarbital: ~ 9.6 min

4.6 Calculations

1. The density of each formulation was calculated by dividing the weight of each formulation aliquot by the aliquot volume. The mean density of each formulation was calculated.

2. The density of corn oil was calculated by dividing the weight of each corn oil aliquot by the aliquot volume. The mean density of corn oil was calculated.

3. A peak area ratio (PAR) was calculated for phenobarbital free acid as follows:

$$\text{Par} = \frac{\text{Peak Area}_{\text{Phenobarbital Free Acid}}}{\text{Peak Area}_{\text{Internal Standard}}}$$

4. The slope, y-intercept, and correlation coefficient were calculated using a weighted (1/y) linear regression analysis of the spiked vehicle standard curve by relating the PAR of each spiked vehicle standard to its corresponding expected analytical concentration (µg/mL).

5. The determined concentration of each sample was calculated using the regression equation obtained for the spiked vehicle standard curve and the PAR for each sample by using the following equation:

$$\text{Determined conc. (mg/mL)} = \frac{[\text{Par - (y - intercept}]}{\text{slope}} \times \frac{1\text{ mg}}{1000\ \mu\text{g}} \times \frac{\text{dilution volume (mL)}}{\text{sample wt (g)}} \times \text{Density (g/mL)}$$

where: the dilution volume for the 0, 10 and 20 mg/mL formulation = 50 mL
the dilution volume for the 40 mg/mL formulation = 100 mL

6. The percent target concentration was calculated as follows:

$$\%\,\text{Target} = \frac{D}{T} \times 100$$

where: D = determined concentration (mg/mL)
T = target concentration (mg/mL)

7. The average percent target concentration with the corresponding statistical data was calculated for each concentration level.

8. The system suitability parameters for instrumental precision, peak tailing (T), theoretical plates (N), and peak resolution (R) were calculated for phenobarbital free acid and the internal standard peaks according to USP guidelines.[2] The instrumental precision (% RSD) for system suitability was calculated from six replicate injections of a mid-range spiked vehicle standard.

9. Sample mean ($\bar{x}$), standard deviation (s), and percent relative standard deviation (% RSD) were calculated using commonly accepted techniques.

10. The response ratio (smallest value/largest value) of the duplicate analysis was calculated for each formulation.

[2] *United States Pharmacopeia*, 25th edition, p. 1990-1991.

4.7 Results

The density for the 0, 10, 20, and 40 mg/mL formulations were determined to be 0.91973, 0.92074, 0.92313, and 0.93547 g/mL, respectively. The data for the determined concentrations of the formulations of phenobarbital free acid in corn oil are presented in Table 5.

The 10 mg/mL and 40 mg/mL phenobarbital free acid in corn oil were evaluated for homogeneity, and the results indicated that the both formulations were homogeneous with % RSDs of 1.8 and 3.7, respectively; determined recoveries were of 101.8 ± 1.8 (s)% and 100.3 ± 3.7 (s)%, respectively, of the target concentration. The mean (n = 6) concentration of the 10 mg/mL and 40 mg/mL formulations were 10.17 mg/mL and 40.13 mg/mL, respectively.

The linear regression equation derived from the data for the spiked vehicle standard curve used for quantitation of the formulation concentrations had a linear correlation of ≥ 0.999. The values for the weighted (1/y) linear equation were calculated as 0.00536030 for the slope and –0.0143390 for the y-intercept. The data for system suitability are presented in Table 6.

Table 5. Results of the Formulation Analysis of Phenobarbital Free Acid in Corn Oil for TherImmune Research Corporation—Formulation Date: December 3, 2002

Study No.	Target concentration (mg/mL)	Determined concentration (mg/mL)	Average (n=2) determined concentration (mg/mL)	% Target	Average (n=2) % target
7244-600	0	0 0	0	N/A	N/A
7244-600	10	10.02 10.02	10.02 ± 0.00 0.0% RAD	100.2 100.2	100.2 ± 0.0 0.0% RAD
7244-600	20	18.94 19.54	19.24 ± 0.30 1.6% RAD	94.7 97.7	96.2 ± 1.5 1.6% RAD
7244-600	40	42.34 41.84	42.09 ± 0.25 0.6% RAD	105.9 104.6	105.3 ± 0.7 0.7% RAD
7244-600	10-T	10.31 10.01	10.16 ± 0.15 1.5% RAD	103.1 100.1	101.6 ± 1.5 1.5% RAD
7244-600	10-M	10.09 10.43	10.26 ± 0.17 1.7% RAD	100.9 104.3	102.6 ± 1.7 1.7% RAD
7244-600	10-B	9.975 10.24	10.11 ± 0.13 1.3% RAD	99.8 102.4	101.1 ± 1.3 1.3% RAD
7244-600	40-T	41.69 39.38	40.54 ± 1.16 2.9% RAD	104.2 98.5	101.4 ± 2.9 2.9% RAD
7244-600	40-M	37.85 39.47	38.66 ± 0.81 2.1% RAD	94.6 98.7	96.7 ± 2.1 2.2% RAD
7244-600	40-B	40.88 41.49	41.19 ± 0.31 0.8% RAD	102.2 103.7	103.0 ± 0.8 0.8% RAD
Results of Homogeneity Study					
Study No.	Target concentration (mg/mL)	Average (n=6) determined concentration (mg/mL)		Average (n=6) % target	
7244-600	Homogeneity 10 mg/mL	10.17 ± 0.18 1.8 % RSD		101.8 ± 1.8 1.8 % RSD	
7244-600	Homogeneity 40 mg/mL	40.13 ± 1.49 3.7 % RSD		100.3 ± 3.7 3.7 % RSD	

Table 6. System Suitability Criteria

System precision	Theoretical plates	Tailing factor	Resolution[a]
Analytical results: 2.9	Phenobarbital free acid = 32,845 IS = 31,356	Phenobarbital free acid = 1.5 IS = 2.0	11.9
Method criteria:[b] ≤ 8.0	Phenobarbital free acid ≥ 10,000 IS ≥ 10,000	Phenobarbital free acid ≤ 2.0 IS ≤ 2.0	≥ 7.0

[a] Between Phenobarbital free acid and IS peak.
[b] Acceptable system suitability criteria was established for this method.

5. Conclusions

The purpose of this study was to perform a formulation analysis of phenobarbital free acid in corn oil received from TherImmune Research Corporation. Samples included dose verification samples (one vial each) at concentration of 0 (control) 10, 20, 40 mg/mL, and homogeneity samples (top, middle and bottom) for formulations prepared at target concentration 10 and 40 mg/mL phenobarbital free acid in corn oil. The concentrations of the dose formulations were determined with TherImmune-supplied test article as an analytical standard.

The average concentrations of the dose verification samples were determined to be 0, 10.02, 19.24, and 42.09 mg/mL phenobarbital free acid in corn oil. The average percentages of the target concentrations were 100.2%, 96.2%, and 105.3% for the 10, 20, and 40 mg/mL formulations, respectively. All of the samples were determined to be within 5.3% of the respective target concentrations.

The 10 mg/mL and 40 mg/mL phenobarbital free acid in corn oil were evaluated for homogeneity, and the results indicated that the both formulations were homogeneous with % RSDs of 1.8 and 3.7, and recoveries of 101.8% and 100.3%, respectively. The mean (n = 6) concentration of the 10 mg/mL and 40 mg/mL formulations were 10.17 mg/mL and 40.13 mg/mL, respectively.

6. Contributors

Personnel contributing to this study were Kathryn Brackman and Sherry Ferguson.

MIDWEST RESEARCH INSTITUTE

Amir Kazerooni
Study Director

Dale Messer, Ph.D.
Group Leader

Approved:

Roger K. Harris, Ph.D.
Principal Investigator
Environmental Toxicology Program

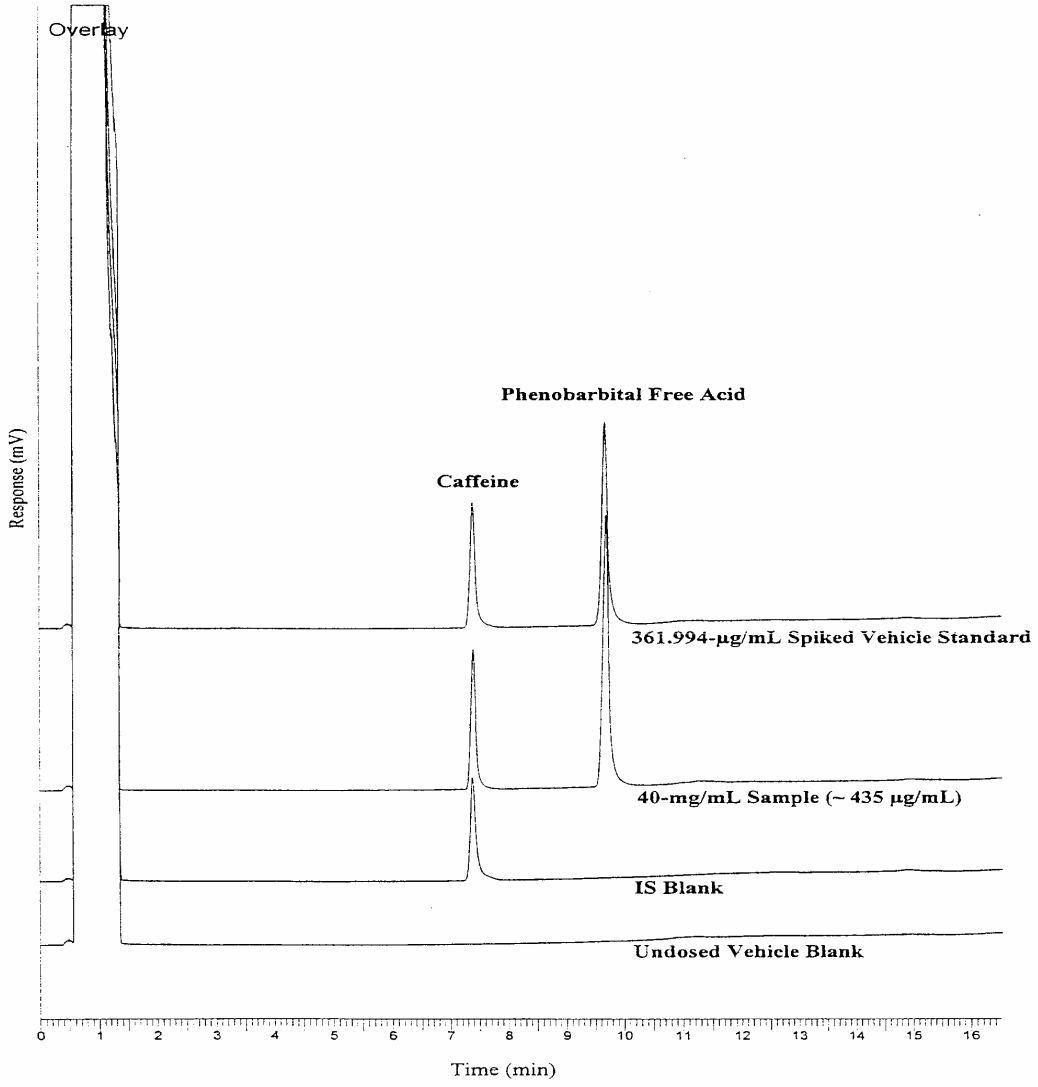


Figure 1. Representative Chromatograms of Phenobarbital Free Acid in Corn Oil; 361.944 µg/mL Spiked Vehicle Standard, 40 mg/mL Sample (~ 435 µg/mL), IS Blank, and Undosed Vehicle Blank

Formulation Analysis of Phenobarbital Free Acid in Corn Oil—Formulation Date: December 13, 2002

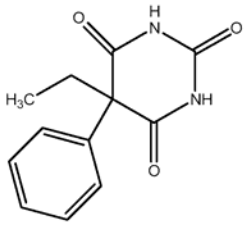
NIEHS Contract No. N01-ES-05457
ETP Task No. CHEM07227
MRI Project No. 110100
MRI Task No. 604

Midwest Research Institute
425 Volker Boulevard
Kansas City, Missouri 64110

February 4, 2003

Formulation Analysis (FA)

Chemical Information: Phenobarbital Free Acid

CAS No.: 50-06-6 MRI Task No.: 604 ETP Task No.: CHEM07227 Program Supported: RTX Analysis Date: December 18, 2002 Interim Report Date: December 20, 2002	Lot No.: QI0645 (Supplied by TherImmune Research Corporation) Samples Received: Dose verification samples at 0, 10, 20, and 40 mg/mL phenobarbital free acid in corn oil Sample Receipt Dates: December 5, 2002 (neat chemical) and December 17, 2002 (formulations) Submitter: TherImmune Research Corporation Study Lab: TherImmune Research Corporation Formulation Date: December 13, 2002 Receipt Condition: Intact, ice packs (formulation) Storage Conditions (@ MRI): Formulation samples, refrigerated (~ 5°C) and neat test article, frozen (~ –20°C, protected from heat)
Structure 	Molecular weight: 232.2 Molecular formula: $C_{12}H_{12}N_2O_3$

Quality Assurance Statement

Formulation Analysis of Phenobarbital Free Acid in Corn Oil—Formulation
Date: December 13, 2002

ETP Task No. CHEM07227
MRI Project No. 110100
MRI Task No. 604

This study was inspected by the Quality Assurance Unit of MRI (QAU) and the findings reported to the Study Director and Management as follows:

Phase inspected	Date inspected	Date reported
Protocol Audit	12/18/02	12/20/02
In-Life Audit; Formulation Analysis	12/18/02	12/20/02
Protocol Amendment No.1 Audit	2/3/03	2/3/03
Data Audit	2/3/03	2/3/03
Report Audit	2/3/03	2/3/03

The raw data and report will be stored in the MRI Archives.

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

Facility/equipment	Date inspected	Date reported
GC facility	11/18/02	11/19/02
PCA laboratory complex	9/13/02	9/16/02

MIDWEST RESEARCH INSTITUTE

Carlos Castro
Senior Quality Assurance Officer

Approved:

James Dworak, Ph.D.
Manager, Quality Assurance

February 4, 2003

MRI-ETP\TASK 604.DOC

Good Laboratory Practice Compliance Statement

Formulation Analysis of Phenobarbital Free Acid in Corn Oil—Formulation Date: December 13, 2002

ETP Task No. CHEM07227
MRI Project No. 110100
MRI Task No. 604

This formulation analysis study of phenobarbital free acid in corn oil was conducted in compliance with the Good Laboratory Practice Regulations of the U.S. Food and Drug Administration (21 *CFR* Part 58).

Amir Kazerooni
Study Director

2/4/03
Date

Executive Summary

The purpose of this study was to perform a formulation analysis of phenobarbital free acid in corn oil received from TherImmune Research Corporation. Samples included dose verification samples (one vial each) at concentrations of 0 (control), 10, 20, and 40 mg/mL, phenobarbital free acid in corn oil. The concentrations of the dose formulations were determined with TherImmune-supplied test article received on December 5, 2002, for MRI Task No. 591 (ETP Task No. CHEM07192) as an analytical standard.

The average concentrations of the dose verification samples (Mix No. 2) were determined to be 9.037, 16.56, and 38.31 mg/mL phenobarbital free acid in corn oil. The average percentages of the target concentrations were 90.4%, 82.8%, and 95.8%, respectively. All of the samples were determined to be within 9.6% of the respective target concentrations with the exception of the 20 mg/mL sample, which was within 17.2% of the target concentration. No test article was detected in the 0 mg/mL sample submitted for the formulation analysis.

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Formulation Analysis of Phenobarbital Free Acid in Corn Oil—Formulation Date: December 13, 2002

1. Introduction

The purpose of this study was to perform a formulation analysis of phenobarbital free acid in corn oil received from TherImmune Research Corporation. Samples included dose verification samples (one vial each) at concentrations of 0 (control) 10, 20, and 40 mg/mL phenobarbital free acid in corn oil. The concentrations of the dose formulations were determined with TherImmune-supplied test article received on December 5, 2002, for MRI Task No. 591 (ETP Task No. CHEM07192) as an analytical standard. This study was initiated on December 17, 2002.

The formulation analysis was based on the validated method as described in MRI report for "Dose Formulation Development of Phenobarbital Free Acid in Corn Oil," NIEHS Contract No. N01-ES-05457, ETP Task No. CHEM06833, MRI Project No. 110100, MRI Task No. 496, issued on December 3, 2002.

2. Chemical and Sample Information

Test Article:	Phenobarbital Free Acid[1]
Lot No.:	QI0645
Supplier:	TherImmune Research Corporation
Molecular Formula:	$C_{12}H_{12}N_2O_3$
Molecular Weight:	232.2
CAS No.:	50-06-6

The formulations submitted for formulation analysis by TherImmune Research Corporation were received at MRI on December 17, 2002, in amber glass bottles and are identified in Table 1 using TherImmune Research Corporation designations. Neat phenobarbital free acid was not received; therefore, the neat chemical from ETP Task No. CHEM07192, MRI Task No. 591, received from TherImmune Research Corporation and stored at MRI under frozen conditions (~ –20°C) was used in this study. The formulation samples were stored under refrigerated conditions (~ 5°C) prior to analysis.

[1] An analysis of this aliquot of neat chemical, submitted by TherImmune Research Corporation, was performed at MRI; see MRI report, "Bulk Chemical Reanalysis for Phenobarbital Free Acid," NIEHS Contract No. NOLES-05457, ETP Task No. CHEM07191, MRI Project No. 110100, MRI Task No. 590, January 23, 2003.

Table 1. Formulations From TherImmune Research Corporation—Formulation
Date: December 13, 2002

Study No.	Concentration (mg/mL)	Volume (~ mL)
Dose verification samples		
7244-600	0	35
7244-600	10	35
7244-600	20	35
7244-600	40	35

3. Materials and Equipment

Corn oil, Spectrum Chemicals
Caffeine (IS), Aldrich
Acetone, Burdick and Jackson, High Purity
Vortex Mixer, VWR Scientific
Ultra Sonic Bath, Branson Model 5510
Agilent 6890N Gas Chromatograph with Flame Ionization Detector (FID) and Turbochrom data system, Version 6.1.1

4. Formulation Analysis

A formulation analysis was performed to determine the concentration of phenobarbital free acid in corn oil formulations received from TherImmune Research Corporation. The concentrations of the formulations were determined using neat phenobarbital free acid standard supplied by TherImmune Research Corporation. All formulations were analyzed in duplicate and quantitation was performed using a six-point spiked vehicle standard curve prepared with neat chemical and blank corn oil supplied by MRI.

4.1 Determination of Vehicle and Formulation Density

The density of the MRI corn oil (vehicle) and of the control (0 mg/mL), 10 mg/mL, 20 mg/mL, and 40 mg/mL dose formulations was determined by transferring triplicate aliquots of each into individual 10-mL volumetric flasks and obtaining the weight. The density and average density were calculated using commonly accepted techniques.

4.2 Preparation of Standard Solutions

4.2.1 Internal Standard Solution

An internal standard (IS) solution was prepared by accurately weighing ~ 600 mg of caffeine into a 200-mL volumetric flask. An aliquot of acetone was added to the

volumetric flask and the contents of the flask were mixed by vortexing for ~ 2 min, mixed by sonication for ~ 2 min, diluted to volume with acetone, and mixed by inversion.

4.2.2 Stock Solutions

Two stock solutions of phenobarbital free acid were prepared by accurately weighing 196.908 mg (Stock A) and 104.953 mg (Stock B) of phenobarbital free acid into respective 100-mL volumetric flasks. The contents of each flask were diluted to volume with acetone and mixed by inversion. Expected concentrations: Stock A, 1.96908 mg/mL; Stock B, 1.04953 mg/mL.

4.2.3 Spiked Vehicle Standards

The spiked vehicle standards for phenobarbital free acid were prepared by transferring aliquots of either of the two stock solutions into individual 50-mL volumetric flasks containing ~ 1-g aliquot of corn oil (vehicle) and 5 mL of IS solution, diluting to volume with acetone, and mixing by inversion (see Table 2). The final analytical concentration of the spiked vehicle standard curve covered a range of 20.9906 to 551.342 µg/mL.

NOTE: Due to lack of availability of recently purchased corn oil, expired corn oil (Spectrum Chemicals, Lot No. QT0668, MRI-Assigned Batch No. 01, expiration date, 8/31/02) was used for the preparation of the spiked vehicle standards. However, analysis of this corn oil (Dose vehicle analysis, MRI Task No. 595[2]) determined that the corn oil was below (2.44 meq/Kg) the ETP criteria for maximum peroxide content (< 3.0 meq/Kg) and therefore, still viable. Inspection of the chromatography indicated that the corn oil contributed no interferences to the analysis, the use of this corn oil did not affect the integrity of the study.

Table 2. Preparation of Spiked Vehicle Standards

Spiked vehicle standard	Stock aliquot A or B (mL)	Corn oil aliquot (~ g)[a]	IS (mL)	Final volume (mL)	Expected concentration in corn oil (mg/mL)	Expected analytical concentration (µg/mL)
B_{11}	B, 1	1	5	50	0.96121	20.9906
A_{21}	A, 2	1	5	50	3.6068	78.7632
B_{31}	B, 10	1	5	50	9.6121	209.906
A_{41}	A, 9	1	5	50	16.230	354.434
B_{51}	B, 23	1	5	50	22.108	482.784
A_{61}	A, 14	1	5	50	25.247	551.342

[a] Average determined density of corn oil: 0.91585 g/mL.

[2] MRI Report, "Dose Vehicle Analysis on Corn Oil, Lot No. QT0668," NIEHS Contract No. N01-ES-05457, ETP Task No. CHEM07197, MRI Project No. 110100, MRI Task No. 595, submitted on January 15, 2003.

4.2.4 Blanks

4.2.4.1 IS Blank

An internal standard blank was prepared by volumetrically transferring 5 mL of IS solution into a 50-mL volumetric flask, diluting to volume with acetone, and mixing by inversion.

4.2.4.2 Reagent Blank (D0)

A reagent blank (D0) was prepared by transferring an aliquot of acetone into an autosampler vial for analysis.

4.2.4.3 Undosed Vehicle Blanks (C01 and C02)

An undosed vehicle blank (C01) was prepared by accurately weighing ~ 1 g of corn oil into a 50-mL volumetric flask, diluting the contents to volume with acetone, and mixing by inversion.

A second undosed vehicle blank (C02) was prepared with IS by accurately weighing ~ 1 g of corn oil into a 50-mL volumetric flask, and adding 5 mL of IS solution. The contents of the flask were diluted to volume with acetone and mixed by inversion.

4.3 Preparation of Formulation Samples

Each formulation was prepared in duplicate by transferring accurately weighed ~ 1.0-g aliquots of each formulation into individual volumetric flasks containing IS solution (see Table 3). The contents of each flask were diluted to volume with acetone and mixed by inversion.

Table 3. Preparation of Formulation Samples

Expected formulation concentration (mg/mL)	Formulation aliquot (~ g)	IS (mL)	Final dilution (mL)	Expected analytical concentration (~ µg/mL)
0 (Control)	1.0	5	50	0.0
10	1.0	5	50	217
20	1.0	5	50	435
40	1.0	10	100	435

4.4 Instrumental System and Parameters

Aliquots of the spiked vehicle standards, blanks, and formulation preparations were transferred to autosampler vials and analyzed using the instrumentation outlined in Table 4. Representative chromatograms for the formulation analysis of phenobarbital free acid in corn oil are presented in Figure 1.

Table 4. GC Conditions

Instrument:	Agilent 6890N Gas Chromatograph
Injector:	Agilent 7683 Autosampler
Injection Temperature:	250°C
Injector Mode:	Splitless, purge on at 0.75 min
Injector Volume:	1 µL
Detector:	Flame Ionization Detector (FID)
Detector Temperature:	275°C
Carrier Gas, Flow Rate:	Helium, ~ 10 mL/min
Purge Gas, Flow Rate:	Nitrogen, ~ 30 mL/min
Air, Flow Rate:	~ 300 mL/min
Hydrogen, Flow Rate:	~ 30 mL/min
Range:	5
Column:	DB-5 (J&W Scientific) 30 m x 0.53 mm ID, 1.5-µm film thickness
Data System:	PE Nelson Interface; Turbochrom, Version 6.1.1
Oven Program:	Initial temp: 185°C, 5-min hold, ramp at 10°C/min to 250°C, 5-min hold
Retention Time:	Caffeine (IS): ~ 7.4 min Phenobarbital Free Acid: ~ 9.6 min

4.5 Calculations

1. The density of each formulation was calculated by dividing the weight of each formulation aliquot by the aliquot volume. The mean density of each formulation was calculated.
2. The density of corn oil was calculated by dividing the weight of each corn oil aliquot by the aliquot volume. The mean density of corn oil was calculated.
3. A peak area ratio (PAR) was calculated for phenobarbital free acid as follows:

$$PAR = \frac{Peak\ Area_{Phenobarbital\ Free\ Acid}}{Peak\ Area_{Internal\ Standard}}$$

4. The slope, y-intercept, and correlation coefficient were calculated using a weighted (1/y) linear regression analysis of the spiked vehicle standard curve by relating the PAR of each spiked vehicle standard to its corresponding expected analytical concentration (µg/mL).
5. The determined concentrations of each sample were calculated using the regression equation obtained for the spiked vehicle standard curve and the PAR for each sample by using the following equation:

$$\text{Determined Conc. (mg/mL)} = \frac{[\text{PAR} - (\text{Y} - \text{intercept})]}{\text{slope}} \times \frac{1\text{ mg}}{1000\ \mu\text{g}} \times \frac{\text{dilution volume (mL)}}{\text{sample wt (g)}} \times \text{Density (g/mL)}$$

where: the dilution volume for the 0, 10, and 20 mg/mL formulation = 50 mL
the dilution volume for the 40 mg/mL formulation = 100 mL

6. The percent target concentration was calculated as follows:

$$\%\,\text{Target} = \frac{D}{T} \times 100$$

where: D = determined concentration (mg/mL in corn oil)
T = target concentration (mg/mL in corn oil)

7. The average percent target concentration with the corresponding statistical data was calculated for each concentration level.
8. The system suitability parameters for instrumental precision, peak tailing (T), theoretical plates (N), and peak resolution (R) were calculated for phenobarbital free acid and the internal standard peaks according to USP guidelines.[3] The instrumental precision (% RSD) for system suitability was calculated from six replicate injections of a midrange spiked vehicle standard.
9. Sample mean ($\bar{x}$), standard deviation (s), and percent relative standard deviation (% RSD) were calculated using commonly accepted techniques.
10. The response ratio (smallest value/largest value) of the duplicate analysis was calculated for each formulation.

4.6 Results

The density for the vehicle 0, 10, 20, and 40 mg/mL formulations was determined to be 0.91585, 0.91561, 0.91717, 0.91586, and 0.92330 g/mL, respectively. The data for the determined concentrations of the formulations of phenobarbital free acid in corn oil are presented in Table 5.

The linear regression equation derived from the data for the spiked vehicle standard curve used for quantitation of the formulation concentrations had a linear correlation of ≥ 0.9999. The values for the weighted (1/y) linear equation were calculated as 0.00512314 for the slope and –0.00466068 for the y-intercept. The data for the system suitability are presented in Table 6.

[3] *United States Pharmacopeia*, 25th Edition, p. 1990-1991.

Table 5. Results of the Formulation Analysis of Phenobarbital Free Acid in Corn Oil for TherImmune Research Corporation—Formulation Date: December 13, 2002

Study No.	Target concentration (mg/mL)	Determined concentration (mg/mL)	Average (n = 2) determined concentration (mg/mL)	% Target	Average (n = 2) % target
7244-600	0	0 0	0	N/A	N/A
7244-600	10	8.926 9.147	9.037 ± 0.111 1.2% RAD	89.3 91.5	90.4 ± 1.1 1.2% RAD
7244-600	20	16.71 16.40	16.56 ± 0.16 1.0% RAD	83.6 82.0	82.8 ± 0.8 1.0% RAD
7244-600	40	38.74 37.87	38.31 ± 0.44 1.1% RAD	96.9 94.7	95.8 ± 1.1 1.1% RAD

Table 6. System Suitability Results

System precision	Theoretical plates	Tailing factor	Resolution[a]
Analytical results: ≤ 2.3	Phenobarbital free acid = 37,806 IS = 34,193	Phenobarbital free acid = 1.2 IS = 1.2	12.6
Method criteria:[b] ≤ 8.0	Phenobarbital free acid ≥ 10,000 IS ≥ 10,000	Phenobarbital free acid ≤ 2.0 IS ≤ 2.0	≥ 7.0

[a] Between Phenobarbital free acid and IS peak.
[b] Acceptable system suitability criteria was established for this method.

5. Conclusions

The purpose of this study was to perform a formulation analysis of phenobarbital free acid in corn oil received from TherImmune Research Corporation. Samples included dose verification samples (one vial each) at concentrations of 0 (control), 10, 20, and 40 mg/mL phenobarbital free acid in corn oil. The concentrations of the dose formulations were determined with TherImmune-supplied test article received on December 5, 2002, for MRI Task No. 591 (ETP Task No. CHEM07192) as an analytical standard.

The average concentrations of the dose verification samples (Mix No. 2) were determined to be 9.037, 16.56, and 38.31 mg/mL phenobarbital free acid in corn oil. The average percentages of the target concentrations were 90.4%, 82.8%, and 95.8%, respectively. All of the samples were determined to be within 9.6% of the respective target concentrations with the exception of the 20 mg/mL sample, which was within

17.2% of the target concentration. No test article was detected in the 0 mg/mL sample submitted for the formulation analysis.

6. Contributors

Personnel contributing to this study were Kathryn Brackman and Sarah Barnes.

MIDWEST RESEARCH INSTITUTE

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MRI-ETP\TASK 604.DOC

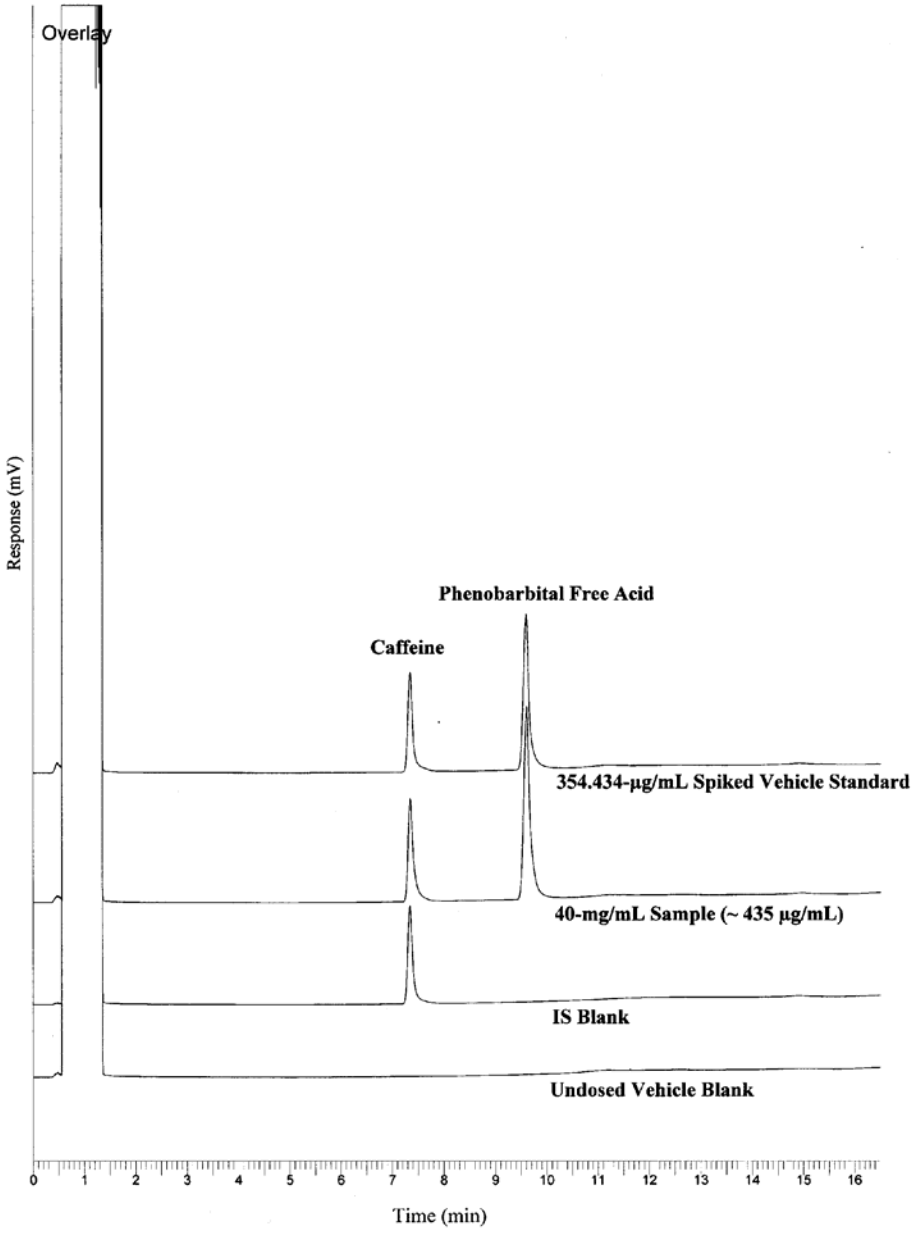


Figure 1. Representative Chromatograms of Phenobarbital Free Acid in Corn Oil; 354.434 µg/mL Spiked Vehicle Standard, 40 mg/mL Sample (~ 435 µg/mL), IS Blank, and Undosed Vehicle Blank

Formulation Analysis of Phenobarbital Free Acid in Corn Oil—Formulation Dates: December 23, 2002, and January 3, 2003

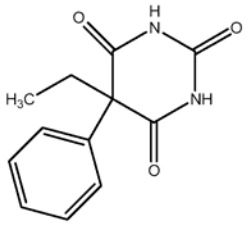
NIEHS Contract No. N01-ES-05457
ETP Task No. CHEM07229
MRI Project No. 110100
MRI Task No. 606

Midwest Research Institute
425 Volker Boulevard
Kansas City, Missouri 64110

February 13, 2003

Formulation Analysis (FA)

Chemical Information: Phenobarbital Free Acid

CAS No.: 50-06-6 MRI Task No.: 606 ETP Task No.: CHEM07229 Program Supported: RTX Analysis Date: January 13, 2003 Interim Report Date: January 16, 2003	Lot No.: QI0645 (Supplied by TherImmune Research Corporation) Samples Received: Dose verification samples for Mix No.: 3 at 0, 10, 20, and 40 mg/mL and Mix No.: 4 at 0, 10, 20, and 40 mg/mL phenobarbital free acid in corn oil Sample Receipt Date: December 5, 2002 (neat chemical) and January 10, 2003 (formulations) Submitter: TherImmune Research Corporation Study Lab: TherImmune Research Corporation Formulation Dates: December 23, 2002 (Mix No.: 3) and January 3, 2003 (Mix No.: 4) Receipt Condition: Intact, cold-ice packs Storage Conditions (@ MRI): Formulation samples, refrigerated (~ 5°C) and neat test article, frozen (~ –20°C), protected light
Structure  	Molecular Weight: 232.2 Molecular Formula: $C_{12}H_{12}N_2O_3$

Quality Assurance Statement

Formulation Analysis of Phenobarbital Free Acid in Corn Oil—Formulation Dates: December 23, 2002, and January 3, 2003

ETP Task No. CHEM07229
MRI Project No. 110100
MRI Task No. 606

This study was inspected by the Quality Assurance Unit of MRI (QAU) and the findings reported to the Study Director and Management as follows:

Phase inspected	Date inspected	Date reported
Protocol Audit	1/13/03	1/13/03
In-Life Audit; Formulation Analysis	1/13/03	1/13/03
Protocol Amendment No. 1 Audit	2/11/03	2/12/03
Data Audit	2/11/03	2/12/03
Report Audit	2/11/03	2/12/03

The raw data and report will be stored in the MRI Archives.

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

Facility/equipment	Date inspected	Date reported
GC facility	11/18/02	11/19/02
PCA laboratory complex	9/13/03	9/16/02

MIDWEST RESEARCH INSTITUTE
Rosemary Moran
Rosemary Moran
Quality Assurance Officer

Approved:
James Dworak
James Dworak, Ph.D.
Manager, Quality Assurance

February 13, 2003

Good Laboratory Practice Compliance Statement

Formulation Analysis of Phenobarbital Free Acid in Corn Oil—Formulation Dates: December 23, 2002, and January 3, 2003

ETP Task No. CHEM07229
MRI Project No. 110100
MRI Task No. 606

This formulation analysis study of phenobarbital free acid in corn oil was conducted in compliance with the Good Laboratory Practice Regulations of the U.S. Food and Drug Administration (21 *CFR* Part 58).

Amir Kazerooni
Amir Kazerooni
Study Director

2/13/03
Date

MRI-ETP\TASK 606.DOC

Executive Summary

The purpose of this study was to perform a formulation analysis of phenobarbital free acid in corn oil received from TherImmune Research Corporation. Samples (Mix No.: 3) included dose verification samples (one vial each) at concentrations of 0 (control), 10, 20, and 40 mg/mL phenobarbital free acid in corn oil. Samples (Mix No.: 4) included dose verification samples (one vial each) at concentrations of 0 (control), 10, 20, and 40 mg/mL phenobarbital free acid in corn oil. The concentration of the dose formulations was determined with TherImmune-supplied test article received on December 5, 2002, for MRI Task No. 591 (ETP Task No. CHEM07192) as an analytical standard.

The average concentrations of the dose verification samples (Mix No.: 3) were determined to be 8.691, 19.25, and 32.12 mg/mL phenobarbital free acid in corn oil. The average percentages of the target concentrations were 87.0%, 96.3%, and 80.3%, respectively. The 20 mg/mL sample was determined to be within 3.7% of the respective target concentration. The 10 and 40 mg/mL samples were within 13.0% and 19.7% of the target concentrations, respectively. No test article was detected in the 0 mg/mL (control) sample.

The average concentrations of the dose verification samples (Mix No.: 4) were determined to be 8.997, 18.33, and 30.86 mg/mL phenobarbital free acid in corn oil. The average percentages of the target concentrations were 90.0%, 91.7%, and 77.2%, respectively. All of the samples were determined to be within 10.0% of the respective target concentrations, with the exception of the 40 mg/mL sample, which was within 22.8% of the target concentration. No test article was detected in the 0 mg/mL (control) sample.

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Figure 1. Representative Chromatograms of Phenobarbital Free Acid in Corn Oil; 355.912 µg/mL Spiked Vehicle Standard, 40 mg/mL Sample (~ 435 µg/mL), IS Blank, and Undosed Vehicle Blank 9

Formulation Analysis of Phenobarbital in Corn Oil— Formulation Dates: December 23, 2002, and January 3, 2003

1. Introduction

The purpose of this study was to perform a formulation analysis of phenobarbital free acid in corn oil received from TherImmune Research Corporation. Samples (Mix No.: 3) included dose verification samples (one vial each) at concentrations of 0 (control), 10, 20, and 40 mg/mL phenobarbital free acid in corn oil. Samples (Mix No.: 4) included dose verification samples (one vial each) at concentrations of 0 (control), 10, 20, and 40 mg/mL phenobarbital free acid in corn oil. The concentration of the dose formulations was determined with TherImmune-supplied test article received on December 5, 2002, for MRI Task No. 591 (ETP Task No. CHEM07192) as an analytical standard. This study was initiated on January 10, 2003.

The formulation analysis was based on the validated method as described in the MRI dose formulation development report.[1]

2. Chemical and Sample Information

Test Article:	Phenobarbital Free Acid
Lot No.:	QI0645
Supplier:	TherImmune Research Corporation
Molecular Formula:	$C_{12}H_{12}N_2O_3$
Molecular Weight:	232.2
CAS No.:	50-06-6
Purity:	99.4% Per BCR at MRI[2]

The formulations submitted for formulation analysis from TherImmune Research Corporation were received at MRI on January 10, 2003, in amber glass bottles and are identified in Table 1 using TherImmune Research Corporation designations. Neat phenobarbital free acid was not received; therefore, the neat chemical from ETP Task No. CHEM07192, MRI Task No. 591, received from TherImmune Research Corporation on December 5, 2002, stored under frozen conditions (~ –20°C) was used in this study . The formulation samples were stored under refrigerated conditions (~ 5ºC) prior to analysis.

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[1] MRI Report, "Dose Formulation Development of Phenobarbital Free Acid in Corn Oil," NIEHS Contract No. N01-ES-05457, ETP Task No. CHEM06833, MRI Project No. 110100, MRI Task No. 496, issued on December 3, 2002.

[2] MRI Report, "Bulk Chemical Reanalysis for Phenobarbital Free Acid," NIEHS Contract No. N01-ES-05457, ETP Task No. CHEM07191, MRI Project No. 110100, MRI Task No. 590, issued on January 23, 2003.

Table 1. Formulations Received From TherImmune Research Corporation—
Formulation Dates: December 23, 2002, and January 3, 2003

Study No.	Concentration (mg/mL)	Volume (~ mL)
Dose verification samples		
7244-600 (mix 3)[1]	0	50
7244-600 (mix 3)	10	35
7244-600 (mix 3)	20	35
7244-600 (mix 3)	40	35
7244-600 (mix 4)[2]	0	50
7244-600 (mix 4)	10	35
7244-600 (mix 4)	20	35
7244-600 (mix 4)	40	35

[1] Prep date: 12/23/02; reported as "(mix 3)".
[2] Prep date: 1/3/03; reported as "(mix 4)".

3. Materials and Equipment

Corn oil, Spectrum Chemicals
Caffeine (IS), Aldrich
Acetone, Burdick and Jackson, High Purity
Vortex Mixer, VWR Scientific
Ultra Sonic Bath, Branson Model 5510
Agilent 6890N Gas Chromatograph with Flame Ionization Detector (FID) and Turbochrom data system, version 6.1.1

4. Formulation Analysis

A formulation analysis was performed to determine the concentration of phenobarbital free acid in corn oil formulations received from TherImmune Research Corporation. The concentrations of the formulations were determined using neat phenobarbital free acid standard supplied by TherImmune Research Corporation. All formulations were analyzed in duplicate, and quantitation was performed using a six-point spiked vehicle standard curve.

4.1 Determination of Corn Oil Density

The density of corn oil was determined by transferring triplicate aliquots of corn oil into individual 10-mL volumetric flasks and obtaining the weight.

4.2 Preparation of Standard Solutions

4.2.1 Internal Standard Solution

An internal standard (IS) solution was prepared by accurately weighing ~ 600 mg of caffeine into a 200-mL volumetric flask. An aliquot of acetone was added to the volumetric flask, and the contents of the flask were mixed by vortexing for ~ 2 min, mixed by sonication for ~ 2 min, diluted to volume with acetone, and mixed by inversion.

4.2.2 Stock Solutions

Two stock solutions of phenobarbital free acid were prepared by accurately weighing and quantitatively transferring 197.729 mg (Stock A) and 103.790 mg (Stock B) of phenobarbital free acid into respective 100-mL volumetric flasks. The contents of each flask were diluted to volume with acetone and mixed by inversion. Expected concentrations: Stock A, 1.97729 mg/mL; and Stock B, 1.03790 mg/mL.

4.2.3 Spiked Vehicle Standards

The spiked vehicle standards for phenobarbital free acid were prepared by transferring aliquots of stock solutions into individual 50-mL volumetric flasks containing ~ 1 g aliquot of corn oil (vehicle) and 5 mL of IS solution, diluting to volume with acetone, and mixing by inversion (see Table 2). The final analytical concentration of the spiked vehicle standard curve covered a range of 20.7580 to 553.641 µg/mL.

Table 2. Preparation of Spiked Vehicle Standards

Spiked vehicle standard	Stock aliquot A or B (mL)	Corn oil aliquot (~ g)[a]	IS (mL)	Final volume (mL)	Expected concentration in corn oil (mg/mL)	Expected analytical concentration (µg/mL)
B_{11}	B, 1	1	5	50	0.95138	20.7580
A_{21}	A, 2	1	5	50	3.6249	79.0916
B_{31}	B, 10	1	5	50	9.5138	207.580
A_{41}	A, 9	1	5	50	16.312	355.912
B_{51}	B, 23	1	5	50	21.882	477.434
A_{61}	A, 14	1	5	50	25.374	553.641

[a] Density of corn oil: 0.91664 g/mL.

4.2.4 Blanks

4.2.4.1 IS Blank

An internal standard blank was prepared by volumetrically transferring 5 mL of IS solution into a 50-mL volumetric flask, diluting to volume with acetone, and mixing by inversion.

4.2.4.2 Reagent Blank (D0)

A reagent blank (D0) was prepared by transferring an aliquot of acetone into an autosampler vial for analysis.

4.2.4.3 Undosed Vehicle Blanks (C01 and C02)

An undosed vehicle blank (C01) was prepared by accurately weighing ~ 1 g of corn oil into a 50-mL volumetric flask, diluting the contents to volume with acetone, and mixing by inversion.

A second undosed vehicle blank (C02) was prepared with IS by accurately weighing ~ 1 g of corn oil into a 50-mL volumetric flask and adding 5 mL of IS solution. The contents of the flask were diluted to volume with acetone and mixed by inversion.

4.3 Determination of Formulation Density

The density of each formulation was determined by transferring triplicate aliquots of the formulation into individual 10-mL volumetric flasks and obtaining the weight.

4.4 Preparation of Formulation Samples

Each formulation was prepared in duplicate by transferring ~ 1.0-g aliquot of formulation into individual volumetric flasks and adding IS solution (see Table 3). The contents of each flask were diluted to volume with acetone and mixed by inversion.

Table 3. Preparation of Formulation Samples

Expected formulation concentration (mg/mL)	Formulation aliquot (~ g)	IS (mL)	Final dilution (mL)	Expected analytical concentration (~µg/mL)
0 (Control)	1.0	5	50	0.0
10	1.0	5	50	217
20	1.0	5	50	435
40	1.0	10	100	435

4.5 Instrumental System and Parameters

Aliquots of the spiked vehicle standards, blanks, and formulation preparations were transferred into autosampler vials and analyzed using the instrumentation outlined in Table 4. Representative chromatograms for the formulation analysis of phenobarbital free acid in corn oil are presented in Figure 1.

Table 4. GC Conditions

Instrument:	Agilent 6890N Gas Chromatograph
Injector:	Agilent 7683 Autosampler
Injection Temperature:	250°C
Injector Mode:	Splitless, purge on at 0.75 min
Injector Volume:	1 µL
Detector:	Flame Ionization Detector (FID)
Detector Temperature:	275°C
Carrier Gas, Flow Rate:	Helium, ~ 10 mL/min
Purge Gas, Flow Rate:	Nitrogen, ~ 30 mL/min
Air, Flow Rate:	300 mL/min
Hydrogen, Flow Rate:	30 mL/min
Range:	5
Column:	DB-5 (J&W Scientific) 30 m x 0.53 mm ID, 1.5-µm film
Data System:	PE Nelson Turbochrom, version 6.1.1
Oven Program:	Initial temp: 185°C, 5 min hold, ramp at 10°C/min to 250°C, 5 min hold
Retention Time:	Caffeine (IS): ~ 7.4 min
	Phenobarbital:~ 9.6 min

4.6 Calculations

1. The density of each formulation was calculated by dividing the weight of each formulation aliquot by the aliquot volume. The mean density of each formulation was calculated.

2. The density of corn oil was calculated by dividing the weight of each corn oil aliquot by the aliquot volume. The mean density of corn oil was calculated.

3. A peak area ratio (PAR) was calculated for phenobarbital free acid as follows:

$$PAR = \frac{\text{Peak Area}_{\text{Phenobarbital Free Acid}}}{\text{Peak Area}_{\text{Internal Standard}}}$$

4. The slope, y-intercept, and correlation coefficient were calculated using a weighted (1/y) linear regression analysis of the spiked vehicle standard curve by relating the PAR of each spiked vehicle standard to its corresponding expected analytical concentration (µg/mL).

5. The determined concentrations of each sample was calculated using the regression equation obtained for the spiked vehicle standard curve and the PAR for each sample by using the following equation:

$$\text{Determined conc.(mg/mL)} = \frac{[\text{PAR} - (\text{y - intercept})]}{\text{slope}} \times \frac{1\ \text{mg}}{1000\ \mu\text{g}} \times \frac{\text{dilution volume (mL)}}{\text{sample wt (g)}} \times \text{Density (g/mL)}$$

where: the dilution volume for the 0, 10, and 20 mg/mL formulation = 50 mL
the dilution volume for the 40 mg/mL formulation = 100 mL

6. The percent target concentration was calculated as follows:

$$\%\,\text{Target} = \frac{D}{T} \times 100$$

where: D = determined concentration (mg/mL corn oil)
T = target concentration (mg/mL corn oil)

7. The average percent target concentration with the corresponding statistical data was calculated for each concentration level.

8. The system suitability parameters for instrumental precision, peak tailing (T), theoretical plates (N), and peak resolution (R) were calculated for phenobarbital free acid and the internal standard peaks according to USP guidelines.[3] The instrumental precision (% RSD) for system suitability was calculated from six replicate injections of a mid-range spiked vehicle standard.

9. Sample mean ($\bar{x}$), average deviation (d), and percent relative average deviation (% RAD) were calculated using commonly accepted techniques.

10. The response ratio (smallest value/largest value) of the duplicate analysis was calculated for each formulation.

4.7 Results

The density of corn oil (vehicle) was determined to be 0.91664 g/mL. The density for mix three formulations at 0, 10, 20, and 40 mg/mL was determined to be 0.91674, 0.91742, 0.91964, and 0.92267 g/mL, respectively. The data for the determined concentrations of the formulations of phenobarbital free acid in corn oil are presented in Table 5.

[3] *United States Pharmacopeia*, 25th edition, pp. 1990-1991.

The density for mix four formulations at 0, 10, 20, and 40 mg/mL was determined to be 0.91593, 0.91363, 0.91769, and 0.92540 g/mL, respectively. The data for the determined concentrations of the formulations of phenobarbital free acid in corn oil are presented in Table 5.

The linear regression equation derived from the data for the spiked vehicle standard curve used for quantitation of the formulation concentrations had a linear correlation of ≥ 0.999. The y-intercept and slope values calculated from the linear regression equation were –0.0135748 and 0.00507450, respectively. The system suitability data are presented in Table 6.

Table 5. Results of the Formulation Analysis of Phenobarbital Free Acid in Corn Oil for TherImmune Research Corporation—Formulation Dates: December 23, 2002, and January 3, 2003

Study No.	Target concentration (mg/mL)	Determined concentration (mg/mL)	Average (n=2) determined concentration (mg/mL)	% Target	Average (n=2) % target
7244-600 (mix 3)	0	0 0	0	NA	NA
7244-600 (mix 3)	10	8.586 8.792	8.691 ± 0.105 (d) 1.2% RAD	85.9 88.0	87.0 ± 1.1 (d) 1.3% RAD
7244-600 (mix 3)	20	19.02 19.47	19.25 ± 0.23 (d) 1.2% RAD	95.1 97.4	96.3 ± 1.2 (d) 1.2% RAD
7244-600 (mix 3)	40	32.39 31.85	32.12 ± 27 (d) 0.8%RAD	81.0 79.6	80.3 ± 0.7 (d) 0.9% RAD
7244-600 (mix 4)	0	0 0	0	NA	NA
7244-600 (mix 4)	10	9.011 8.983	8.997 ± 0.014 (d) 0.2% RAD	90.1 89.8	90.0 ± 0.2 (d) 0.2% RAD
7244-600 (mix 4)	20	17.37 19.29	18.33 ± 0.96 (d) 5.2% RAD	86.9 96.5	91.7 ± 4.8 (d) 5.2% RAD
7244-600 (mix 4)	40	31.73 29.99	30.86 ± 0.87 (d) 2.8% RAD	79.3 75.0	77.2 ± 2.2 (d) 2.8% RAD

Table 6. System Suitability Results

System precision	Theoretical plates	Tailing factor	Resolution [a]
Analytical results: 2.1	Phenobarbital free acid = 38,126 IS = 32,311	Phenobarbital free acid = 1.2 IS = 1.6	12.7
Method criteria [b]: ≤ 8.0	Phenobarbital free acid ≥ 10,000 IS ≥ 10,000	Phenobarbital free acid ≤ 2.0 IS ≤ 2.0	≥ 7.0

[a] Between Phenobarbital free acid and IS peak.
[b] All system suitability criteria were met.

5. Conclusions

The purpose of this study was to perform a formulation analysis of phenobarbital free acid in corn oil received from TherImmune Research Corporation. Samples (Mix No.: 3) included dose verification samples (one vial each) at concentrations of 0 (control), 10, 20, and 40 mg/mL phenobarbital free acid in corn oil. Samples (Mix No.: 4) included dose verification samples (one vial each) at concentrations of 0 (control), 10, 20, and 40 mg/mL phenobarbital free acid in corn oil. The concentration of the dose formulations was determined with TherImmune-supplied test article received on December 5, 2002, for MRI Task No. 591 (ETP Task No. CHEM07192) as an analytical standard. This study was initiated on January 10, 2003.

The average concentrations of the dose verification samples (Mix No.: 3) were determined to be 8.691, 19.25, and 32.12 mg/mL phenobarbital free acid in corn oil. The average percentages of the target concentrations were 87.0%, 96.3%, and 80.3%, respectively. The 20 mg/mL sample was determined to be within 3.7% of the respective target concentration. The 10 and 40 mg/mL samples were within 13.0% and 19.7% of the target concentrations, respectively. No test article was detected in the 0 mg/mL (control) sample.

The average concentrations of the dose verification samples (Mix No.: 4) were determined to be 8.997, 18.33, and 30.86 mg/mL phenobarbital free acid in corn oil. The average percentages of the target concentrations were 90.0%, 91.7%, and 77.2%, respectively. All of the samples were determined to be within 10.0% of the respective target concentrations, with the exception of the 40 mg/mL sample, which was within 22.8% of the target concentration. No test article was detected in the 0 mg/mL (control) sample.

6. Contributors

Personnel contributing to this study were Kathryn Brackman, Janet Irungu, and Sherry Ferguson.

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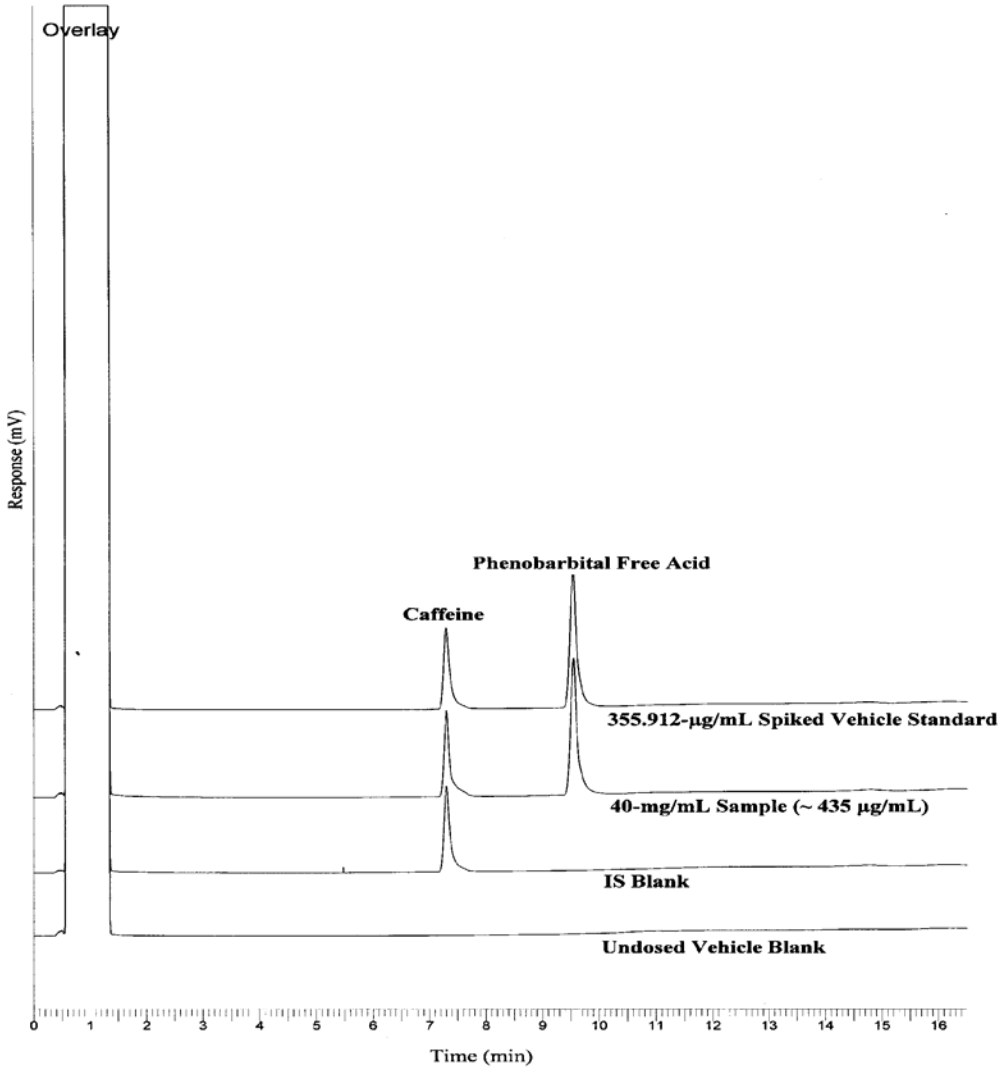

Figure 1. Representative Chromatograms of Phenobarbital Free Acid in Corn Oil; 355.912 µg/mL Spiked Vehicle Standard, 40 mg/mL Sample (~ 435 µg/mL), IS Blank, and Undosed Vehicle Blank

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Chemistry Support Services for the NTP
NIH Contract No.: N01-ES-05456
Battelle Project No.: G004110-BPU
NTP ChemTask No.: CHEM07220
CAS No.: 50471-44-8

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FORMULATION ANALYSIS REPORT

VINCLOZOLIN

8-039-FA-197

March 19, 2003

Approved By:

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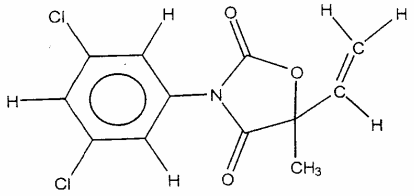
FORMULATION ANALYSIS REPORT

VINCLOZOLIN

CAS No.: 50471-44-8	Lot No.: 102996 (Battelle's Organic Synthesis Group, 99%)
Battelle Chemical ID Code: 039	Samples Received: 0, 4, 12, and 40 mg/mL formulations
Battelle Task No.: 8-039-FA-197	Sample Receipt Date: 12/5/02
NTP Task No.: CHEM07220	Submitter: Gary Holley
Program Supported: RDGT	Study Lab: TherImmune Research Corporation
Analysis Dates: 1/8-1/9/03	Receipt Condition: Good
Interim Results Date: 1/10/03	Shipping Container: Nine 60 mL amber glass serum vials on refrigerant packs.
SOP: CSCSPEC.II-015-00, "Standard Operating Procedure (SOP) for the Analysis of Vinclozolin Formulations in Corn Oil"	Storage Conditions (@ Battelle): Refrigerated (~5°C)

STRUCTURE	Mol. Wt.	Mol. Formula
	286.1 g/mol	$C_{12}H_9NO_3Cl_2$

EXECUTIVE SUMMARY

Formulations of vinclozolin in corn oil at target concentrations of 0, 4, 12, and 40 mg/mL were received from TherImmune and analyzed in support of an RDGT study.

The 0 mg/mL formulation contained no detectable vinclozolin and the average concentration of the 40 mg/mL formulation was within 10% of target, the NTP acceptance limit. The average concentrations of the 4 mg/mL and 12 mg/mL formulations were 5.064 and 13.5 mg/mL, respectively. These values are 26.6% and 13.2% above target. The 4 and 40 mg/mL formulations were homogenous, although the average concentrations were 31.1% and 11.5%, respectively, above target.

QUALITY ASSURANCE STATEMENT

FORMULATION ANALYSIS REPORT

VINCLOZOLIN

NTP ChemTask No.: CHEM07220
Battelle Project No.: G004110-BPU
Battelle Task No.: 8-039-FA-197

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

Critical Phase Inspected	Date Inspected	Date Reported to Study Director and Management
Audit study file	3/11/03	3/11/03
Audit analytical report	3/11/03	3/11/03

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

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

Facility/Equipment	Date Inspected	Date of Report to Management
Chemistry Technical Center inspection	4/23/00	4/24/00
	12/8-12/12/00	12/13/00
	10/17/01	10/19/01
	9/6/02	9/6/02

3-18-03

Quality Assurance Unit Date
Battelle

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1 INTRODUCTION

This report contains:

- a description of the analysis of formulations of vinclozolin in corn oil,
- the results of the analysis, and
- conclusions.

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

2 TEST ARTICLE AND FORMULATION SAMPLES

Samples of corn oil formulations containing vinclozolin were received from TherImmune on December 5, 2002. They were identified as being from Study # 7244-600, Mix 1, and had the following group numbers and target concentrations: Group 5, 4 mg/mL; Group 6, 12 mg/mL; Group 7, 40 mg/mL. A verification sample for each group was received. In addition, three homogeneity samples (top, middle, and bottom) were received for the 4 and 40 mg/mL formulations. The control sample was submitted with the Flutamide formulations under Battelle Study No. G004110-BPR.

All formulations were prepared using vinclozolin, Lot No. 102996, according to information provided with the shipment. The identity of this lot was confirmed and its purity determined previously under NTP ChemTask No. CHEM05774, Battelle Study No. G004110-AWB. A sample of the same lot was used as the analytical standard for this work.

3 FORMULATION ANALYSIS

The formulations were analyzed according to CSCSPEC.II-015-00, "Standard Operating Procedure (SOP) for the Analysis of Vinclozolin Formulations in Corn Oil." The methodology in this SOP is based on work originally conducted in the DFD task for this formulation, NTP ChemTask No. CHEM05773, Battelle Study No. G004110-AWA. This section describes the methodology, the results, and our conclusions.

3.1 Preparation of Standards

3.1.1 Solvent Stocks

Two solvent stock standards (A and B) were made at target concentrations of 28 and 23 mg/mL, respectively, by dissolving approximately 280 and 575 mg of accurately weighed vinclozolin in 10 and 25 mL of ethyl acetate.

3.1.2 Intermediate Standards

Four additional intermediate standards (the stocks were used as the two highest concentration intermediate standards) were made by pipetting the volumes of Stocks A and B into volumetric flasks and diluting to volume with ethyl acetate as described in Table 1.

Table 1 – Intermediate Standards

Intermediate Standard	Nominal Conc (mg/mL)	Source Stock	Source Volume (mL)	Final Volume (mL)
1	28	A	NA	NA
2	23	B	NA	NA
3	11.2	A	2	5
4	6.9	B	3	10
5	1.68	A	3	50
6	0.92	B	1	25

3.1.3 Vehicle/Calibration Standards

Vehicle standards were prepared by pipetting 1 mL of each intermediate standard and 1 mL of vehicle (corn oil) into individual 50-mL volumetric flasks. They were then diluted to volume with ethyl acetate, sealed, and mixed.

Calibration standards were prepared by pipetting 1 mL each of vehicle standard and 1 mL of internal standard (400 mg of isodrin dissolved in 100 mL of ethyl acetate and then diluted 1:10 with ethyl acetate) into individual 10-mL volumetric flasks. They were then diluted to volume with ethyl acetate, sealed, and mixed. Single vehicle/calibration standards were prepared from each intermediate standard. This produced calibration standards with target concentrations of 56, 46, 22.4, 13.8, 3.36, and 1.84 µg/mL.

3.1.4 Blanks

Two blanks were prepared by diluting 1 mL of corn oil to 50 mL with ethyl acetate. A single blank with internal standard (IS) was prepared by pipetting 1 mL of the above prepared blank and 1 mL of IS into a 10-mL volumetric flask and diluting to volume with ethyl acetate. A single blank without internal standard was prepared by pipetting 1 mL of the above prepared blank into a 10-mL volumetric flask and diluting to volume with ethyl acetate.

3.2 Preparation of Formulation Samples for Analysis

The 4 and 12 mg/mL formulations were prepared by first pipetting 1 mL aliquots (duplicate for verification samples and triplicate for homogeneity samples) into individual 50-mL volumetric flasks and diluting to volume with

ethyl acetate. Each diluted formulation was further diluted by pipetting 1 mL of diluted formulation and 1 mL of IS into individual 10-mL volumetric flasks and diluting to volume with ethyl acetate.

The 40 mg/mL formulations were prepared using a three-stage dilution scheme designed to keep the vehicle concentration in the final analysis sample approximately the same as the other formulations. A 1 mL aliquot of formulation was pipetted into a 10-mL volumetric flask and diluted to volume with ethyl acetate. Aliquots (1 mL each; duplicate for the verification sample and triplicate for the homogeneity samples) of the first stage diluted formulation were combined with 900 µL of corn oil into a 50-mL volumetric flask and diluted to volume with ethyl acetate. Each second stage diluted formulation was further diluted by pipetting 1 mL of diluted formulation and 1 mL of IS into individual 10-mL volumetric flasks and diluting to volume with ethyl acetate.

3.3 Analysis

Single injections were made from each vial using the GC system shown in Table 2. Typical chromatograms (overlaid) from a blank, a blank with internal standard, and low and high vehicle standards are shown in Figure 1.

Table 2 – GC System

Instrument	Agilent 6890 (Palo Alto, CA)
Column	Restek RTX-5. 30 m x 0.25 mm (ID), 0.25 µm film thickness (Bellefonte, PA)
Carrier Gas/Flow rate	Helium @ 2 mL/minute
Oven Temperature Program	120°C, hold 1 min., increase at 15°C/minute to 315°C, hold 3 minutes
Detector Type/Gas Settings	Flame Ionization; 30 mL/minute Hydrogen, 380 mL/minute Air
Detector Temperature	225°C
Injection Volume/Mode	1 µL/splitless
Injector Temperature	200°C
Run Time	17 minutes
Retention Times	
Vinclozolin	~7.5 minutes
IS	~8.5 minutes

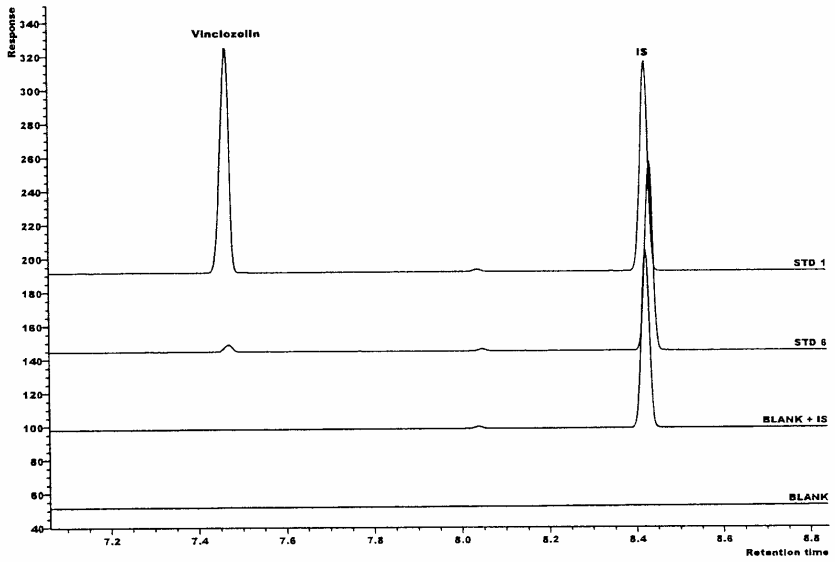

Figure 1 – Representative Overlaid Chromatograms of a Blank, Blank plus IS, Low Standard, and High Standard

3.4 Calculations

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

3.5 Results

The results from the analysis of the formulations are shown in Table 3 and 4. The standard curve and the results of the regression analysis of the standards are shown in Figure 2.

Table 3 – Formulation Analysis Results

Group No	Mix No	Target Conc (mg/mL)	Det'd Conc (mg/mL)		Avg Det'd Conc (mg/mL)	Avg RE
NA	NA	0	BLOQ[a]	BLOQ	BLOQ	BLOQ
5	1	4	5.069	5.059	5.064	26.6
6	1	12	13.85	13.31	13.58	13.2
7	1	40	41.62	40.61	41.11	1.02

a. BLOQ = Below Limit of Quantitation.

Table 4 – Formulation Homogeneity Analysis Results

Group No	Mix No	Target Conc (mg/mL)	Sampling Location	Determined Conc (mg/mL)	Average Determined Conc (mg/mL)	% RSD	Grand Avg Determined Conc (mg/mL)	Grand % RSD	Grand % RE
5	1	4	Top	5.255	5.270	0.32	5.242	0.6	31.1
				5.288					
				5.266					
			Middle	5.241	5.211	1.33			
				5.132					
				5.261					
			Bottom	5.254	5.247	0.14			
				5.239					
				5.247					
7	1	40	Top	45.41	45.48	0.59	44.61	3.0	11.5
				45.25					
				45.77					
			Middle	42.74	43.07	1.19			
				42.80					
				43.66					
			Bottom	44.82	45.29	0.91			
				45.49					
				45.57					

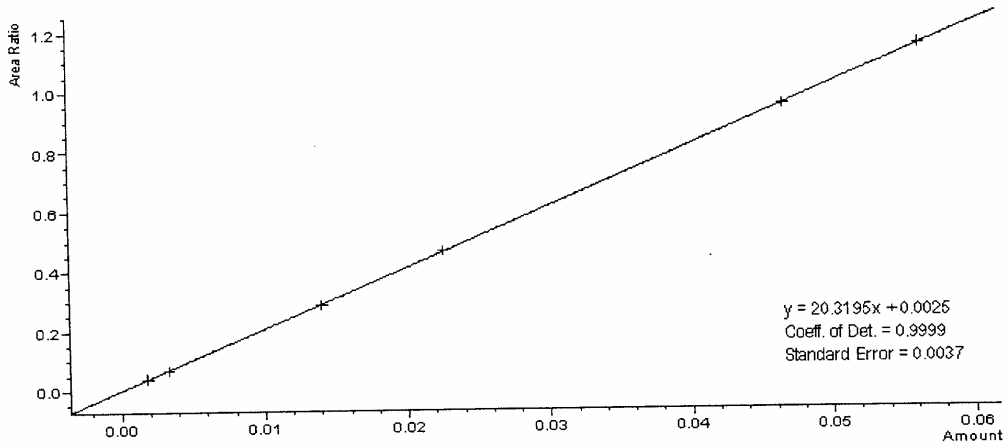

Figure 2 – Standard Curve

3.6 Conclusions

The 0 mg/mL formulation contained no detectable vinclozolin and the average concentration of the 40 mg/mL formulation was within 10% of target, the NTP acceptance limit. The average concentrations of the 4 mg/mL and 12 mg/mL formulations were 5.064 and 13.58 mg/mL, respectively. These values are 26.6% and 13.2% from target. The 4 and 40 mg/mL formulations were homogenous, although the average concentrations were 31.1% and 11.5%, respectively, above target.

4 ACKNOWLEDGMENTS

Kevin Carrico conducted the analytical work. Tim Cristy wrote the report. Padmini Fernando reviewed the raw data for completeness and accuracy.

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Chemistry Support Services for the NTP
NIH Contract No.: N01-ES-05456
Battelle Project No.: G004110-BPV
NTP ChemTask No.: CHEM07221
CAS No.: 50471-44-8

COPY

FORMULATION ANALYSIS REPORT

VINCLOZOLIN

8-039-FA-198

March 19, 2003

Approved By:

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Approved By:

Steven W. Graves, B.S.
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FORMULATION ANALYSIS REPORT

VINCLOZOLIN

CAS No.: 50471-44-8	Lot No.: 102996 (Battelle's Organic Synthesis Group, 99%)
Battelle Chemical ID Code: 039	Samples Received: 0, 4, 12, and 40 mg/mL formulations
Battelle Task No.: 8-039-FA-198	Sample Receipt Date: 12/17/02
NTP Task No.: CHEM07221	Submitter: Gary Holley
Program Supported: RDGT	Study Lab: TherImmune Research Corporation
Analysis Dates: 1/8-1/9/03	Receipt Condition: Good
Interim Results Date: 1/10/03	Shipping Container: Three 35 mL amber glass serum vials on refrigerant packs
SOP: CSCSPEC.II-015-00, "Standard Operating Procedure (SOP) for the Analysis of Vinclozolin Formulations in Corn Oil"	Storage Conditions (@ Battelle): Refrigerated (~5°C)

A full description of the formulation analysis method can be found in the report for NTP ChemTask No. CHEM07220, Battelle Study No. G004110-BPU. This method is based on work originally conducted in the DFD task for this formulation, NTP ChemTask No. CHEM05773, Battelle Study No. G004110-AWA.

The formulation analysis involves the preparation of a standard curve containing 6 standards from two independently prepared stock standards and the analysis of the standards and samples by gas chromatography with flame ionization detection. A linear regression equation was calculated relating the response ratios of the vinclozolin/internal standard to the concentration of the standards. The regression equation and the response ratios and dilution factors of the samples were used to calculate the concentration of the samples.

Representative chromatograms (overlaid) from the high and low standards and blanks are shown in Figure 1. The standard curve is shown in Figure 2.

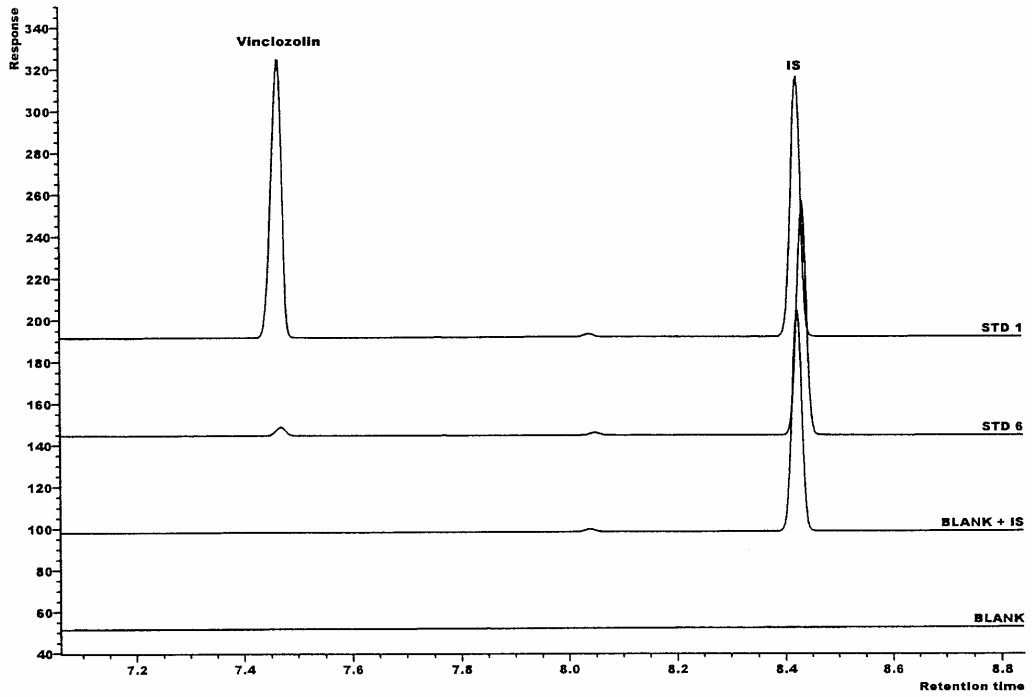

Figure 1 – Representative Overlaid Chromatograms from High Standard, Low Standard, Blank with IS, and Blank (shown top to bottom)

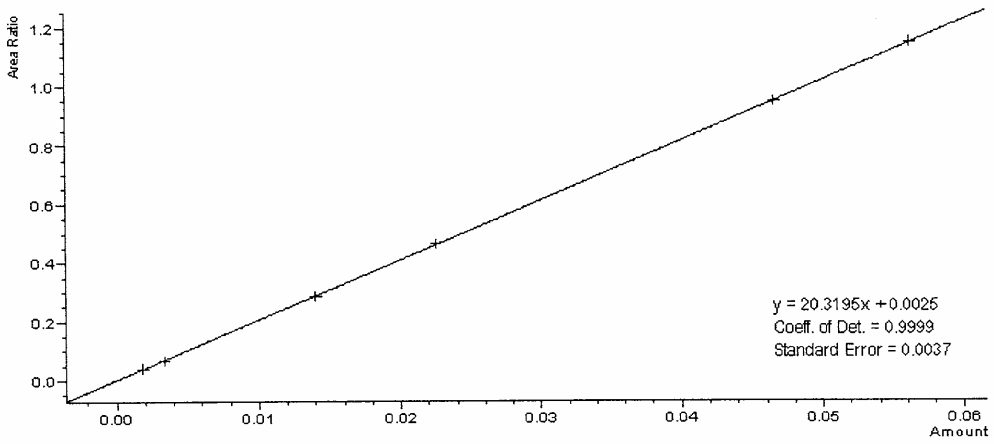

Figure 2 – Standard Curve

The 0 mg/mL formulation did not contain any detectable vinclozolin. The average concentrations of the 4, 12, and 40 mg/mL formulations were within 10% of target, which is within the normal acceptance limit. The results of the analysis are shown in Table 1.

Table 1 – Formulation Analysis Results

Group No	Mix No	Target Conc (mg/mL)	Det'd Conc (mg/mL)		Avg Det'd Conc (mg/mL)	Avg RE
NA	NA	0	BLOQ[a]	BLOQ	BLOQ	NA
5	2	4	3.832	3.883	3.858	-3.6
6	2	12	12.46	12.22	12.34	2.9
7	2	40	41.91	42.30	42.10	5.3

a. BLOQ = below limit of quantitation.

Kevin Carrico conducted the analytical work. Tim Cristy wrote the report. Padmini Fernando reviewed the raw data for completeness and accuracy.

QUALITY ASSURANCE STATEMENT

FORMULATION ANALYSIS REPORT

VINCLOZOLIN

NTP ChemTask No.: CHEM07221
Battelle Project No.: G004110-BPV
Battelle Task No.: 8-039-FA-198

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

Critical Phase Inspected	Date Inspected	Date Reported to Study Director and Management
Audit study file	3/11/03	3/11/03
Audit analytical report	3/11/03	3/11/03

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

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

Facility/Equipment	Date Inspected	Date of Report to Management
Chemistry Technical Center inspection	4/23/00	4/24/00
	12/8-12/12/00	12/13/00
	10/17/01	10/19/01
	9/6/02	9/6/02

3-18-03

Quality Assurance Unit Date

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Chemistry Support Services for the NTP
NIH Contract No.: N01-ES-05456
Battelle Project No.: G004110-BPW
NTP ChemTask No.: CHEM07222
CAS No.: 50471-44-8

FORMULATION ANALYSIS REPORT

VINCLOZOLIN

8-039-FA-199

March 12, 2003

Prepared By:

Wendy M. Black
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FORMULATION ANALYSIS REPORT

VINCLOZOLIN

CAS No.: 50471-44-8	Lot No.: 102996 (Battelle's Organic Synthesis Group, 99%)
Battelle Chemical ID Code: 039	Samples Received: 0, 4, 12 and 40 mg/mL formulations
Battelle Task No.: 8-039-FA-199	Sample Receipt Date: 1/9/03
NTP Task No.: CHEM07222	Submitter: Gary Holley
Program Supported: RDGT	Study Lab: TherImmune Research Corporation
Analysis Date: 1/20/03	Receipt Condition: Good
Interim Results Date: 1/21/03	Shipping Container: Four 60 mL amber glass serum bottles on ice packs
SOP: CSCSPEC.II-015-00, "Standard Operating Procedure (SOP) for the Analysis of Vinclozolin Formulations in Corn Oil"	Storage Conditions (@ Battelle): Refrigerated (~5°C)

A full description of the formulation analysis method can be found in the report for NTP ChemTask No. CHEM07220, Battelle Study No. G004110-BPU. This method is based on work originally conducted in the DFD task for this formulation, NTP ChemTask No. CHEM05773, Battelle Study No. G004110-AWA.

The formulation analysis involves the preparation of a standard curve containing 6 standards from two independently prepared stock standards and the analysis of the standards and samples by gas chromatography with flame ionization detection. A linear regression equation was calculated relating the response ratios of the vinclozolin/internal standard to the concentration of the standards. The regression equation and the response ratios and dilution factors of the samples were used to calculate the concentration of the samples.

Representative chromatograms (overlaid) from the high and low standards and blanks are shown in Figure 1. The standard curve is shown in Figure 2.

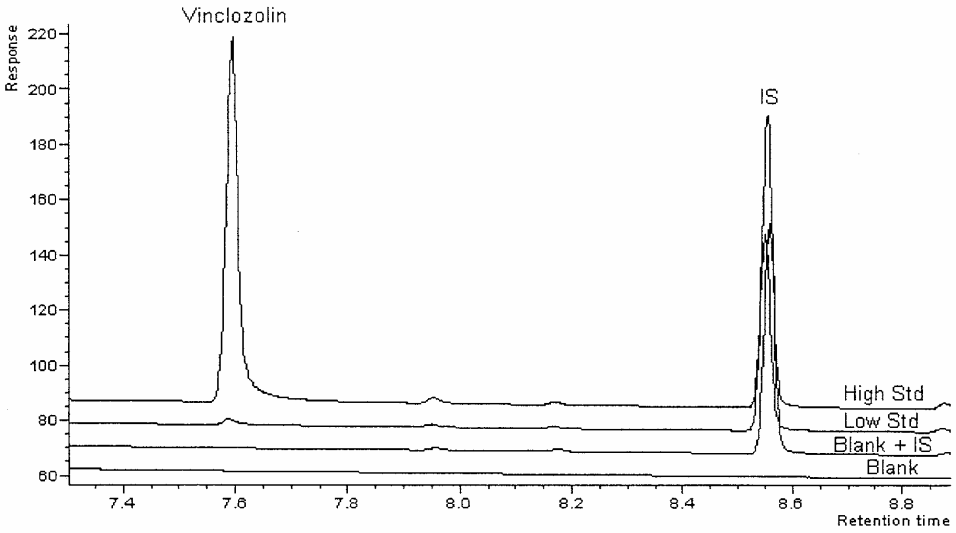

Figure 1 – Representative Overlaid Chromatograms from High and Low Standard, Blank with IS and Blank

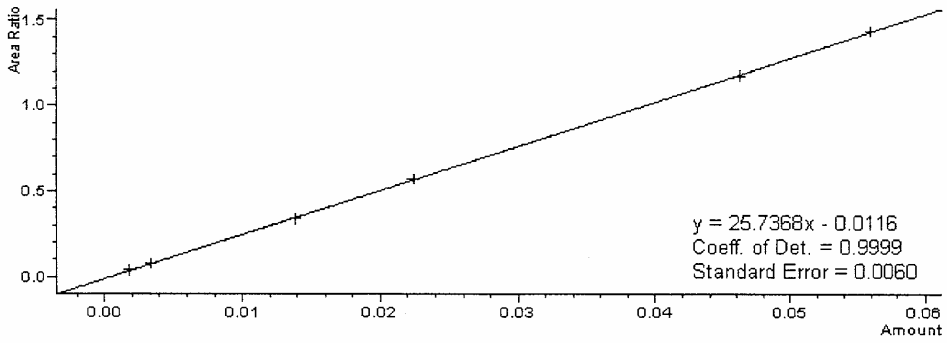

Figure 2 – Standard Curve

The 0 mg/mL formulation did not contain any detectable vinclozolin. The average concentrations of the 4, 12, and 40 mg/mL formulations were within 10% of target, the normal acceptance limit. The results of the analysis are shown in Table 1.

Table 1 – Formulation Analysis Results

Group No	Mix No	Target Conc (mg/mL)	Det'd Conc (mg/mL)		Avg Det'd Conc (mg/mL)	Avg RE
NA	NA	0	BLOQ[a]	BLOQ	BLOQ	NA
5	4	4	3.734	3.798	3.766	-5.9
6	4	12	11.38	11.50	11.44	-4.7
7	4	40	36.99	36.51	36.75	-8.1

a. BLOQ = below limit of quantitation.

David Chang conducted the analytical work. Wendy Black wrote the report. Padmini Fernando reviewed the raw data for completeness and accuracy.

QUALITY ASSURANCE STATEMENT

FORMULATION ANALYSIS REPORT

VINCLOZOLIN

NTP ChemTask No.: CHEM07222
Battelle Project No.: G004110-BPW
Battelle Task No.: 8-039-FA-199

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

Critical Phase Inspected	Date Inspected	Date Reported to Study Director and Management
Formulation analysis	1/16/03	1/17/03
Audit study file	3/6/03	3/6/03
Audit analytical report	3/6/03	3/6/03

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

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

Facility/Equipment	Date Inspected	Date of Report to Management
Chemistry Technical Center inspection	4/23/00	4/24/00
	12/8-12/12/00	12/13/00
	10/17/01	10/19/01
	9/6/02	9/6/02

3/11/03
Quality Assurance Unit Date

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Chemistry Support Services for the NTP
NIH Contract No.: N01-ES-05456
Battelle Project No.: G004110-BPR
NTP ChemTask No.: CHEM07217
CAS No.: 13311-84-7

COPY

FORMULATION ANALYSIS REPORT

FLUTAMIDE

8-139-FA-194

April 23, 2003

Approved By:

Wendy M. Black
Study Director

Approved By:

Steven W. Graves, B.S.
Principal Investigator

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7244-600
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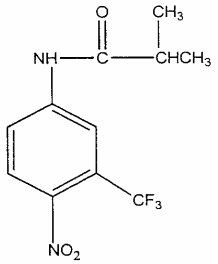
FORMULATION ANALYSIS REPORT

FLUTAMIDE

CAS No.: 13311-84-7	Lot No.: 109H0952 (Sigma, >99.0% by Thin Layer Chromatography)
Battelle Chemical ID Code: 139	Samples Received: 10 mg/L and 20 mg/L formulations (4 vials each of Groups 8 and 9 of Mix 1), Corn Oil
Battelle Task No.: 8-139-FA-194	Sample Receipt Date: 12/5/02
NTP Task No.: CHEM07217	Submitter: Gary Holley
Program Supported: RDGT	Study Lab: TherImmune Reasearch Corporation
Analysis Dates: 1/8-1/9/03	Receipt Condition: Good
Interim Results Date: 1/10/03	Shipping Container: Eight 60-mL amber glass serum vials on refrigerant packs and 1 x 35 mL of corn oil
SOP: CSCSPEC.II-014-00, "Standard Operating Procedure (SOP) for the Analysis of Flutamide Formulations in Corn Oil"	Storage Conditions (@ Battelle): Refrigerated (~5°C)

STRUCTURE	Mol. Wt.	Mol. Formula
	276.2 g/mol	$C_{11}H_{11}F_3N_2O_3$

EXECUTIVE SUMMARY

Formulations of flutamide in corn oil at target concentrations of 0, 10, and 20 mg/mL were received from TherImmune and analyzed in support of an RDGT study.

The 0 mg/mL formulation contained no detectable flutamide and the average concentrations of the 10 and 20 mg/mL formulations were within 10% of target, the NTP acceptance limit. The formulations were homogenous.

QUALITY ASSURANCE STATEMENT

FORMULATION ANALYSIS REPORT

FLUTAMIDE

NTP ChemTask No.: CHEM07217
Battelle Project No.: G004110-BPR
Battelle Task No.: 8-139-FA-194

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Audit analytical report	3/13/03	3/13/03

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	9/6/02	9/6/02

4/22/03
Quality Assurance Unit Date

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1 INTRODUCTION

This report contains:

- a description of the analysis of formulations of flutamide in corn oil,
- the results of the analysis, and
- conclusions.

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

2 TEST ARTICLE AND FORMULATION SAMPLES

Samples of corn oil formulations containing flutamide as well as blank corn oil were received from TherImmune on December 5, 2002. They were identified as being from Study # 7244-600, Mix 1, and had the following group numbers and target concentrations: Group 8/10 mg/mL and Group 9/20 mg/mL. Four samples from each group [one verification sample and three homogeneity samples (top, middle, and bottom)] were received.

All formulations were prepared using flutamide, Lot No. 109H0952, according to information provided with the shipment. The identity of this lot was confirmed and its purity determined previously under NTP ChemTask No. CHEM04550, Battelle Study No. G004110-AXC. A sample of the same lot was used as the analytical standard for this work.

3 FORMULATION ANALYSIS

CSCSPEC.II-014-00, "Standard Operating Procedure (SOP) for the Analysis of Flutamide Formulations in Corn Oil." The methodology in this SOP is based on work originally conducted in the DFD task for this formulation, NTP ChemTask No. CHEM05893, Battelle Study No. G004110-AXG. This section describes the methodology, the results, and our conclusions. This methodology contains two differences from the SOP: (1) the vehicle standards and blanks were mixed and sonicated the same as the samples, and (2) the samples, standards, and blanks were centrifuged to clarify them prior to analysis.

3.1 Preparation of Standards

3.1.1 Solvent Stocks

Two solvent stock standards (A and B) were prepared at target concentrations of 5 and 3 mg/mL, respectively, by dissolving approximately 250 and 150 mg of accurately weighed flutamide in 50 mL of acetone.

3.1.2 Intermediate Standards

Four additional intermediate standards (the stocks were used as the two highest concentration intermediate standards) were prepared by pipetting the volumes of Stocks A and B into volumetric flasks and diluting to volume with acetone as described in Table 1.

Table 1 – Intermediate Standards

Intermediate Standard	Nominal Conc (µg/mL)	Source Stock	Source Volume (mL)	Final Volume (mL)
1	5000	A	NA	NA
2	3000	B	NA	NA
3	2000	A	10	25
4	1200	B	10	25
5	1000	A	10	50
6	600	B	10	50

3.1.3 Vehicle/Calibration Standards

Vehicle/calibration standards were prepared by pipetting 5 mL of each intermediate standard, 1 mL of vehicle (corn oil), and 1 mL of internal standard (IS) solution (400 µL of acetophenone dissolved in 100 mL of HPLC mobile phase) into individual 100-mL volumetric flasks. They were then diluted to volume with HPLC mobile phase, sealed and mixed (vortexed for 1 minute, sonicated for 30 minutes, vortexed for an additional 1 minute, and shaken well). Single vehicle/calibration standards were prepared from each intermediate standard. This produced vehicle/calibration standards with target concentrations of 50, 30, 20, 12, 10, and 6 µg/mL.

3.1.4 Blanks

A single blank with internal standard (IS) was prepared by combining 5 mL of acetone, 1 mL of corn oil and 1 mL of IS in a 100-mL volumetric flask. A single blank without internal standard was prepared by combining 5 mL of acetone and 1 mL of corn oil in a 100-mL volumetric flask. They were then diluted to volume with HPLC mobile phase, sealed and mixed (vortexed for 1 minute, sonicated for 30 minutes, vortexed for an additional 1 minute, and shaken well).

3.2 Preparation of Formulation Samples for Analysis

For the verification samples, duplicate aliquots from each formulation were prepared for analysis. Triplicate aliquots from each homogeneity sample were prepared for analysis. Each formulation sample was prepared by pipetting 1 mL of formulation, 5 mL of acetone, and 1 mL of IS into a 100-mL volumetric flask. They were then diluted to volume with HPLC mobile phase, sealed and mixed (vortexed for 1 minute, sonicated for 30 minutes, vortexed for an additional 1 minute, and shaken well).

3.3 Analysis

A portion of each formulation sample, standard, and blank was transferred to individual microcentrifuge tubes. They were centrifuged at a setting of approximately 14,000 rpm to clarify them. The supernatants were transferred to individual autosampler vials and the vials were sealed.

Single injections were made from each vial using the HPLC system shown in Table 2. Typical overlaid chromatograms from a blank, a blank with internal standard, and low and high vehicle standards are shown in Figure 1.

Table 2 – HPLC System

Instrument	Agilent 1100 (Palo Alto, CA)
Column	Phenomenex Ultramex C-18, 250 x 4.6 mm (ID), 5 µm (Torrance, CA)
Mobile Phase	Water:Acetonitrile (35:65), Isocratic
Flow Rate	0.7 mL/minute
Detector Type and Wavelength	Ultraviolet at 240 nm
Injection Volume	10 µL
Run Time	20 minutes
Retention Times	
Flutamide	~7.6 minutes
IS	~5.7 minutes

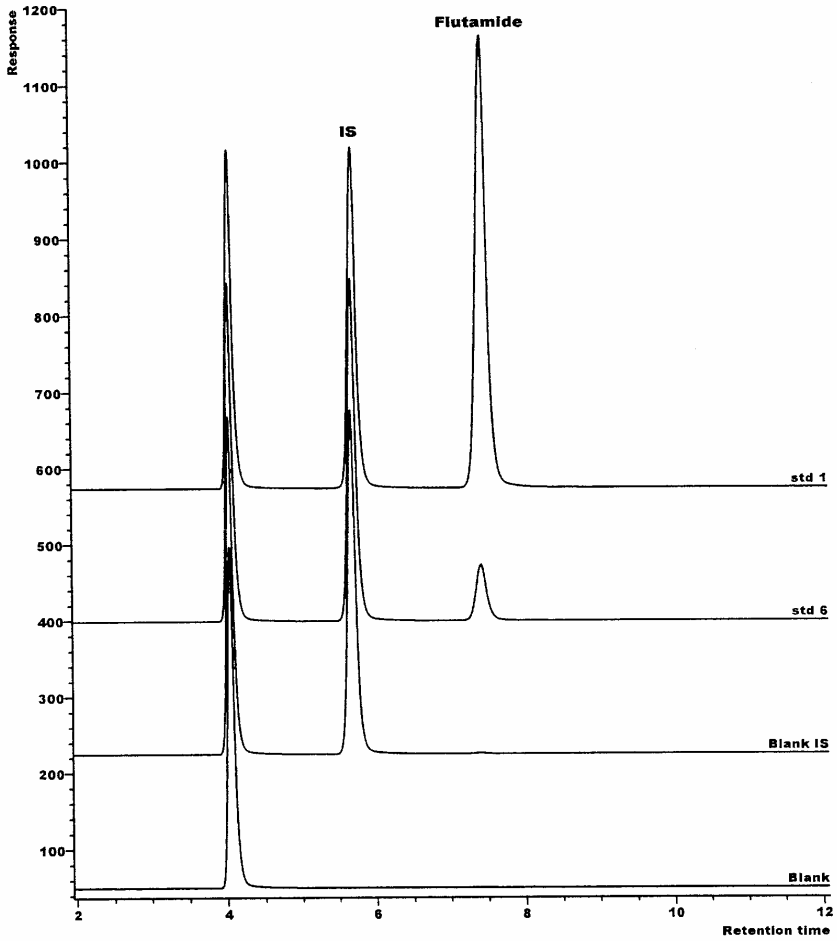

Figure 1 – Representative Overlaid Chromatograms of a Blank, Blank plus IS, Low Standard, and High Standard

3.4 Calculations

The integration of the flutamide and internal standard (IS) peaks as done by the chromatography data system was evaluated and manually adjusted if necessary, to achieve consistent integration. The response ratio of the flutamide peak area divided by the IS peak area was calculated. A linear regression equation was calculated relating the response ratio of the vehicle standards to their nominal concentrations. A determined concentration was

calculated for each standard using the regression equation and the response ratio for that standard. The relative error (RE) for each standard was calculated by subtracting the target concentration from its determined concentration, dividing the difference by the target concentration, and multiplying the result by 100. The concentration of each sample was calculated using its response ratio, the regression equation, and any dilution factor.

3.5 Results

The results from the analysis of the formulations are shown in Tables 3 and 4. The standard curve and the results of the regression analysis of the standards are shown in Figure 2.

Table 3 – Formulation Analysis Results

Group No	Mix No	Target Conc (mg/mL)	Det'd Conc (mg/mL)		Avg Det'd Conc (mg/mL)	Avg RE
NA	NA	0	BLOQ[a]	BLOQ	BLOQ	BLOQ
8	1	10	10.49	10.48	10.49	4.9
9	1	20	20.86	20.42	20.64	3.2

a. BLOQ = Below Limit of Quantitation.

Table 4 – Formulation Homogeneity Analysis Results

Group No	Mix No	Target Conc (mg/mL)	Sampling Location	Determined Conc (mg/mL)	Average Determined Conc (mg/mL)	% RSD	Grand Avg Determined Conc (mg/mL)	Grand % RSD	Grand % RE
8	1	10	Top	10.22	10.11	1.1	10.18	0.8	1.8
				10.11					
				9.995					
			Middle	10.39	10.26	1.3			
				10.13					
				10.27					
			Bottom	10.19	10.17	0.2			
				10.15					
				10.18					
9	1	20	Top	22.56	21.46	5.0	20.73	3.2	3.7
				20.42					
				21.41					
			Middle	20.17	20.16	0.05			
				20.15					
				20.17					
			Bottom	20.50	20.57	0.39			
				20.66					
				20.56					

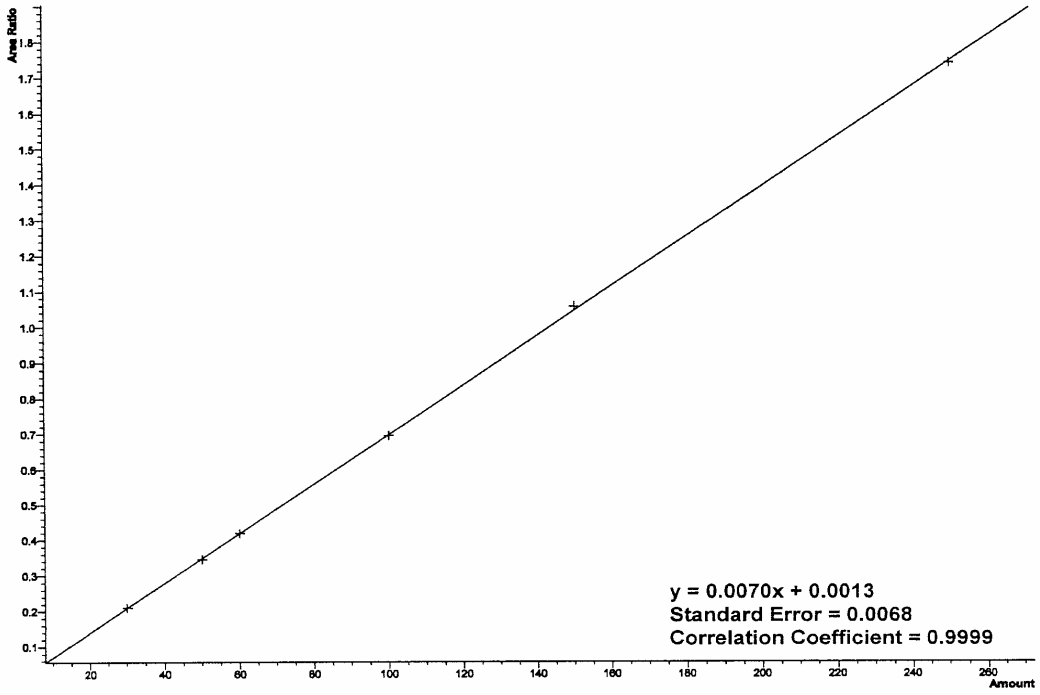

Figure 2 – Standard Curve

3.6 Conclusions

The 0 mg/mL formulation contained no detectable flutamide and the average concentrations of the 10 and 20 mg/mL formulations were within 10% of target, the NTP acceptance limit. The formulations were homogenous.

4 ACKNOWLEDGMENTS

Darren Brown conducted the analytical work. Tim Cristy wrote the report. Patricia Athey reviewed the raw data for completeness and accuracy.

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Chemistry Support Services for the NTP
NIH Contract No.: N01-ES-05456
Battelle Project No.: G004110-BPS
NTP ChemTask No.: CHEM07218
CAS No.: 13311-84-7

COPY

FORMULATION ANALYSIS REPORT

FLUTAMIDE

8-139-FA-195

April 23, 2003

Approved By:

Wendy M. Black
Study Director

Approved By:

Steven W. Graves, B.S.
Principal Investigator

Submitted to:

Dr. Cynthia S. Smith
National Institute of Environmental Health Sciences
Mail Drop: EC-06
4401 Commons Building, Suite 100
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FORMULATION ANALYSIS REPORT

FLUTAMIDE

CAS No.: 13311-84-7	Lot No.: 109H0952 (Sigma, >99.0% by Thin Layer Chromatography)
Battelle Chemical ID Code: 139	Samples Received: 10 mg/L and 20 mg/L formulations (1 vial each of Groups 8 and 9 of Mix 2)
Battelle Task No.: 8-139-FA-195	Sample Receipt Date: 12/17/02
NTP Task No.: CHEM07218	Submitter: Gary Holley
Program Supported: RDGT	Study Lab: TherImmune Reasearch Corporation
Analysis Dates: 1/8-1/9/03	Receipt Condition: Good
Interim Results Date: 1/10/03	Shipping Container: Two 35 mL amber glass serum vials on frozen packs
SOP: CSCSPEC.II-014-00, "Standard Operating Procedure (SOP) for the Analysis of Flutamide Formulations in Corn Oil"	Storage Conditions (@ Battelle): Refrigerated (~2-8°C)

A full description of the formulation analysis method can be found in the report for NTP ChemTask No. CHEM07217, Battelle Study No. G004110-BPR. The methodology in this SOP is based on work originally conducted in the DFD task for this formulation, NTP ChemTask No. CHEM05893, Battelle Study No. G004110-AXG.

The formulation analysis involves the preparation of a standard curve containing 6 standards from two independently prepared stock standards and the analysis of the standards and samples by high performance liquid chromatography with ultraviolet detection. A linear regression equation was calculated relating the response ratios of the flutamide/internal standard to the concentration of the standards. The regression equation and the response ratios and dilution factors of the samples were used to calculate the concentration of the samples.

Representative overlaid chromatograms from the high and low standards and blanks are shown in Figure 1. The standard curve is shown in Figure 2.

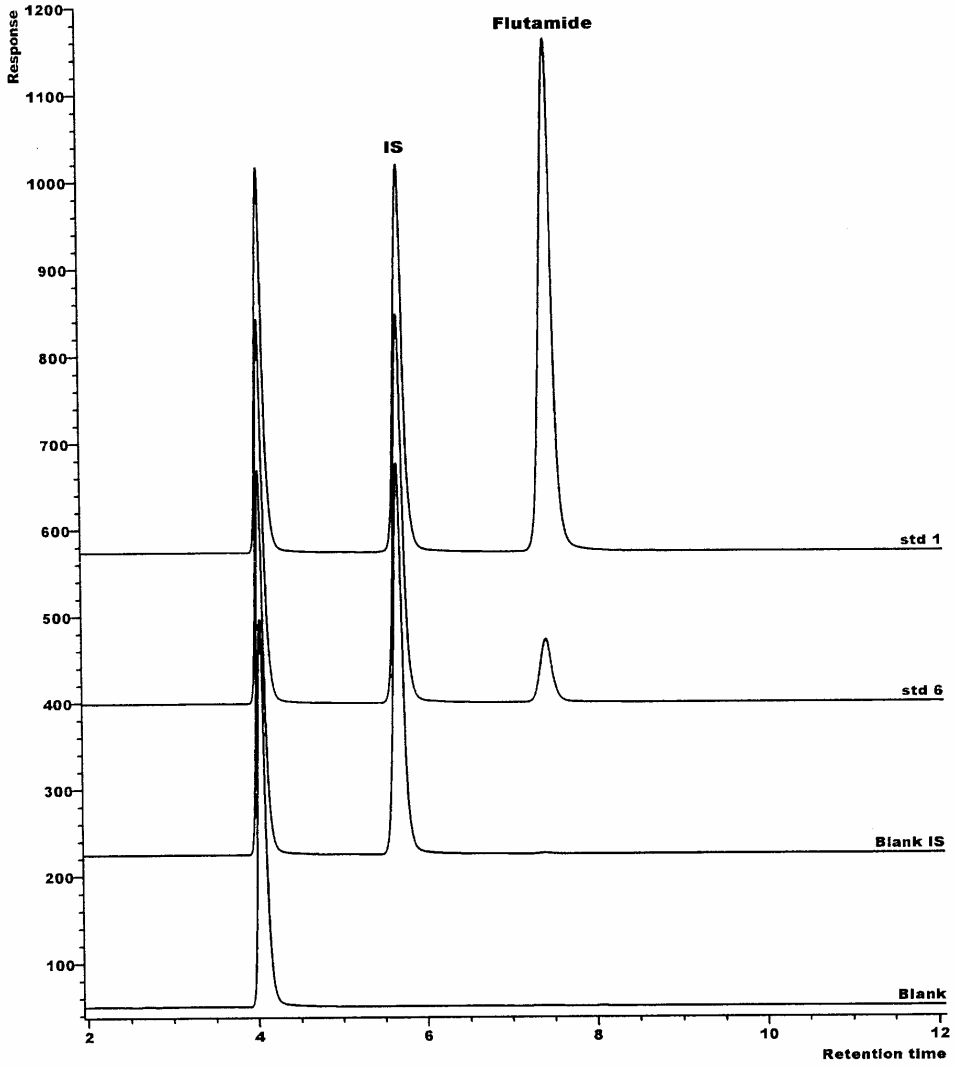

Figure 1 – Representative Overlaid Chromatograms of a Blank, Blank plus IS, Low Standard, and High Standard

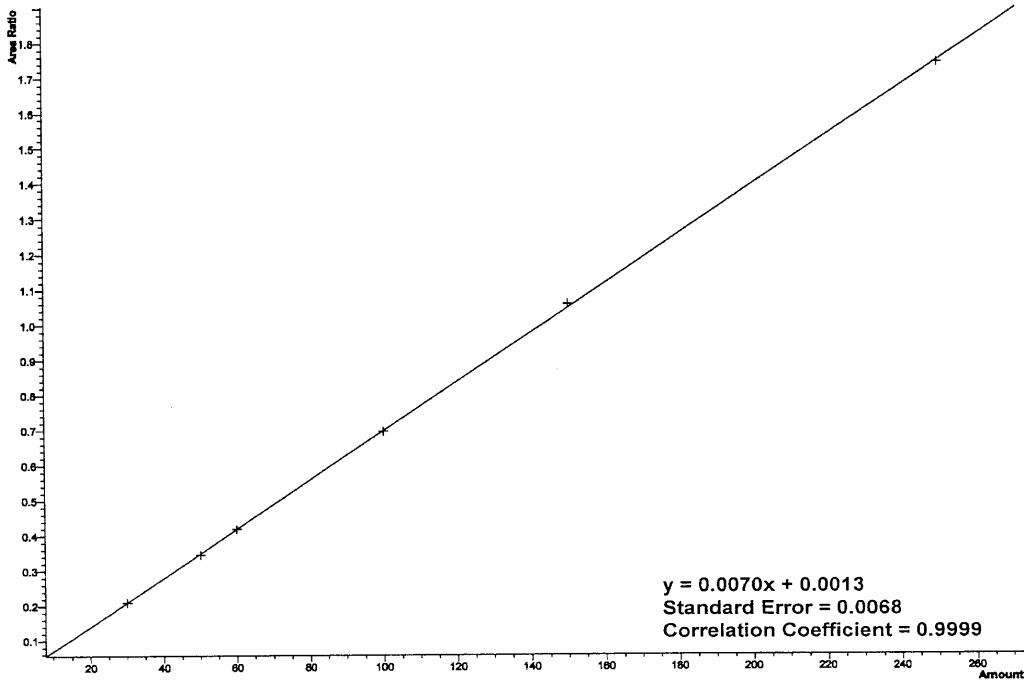

Figure 2 – Standard Curve

The concentrations of the 10 and 20 mg/mL formulations were within the normal acceptance limit of ± 10% of target. The results of the analysis are shown in Table 1.

Table 1 – Formulation Analysis Results

Group No	Mix No	Target Conc (mg/mL)	Det'd Conc (mg/mL)		Avg Det'd Conc (%)	Avg RE
8	2	10	10.11	10.22	10.16	1.6
9	2	20	20.93	21.10	21.02	5.1

a. BLOQ = below limit of quantitation.

Darren Brown conducted the analytical work. Tim Cristy wrote the report. Patricia Athey reviewed the raw data for completeness and accuracy.

QUALITY ASSURANCE STATEMENT

FORMULATION ANALYSIS REPORT

FLUTAMIDE

NTP ChemTask No.: CHEM07218
Battelle Project No.: G004110-BPS
Battelle Task No.: 8-139-FA-195

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

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4/23/03
Quality Assurance Unit Date

Battelle

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Chemistry Support Services for the NTP
NIH Contract No.: N01-ES-05456
Battelle Project No.: G004110-BPT
NTP ChemTask No.: CHEM07219
CAS No.: 13311-84-7

COPY

FORMULATION ANALYSIS REPORT

FLUTAMIDE

8-139-FA-196

April 23, 2003

Prepared By:

Wendy M. Black
Study Director

Approved By:

Steven W. Graves, B.S.
Principal Investigator

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FORMULATION ANALYSIS REPORT

FLUTAMIDE

CAS No.: 13311-84-7	Lot No.: 109H0952 (Sigma, >99.0% by Thin Layer Chromatography)
Battelle Chemical ID Code: 139	Samples Received: 0 mg/mL, 10 mg/mL (Group 8) and 20 mg/mL (Group 9) formulations of Mix 4
Battelle Task No.: 8-139-FA-196	Sample Receipt Date: 1/09/03
NTP Task No.: CHEM07219	Submitter: Gary Holley
Program Supported: RDGT	Study Lab: TherImmune Research Corporation
Analysis Dates: 1/14-1/15/03	Receipt Condition: Good
Interim Results Date: 1/16/03	Shipping Container: Three 60 mL amber glass serum vials on ice packs
SOP: CSCSPEC.II-014-00, "Standard Operating Procedure (SOP) for the Analysis of Flutamide Formulations in Corn Oil"	Storage Conditions (@ Battelle): Refrigerated (~2-8°C)

A full description of the formulation analysis method can be found in the report for NTP ChemTask No. CHEM07217, Battelle Study No. G004110-BPR. The methodology in this SOP is based on work originally conducted in the DFD task for this formulation, NTP ChemTask No. CHEM05893, Battelle Study No. G004110-AXG.

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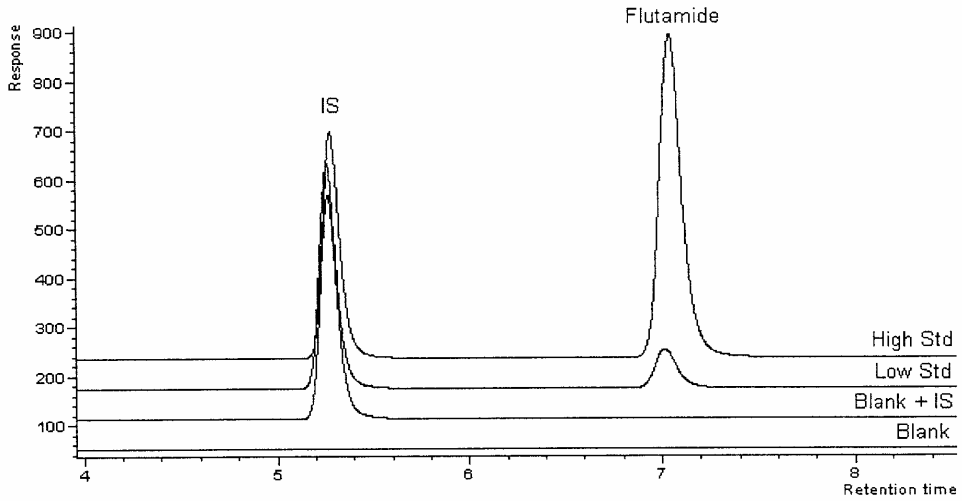

Figure 1 – Representative Overlaid Chromatograms from High and Low Standards, Blank with IS, and Blank

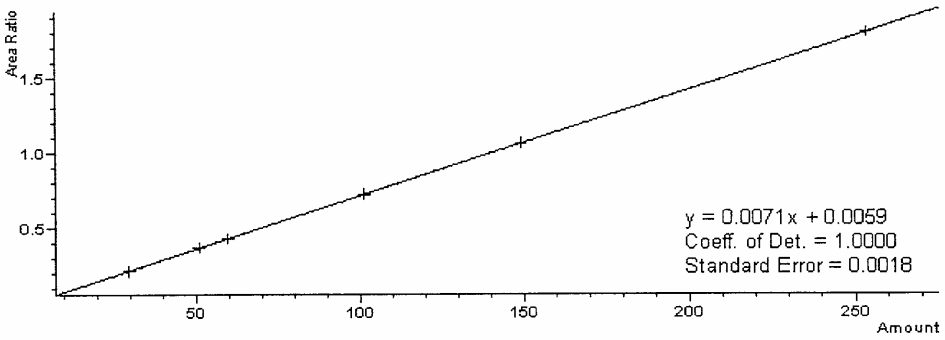

Figure 2 – Standard Curve

The 0 mg/mL formulation did not appear to contain any flutamide. The concentrations of the 10 and 20 mg/mL formulations were within 10% of target, which is the normal acceptance limit. The results of the analysis are shown in Table 1.

Table 1 – Formulation Analysis Results

Group Number	Mix No	Mix Date	Target Conc (mg/mL)	Det'd Conc (mg/mL)		Avg Det'd Conc (mg/mL)	Avg RE
NA	NA	NA	0	BLOQ[a]	BLOQ	BLOQ	BLOQ
8	4	1/3/03	10	9.817	10.68	10.25	2.5
9	4	1/3/03	20	19.96	21.52	20.74	3.7

a. BLOQ = below limit of quantitation.

David Chang conducted the analysis. Wendy Black wrote the report. Patricia Athey reviewed the raw data for completeness and accuracy.

QUALITY ASSURANCE STATEMENT

FORMULATION ANALYSIS REPORT

FLUTAMIDE

NTP ChemTask No.: CHEM07219
Battelle Project No.: G004110-BPT
Battelle Task No.: 8-139-FA-196

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	9/6/02	9/6/02

4/22/03
Quality Assurance Unit Date

Battelle

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Formulation Analysis of Ethinyl Estradiol in Corn Oil— Formulation Date: December 3, 2002

NIEHS Contract No. N01-ES-05457
ETP Task No. CHEM07194
MRI Project No. 110100
MRI Task No. 593

Midwest Research Institute
425 Volker Boulevard
Kansas City, Missouri 64110

January 29, 2003

7244-600
Rocca
Wolfe
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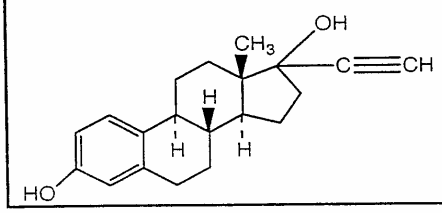
Formulation Analysis (FA)

Chemical Information: Ethinyl Estradiol

CAS No.: 57-63-6 MRI Task No.: 593 ETP Task No.: CHEM07194 Program Supported: RACB Analysis Dates: December 10, 2002, to December 12, 2002 Interim Report Date: December 13, 2002	Lot No.: 45H0716 (Sigma Chemical Co., 98.8% per BCR at MRI) Samples Received: Dose verification samples at 0.001 and 0.002 mg/mL of ethinyl estradiol in corn oil; homogeneity samples at 0.001 and 0.002 mg/mL (top, middle, and bottom at each concentration); and neat ethinyl estradiol Sample Receipt Date: December 5, 2002 (dose verification samples, homogeneity samples, and neat chemical) Submitter: TherImmune Research Corporation Study Lab: TherImmune Research Corporation Formulation Date: December 3, 2002 Receipt Condition: Intact, Ice packs Storage Conditions (@ MRI): Dose verification samples and homogeneity samples, refrigerated (~ 5°C) and neat test article, frozen (~ –20°C)
Structure  	Molecular Weight: 296.4 Molecular Formula: $C_{20}H_{24}O_2$

Quality Assurance Statement

Formulation Analysis of Ethinyl Estradiol in Corn Oil—Formulation Date December 3, 2002

ETP Task No. CHEM07194
MRI Project No. 110100
MRI Task No. 593

This study was inspected by the Quality Assurance Unit of MRI (QAU) and the findings reported to the Study Director and Management as follows:

Phase inspected	Date inspected	Date reported
Protocol Audit	12/10/02	12/12/02
Protocol Amendment No. 1 Audit	1/23/03	1/24/03
In-Life Audit; formulation analysis	12/10/02	12/12/02
Data Audit	1/23/03	1/24/03
Report Audit	1/23/03	1/24/03

The raw data and report will be stored in the MRI Archives.

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

Facility/equipment	Date inspected	Date reported
HPLC facility	11/18/02	11/19/02
PCA laboratory complex	9/13/02	9/16/02

MIDWEST RESEARCH INSTITUTE

Rosemary Moran

Rosemary Moran
Quality Assurance Officer

Approved:

James Dworak, Ph.D.
Manager, Quality Assurance

January 29, 2003

Good Laboratory Practice Compliance Statement

Formulation Analysis of Ethinyl Estradiol in Corn Oil—Formulation Date: December 3, 2002

ETP Task No. CHEM07194
MRI Project No. 110100
MRI Task No. 593

This formulation analysis study (MRI Task No. 593) of ethinyl estradiol in corn oil was conducted in compliance with the Good Laboratory Practice Regulations of the U.S. Food and Drug Administration (21 *CFR* 58).

Amir Kazerooni
Study Director

1/29/03
Date

Executive Summary

The purpose of this study was to perform a formulation analysis of ethinyl estradiol in corn oil received from TherImmune Research Corporation. Samples included dose verification samples (one vial each) at concentrations of 0.001 and 0.002 mg/mL, and control (0 mg/mL), and homogeneity samples (top, middle, and bottom) for formulations prepared at target concentrations 0.001 and 0.002 mg/mL ethinyl estradiol in corn oil. The concentrations of the dose formulations were determined with TherImmune-supplied test article as an analytical standard.

The average concentrations of the dose verification samples were determined to be 0.1985 and 0.9260 µg/mL ethinyl estradiol in corn oil. All of the dose verification samples were not within the respective target concentrations. The 0.001 mg/mL formulation was 19.9% of target concentration. The 0.002 mg/mL formulation was 46.3% of target concentration.

The 0.001 mg/mL and 0.002 mg/mL ethinyl estradiol in corn oil was evaluated for homogeneity. The results indicated that neither formulation was homogeneous as the % RSDs were 22.3 and 28.5, respectively. The mean (n = 6) concentration of the 0.001 mg/mL and 0.002 mg/mL formulations was 0.1969 µg/mL and 0.7235 µg/mL, which were 19.7% and 36.2%, respectively, of the target concentration.

All the determined concentrations for the 0.001 mg/mL formulations were below the lowest point of the calibration curve; therefore, the values were estimated.

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Formulation Analysis of Ethinyl Estradiol in Corn Oil— Formulation Date: December 3, 2002

1. Introduction

The purpose of this study was to perform a formulation analysis of ethinyl estradiol in corn oil received from TherImmune Research Corporation. Samples included dose verification samples (one vial each) at concentration of 0.001 and 0.002 mg/mL, and control (0 mg/mL), and homogeneity samples (top, middle, and bottom) for formulations prepared at target concentrations 0.001 and 0.002 mg/mL ethinyl estradiol in corn oil. The concentrations of the dose formulations were determined with TherImmune-supplied test article as an analytical standard. This study was initiated on December 5, 2002.

The formulation analysis was based on the validated method as described in MRI amended report for "Dose Formulation Development Study for Ethinyl Estradiol in Corn Oil," NIEHS Contract No. N01-ES-05457, ETP Task No. CHEM05958, MRI Project No. 110100, MRI Task No. 197, amended report issued on January 14, 2002.

2. Chemical and Sample Information

Test Article:	Ethinyl Estradiol
Lot No.:	45H0716
Supplier:	Sigma Chemical Company
Molecular Formula:	$C_{20}H_{24}O_2$
Molecular Weight:	296.4
CAS No.:	57-63-6

The formulations submitted for formulation analysis from TherImmune Research Corporation were received at MRI on December 5, 2002, in amber glass injection vials and are identified in Table 1 using TherImmune Research Corporation designations. Neat ethinyl estradiol was also received from TherImmune Research Corporation on December 5, 2002. The formulation samples were stored under refrigerated (~°5°C) conditions and neat test article was stored under frozen (~ –20°C) conditions prior to analysis.

Table 1. Formulations From TherImmune Research Corporation—Formulation Date: December 3, 2002

Sample Identification	Concentration (mg/mL)	Volume (~ mL)
Dose Verification Samples		
Group No. 2, Mix No. 1	0.001	35
Group No. 3, Mix No. 1	0.002	35
Homogeneity Samples		
Group No. 2, Mix No. 1, Top	0.001	35
Group No. 2, Mix No. 1, Middle	0.001	35
Group No. 2, Mix No. 1, Bottom	0.001	35
Group No. 3, Mix No. 1, Top	0.002	35
Group No. 3, Mix No. 1, Middle	0.002	35
Group No. 3, Mix No. 1, Bottom	0.002	35

3. Materials and Equipment

HPLC system equipped with a Waters Alliance 2695 Separations Module, a Waters 474 scanning fluorescence detector, and Turbochrom data system, version 6.1.1
Column, Inertsil ODS-2, 5 µm, 250 x 2.0 mm ID (Metachem)
Guard column, Inertsil ODS-2, 5 µm, 25 x 2.0 mm ID (Metachem)
2-Phenylphenol, > 99% purity, Aldrich, used as internal standard
Acetonitrile (ACN), Burdick and Jackson, Inc., High Purity
ASTM Type I reagent grade water, 18.2 MΩ-cm, from in-house purification system
Sonicator, Branson, model 5510
Vortex mixer, SP, speed control on 10
Centrifuge, Damon IEC Division CU-5000
Syringes, 5-mL disposable
Vials, 2-mL amber autosampler vials
Filters, Gelman, Acrodisc, 0.2-µm, Nylon, syringe type
Centrifuge tubes, 15-mL (plastic)
Vehicle, Corn Oil, Spectrum Quality Products, Inc.
Glacial acetic acid, Mallinckrodt, 99.5% purity
Volumetric glassware, Class A, as needed

4. Formulation Analysis

A formulation analysis was performed to determine the concentration of ethinyl estradiol in corn oil formulations from TherImmune Research Corporation. The concentrations of the formulations were determined using neat ethinyl estradiol standard supplied by TherImmune Research Corporation. All formulations were analyzed in duplicate and quantitation was performed using a six-point spiked vehicle standard curve.

4.1 Preparation of Extraction Solution

A 200-µL aliquot of glacial acetic acid was transferred into a 200-mL volumetric flask, diluted to volume with acetonitrile, and mixed by inversion.

4.2 Determination of Corn Oil Density

The density of corn oil was determined by transferring triplicate aliquots of corn oil into individual 5-mL volumetric flasks and obtaining the weight. The density and average density were calculated using commonly accepted techniques.

4.3 Preparation of Standard Solutions

4.3.1 Internal Standard Solution

An internal standard (IS) stock solution was prepared by accurately weighing ~ 65 mg of 2-phenylphenol into a 50-mL volumetric flask, diluting the contents to volume with acetonitrile, and mixing by inversion. The IS solution was prepared by transferring 1 mL of IS stock solution into a 100-mL volumetric flask, diluting to volume with acetonitrile, and mixing by inversion.

4.3.2 Stock Solutions

Two stock solutions of ethinyl estradiol were prepared by accurately weighing and transferring 64.840 mg (Stock B) and 59.960 mg (Stock A) into 200-mL and 100-mL volumetric flasks, respectively, diluting the contents to volume with acetonitrile, and mixing by inversion. The expected concentrations: Stock A, 599.60 µg/mL; Stock B, 324.20 µg/mL.

4.3.3 Intermediate Solutions

Six intermediate solutions (1 to 6) were prepared by transferring alternate aliquots from stock solution A or B by volumetric pipette into individual 100-mL volumetric flasks, diluting to volume with acetonitrile, and mixing by inversion (see Table 2).

Table 2. Preparation of Intermediate Solutions

Intermediate solution	Aliquot of stock or B (mL)	Final volume (mL)	Intermediate concentration (µg/mL)
1	0.5, B	100	1.6210
2	0.5, A	100	2.9980
3	2, B	100	6.4840
4	2, A	100	11.992
5	6, B	100	19.452
6	4, A	100	23.984

[a] Final dilution contained 1-mL IS solution.

4.3.4 Spiked Vehicle Standards

Six spiked vehicle standards (SVS1 to SVS6) were prepared by transferring a 1-mL aliquot of each intermediate solution into individual 15-mL centrifuge tubes containing ~ 2 g of undosed corn oil. Each centrifuge tube was capped and vortexed for ~ 1 min. An aliquot (7 mL) of extraction solution was added to each tube, and the extraction procedure listed in Table 3 was followed.

NOTE: Due to lack of availability of recently purchased corn oil, expired corn oil was used for the preparation of the spiked vehicle standards. However, analysis of this corn oil (Dose vehicle analysis, MRI Task No. 595)[1] determined that the oil was below (2.44 meq/Kg) the ETP criteria for maximum peroxide content (< 3.0 meq/Kg). Inspection of the chromatography indicated that the corn oil contributed no interferences to the analysis, and the use of this corn oil did not affect the integrity of the study.

Table 3. Extraction Procedure

1.	Vortex for 1 min
2.	Sonicate at 45°C for 2 min
3.	Vortex for 30 seconds
4.	Sonicate at 45°C for 2 min
5.	Vortex for 30 sec
6.	Sonicate at 45°C for 2 min
7.	Vortex for 1 min
8.	Centrifuge for 5 min @ 2200 rpm

A 4-mL aliquot of each clarified extract was transferred into individual 10-mL volumetric flasks containing 1 mL of IS solution, diluted to volume with water, and mixed by inversion (see Table 4).

[1] MRI Report, "Dose Vehicle Analysis of Corn Oil," NIEHS Contract No. N01-ES-05457, ETP Task No. CHEM07197, MRI Project No. 110100, MRI Task No. 595, to be submitted.

Table 4. Preparation of Spiked Vehicle Standards

Spiked vehicle standard	Intermediate solution	Corn oil aliquot (~ g)	Intermediate solution (mL) + extraction solvent (mL)	Extract dilution in water [a] (mL)	Expected conc. in corn oil (~ µg/mL)	Expected analytical conc. in diluent (µg/mL)
SVS1	1	2	1 + 7	4/10	0.74	0.081050
SVS2	2	2	1 + 7	4/10	1.4	0.14990
SVS3	3	2	1 + 7	4/10	3.0	0.32420
SVS4	4	2	1 + 7	4/10	5.6	0.59960
SVS5	5	2	1 + 7	4/10	9.0	0.97260
SVS6	6	2	1 + 7	4/10	11.2	1.1992

[a] Final dilution contained 1-mL IS solution.

4.3.5 Blanks

4.3.5.1 IS Blank

An IS blank was prepared by pipetting 1 mL of IS solution into a 10-mL volumetric flask containing 4 mL of acetonitrile, diluting the contents to volume with water, and mixing by inversion.

4.3.5.2 Undosed Vehicle Blank

An undosed vehicle blank was prepared by transferring a 1-mL aliquot of acetonitrile into a 15-mL centrifuge tube containing ~ 2 g of undosed corn oil. The centrifuge tube was capped and vortexed for ~ 1 min. An aliquot (7 mL) of extraction solution was added to the tube and the extraction procedure listed in Section 4.3.4, Table 3, was followed. After the extraction procedure, a 4-mL aliquot of the clarified extract was transferred into a 10-mL volumetric flask containing 1 mL of IS solution, diluted to volume with water, and mixed by inversion.

4.4 Determination of Formulation Density

The density of each formulation was determined by transferring triplicate aliquots of formulation into individual 5-mL volumetric flasks and obtaining the weight. The density and average density were calculated using commonly accepted techniques.

4.5 Preparation of Formulation Samples

The validated formulation range for the method of analysis of ethinyl estradiol in corn oil was from 0.69 to 11.2 µg/mL. Each formulation was prepared in duplicate by transferring a 1-mL aliquot of acetonitrile into individual 15-mL centrifuge tubes containing ~ 2 g of formulation. Each centrifuge tube was capped and vortexed for

~ 1 min. An aliquot (7 mL) of extraction solution was added to each tube and the extraction procedure listed in Section 4.3.4, Table 3, was followed. After the extraction procedure, a 4-mL aliquot of each clarified extract was transferred into individual 10-mL volumetric flasks containing 1 mL of IS solution, diluted to volume with water, and mixed by inversion (see Table 5).

Table 5. Preparation of Formulation Samples

Formulation	Formulation aliquot (~ g)	Acetonitrile (mL) + extraction solvent (mL)	Extract dilution in water [a] (mL)	Expected analytical conc. in diluent (~ µg/mL)
0.001 mg/mL	2	1 + 7	4/10	0.10747
0.002 mg/mL	2	1 + 7	4/10	0.21623

[a] Final dilution contained 1-mL IS solution.

4.6 Preparation of Mobile Phase

Equal volumes of water and acetonitrile were transferred into an appropriate container and mixed by inversion. Before use, the mobile phase was degassed using sonication under vacuum pressure.

4.7 Instrumental System and Parameters

Aliquots of the spiked vehicle standards, samples, and blanks were filtered through 0.2-µm Acrodisc filters into autosampler vials and analyzed using the HPLC system and parameters described below in Table 6. Representative chromatograms for the formulation analysis of ethinyl estradiol in corn oil are presented in Figure 1.

Table 6. HPLC Conditions

Liquid Chromatograph:	Waters Alliance 2695 Separations Module
HPLC Column:	Metachem Inertsil ODS-2, 5 µm, 250 mm x 2.0 mm ID
Guard Column:	Metachem, Inertsil ODS-2, 5 µm, 25 mm x 2.0 mm ID
Detector:	Waters 474 Scanning Fluorescence (210 nm for excitation & 310 nm for emission), Gain = 100
Mobile Phase:	ACN:H_2O (50:50, v/v)
Flow Rate:	0.2 mL/min
Injection Volume:	10 µL
Data System:	Turbochrom, Version 6.1.1
Run Time:	20 min
Retention Time:	Ethinyl Estradiol: ~ 11.9 min 2-Phenylphenol (IS): ~ 14.8 min

4.8 Calculations

1. The density of each formulation was calculated by dividing the weight of each formulation aliquot by the aliquot volume. The mean density of each formulation was calculated.

2. The density of corn oil was calculated by dividing the weight of each corn oil aliquot by the aliquot volume. The mean density of corn oil was calculated.

3. A peak height ratio (PHR) was calculated for ethinyl estradiol as follows:

$$PHR = \frac{\text{Peak Height}_{\text{Ethinyl Estradiol}}}{\text{Peak Height}_{\text{Internal Standard}}}$$

4. The slope, y-intercept, and correlation coefficient were calculated using a linear regression analysis of the spiked vehicle standard curve by relating the PHR of each spiked vehicle standard to its corresponding expected analytical concentration (µg/mL).

5. The determined concentration of each sample was calculated using the regression equation obtained for the spiked vehicle standard curve and the PHR for each sample by using the following equation:

$$\text{Determined conc.}(\mu g/mL) = \frac{\text{PHR - intercept}}{\text{slope}} \times \frac{\text{Dilution Factor}}{\text{Weight of Sample}} \times \text{Density}$$

where: Dilution factor = 20 mL
Weight of sample = ~ 2 g
Density = ~ 0.92 g/mL

6. The sample average, average standard deviation, and percent relative average deviation (% RAD) were calculated using commonly accepted techniques.

7. The percent target concentration was calculated as follows:

$$\%\,\text{Target} = \frac{D}{T} \times 100$$

where: D = determined concentration (µg/mL in corn oil)
T = target concentration (µg/mL in corn oil)

8. The average percent target concentration with the corresponding statistical data was calculated for each concentration level.

9. The system suitability parameters for instrumental precision, peak tailing (T), theoretical plates (N), and peak resolution (R) were calculated for ethinyl

estradiol and the internal standard peaks according to USP guidelines.[2] The instrumental precision (% RSD) for system suitability was calculated from six replicate injections of a mid-range spiked vehicle standard.

10. Sample mean ($\bar{x}$), standard deviation (s), and percent relative standard deviation (% RSD) were calculated using commonly accepted techniques.

11. The response ratio (smallest value/largest value) of the duplicate analysis was calculated for each formulation.

4.9 Results

The density of corn oil was 0.92086 g/mL. The density of the 0.001 and 0.002 mg/mL formulations was 0.93048 and 0.92495 g/mL, respectively. The data for the determined concentrations of the formulations of ethinyl estradiol in corn oil are presented in Table 7.

The linear regression equation derived from the data on December 11, 2002, for the spiked vehicle standard curve used for quantitation of the formulation concentrations had a linear correlation of ≥ 0.99. The values for the linear equation were calculated as –0.00616065 for the y-intercept and 2.325341 for the slope. The data for system suitability are presented in Table 8.

All the determined concentrations for 0.001 mg/mL formulations were below the lowest point of the calibration curve, and therefore the values were estimated.

[2] *United States Pharmacopeia*, 25th edition, p. 1990-1991.

Table 7. Results of the Formulation Analysis of Ethinyl Estradiol in Corn Oil for TherImmune Research Corporation—Formulation Date: December 3, 2002

Formulation sample	Target concentration (µg/mL)	Determined concentration (µg/mL)	Average (n = 2)determined concentration (µg/mL)	% Target	Average (n = 2) % Target
Group No. 2, Mix No. 1 Dose Verification Sample	1.000	0.2008* 0.1962*	0.1985 ± 0.0023 1.2% RAD	20.1* 19.6*	19.9 ± 0.3* 1.5% RAD
Group No. 3, Mix No. 1 Dose Verification Sample	2.000	0.8525 0.9995	0.9260 ± 0.0735 7.9% RAD	42.6 50.0	46.3 ± 3.7 8.0% RAD
Group No. 2, Mix No. 1 Top	1.000	0.1477* 0.1626*	0.1552 ± 0.0075 4.8% RAD	14.8* 16.3*	15.6 ± 0.8* 5.1% RAD
Group No. 2, Mix No. 1 Middle	1.000	0.1905* 0.1948*	0.1927 ± 0.0022 1.1% RAD	19.1* 19.5*	19.3 ± 0.2* 1.0% RAD
Group No. 2, Mix No. 1 Bottom	1.000	0.2907* 0.1949*	0.2428 ± 0.0479 19.7% RAD	29.1* 19.5*	24.3 ± 4.8* 19.8% RAD
Group No. 3, Mix No. 1 Top	2.000	0.5327 0.4980	0.5154 ± 0.0174 3.4% RAD	26.6 24.9	25.8 ± 0.9 3.5% RAD
Group No. 3, Mix No. 1 Middle	2.000	0.9755 0.8734	0.9245 ± 0.0511 5.5% RAD	48.8 43.7	46.3 ± 2.6 5.6% RAD
Group No. 3, Mix No. 1 Bottom	2.000	0.5804 0.8807	0.7306 ± 0.1502 20.6% RAD	29.0 44.0	36.5 ± 7.5 20.5% RAD
Results of Homogeneity Study					
Formulation sample	Target concentration (µg/mL)	Average (n = 6) determined concentration (µg/mL)		Average (n = 6) % Target	
Group No. 2, Mix No. 1	1.000	0.1969 ± 0.0440 (s) % RSD = 22.3		19.7 ± 4.4 (s) % RSD = 22.3	
Group No. 3, Mix No. 1	2.000	0.7235 ± 0.2046 (s) % RSD = 28.3		36.2 ± 10.3 (s) % RSD = 28.5	

* Values estimated; below the lowest point of the calibration curve.

Table 8. System Suitability Results

System precision	Theoretical plates	Tailing factor	Resolution [a]
Analytical results: % RSD = 5.0 (n = 6)[a]	Ethinyl Estradiol = 4958	1.4	4.4
	IS = 6975	1.1	
Method criteria [b] % RSD ≤ 4.5	Ethinyl Estradiol ≥ 900 IS ≥ 1600	1.0 ≤ T ≤ 1.6	≥ 1.8

[a] Between ethinyl estradiol and IS peaks.
[b] All system suitability criteria were met except the system precision established for this method.

5. Conclusions

The purpose of this study was to perform a formulation analysis of ethinyl estradiol in corn oil received from TherImmune Research Corporation. Samples included dose verification samples (one vial each) at concentration of 0.001 and 0.002 mg/mL, and homogeneity samples (top, middle, and bottom) for formulations prepared at target

concentration 0.001 and 0.002 mg/mL ethinyl estradiol in corn oil. The concentrations of the dose formulations were determined with TherImmune-supplied test article as an analytical standard.

The average concentrations of the dose verification samples were determined to be 0.1985 and 0.9260 µg/mL ethinyl estradiol in corn oil. All of the dose verification samples were not within the respective target concentrations. The 0.001 mg/mL formulation was 19.9% of target concentration. The 0.002 mg/mL formulation was 46.3% of target concentration.

The 0.001 mg/mL and 0.002 mg/mL ethinyl estradiol in corn oil was evaluated for homogeneity. The results indicated that neither formulation was homogeneous as the % RSDs were 22.3 and 28.5, respectively. The mean (n = 6) concentration of the 0.001 mg/mL and 0.002 mg/mL formulations was 0.1969 µg/mL and 0.7235 µg/mL, which was 19.7% and 36.2%, respectively, of the target concentration.

All the determined concentrations for the 0.001 mg/mL formulations were below the lowest point of the calibration curve; therefore the values were estimated.

6. Contributors

Personnel contributing to this study were Sherry Ferguson and Sarah Barnes.

MIDWEST RESEARCH INSTITUTE

Amir Kazerooni
Study Director

Dale Messer, Ph.D.
Group Leader

Approved:

for Roger K. Harris, Ph.D.
Principal Investigator
Environmental Toxicology Program

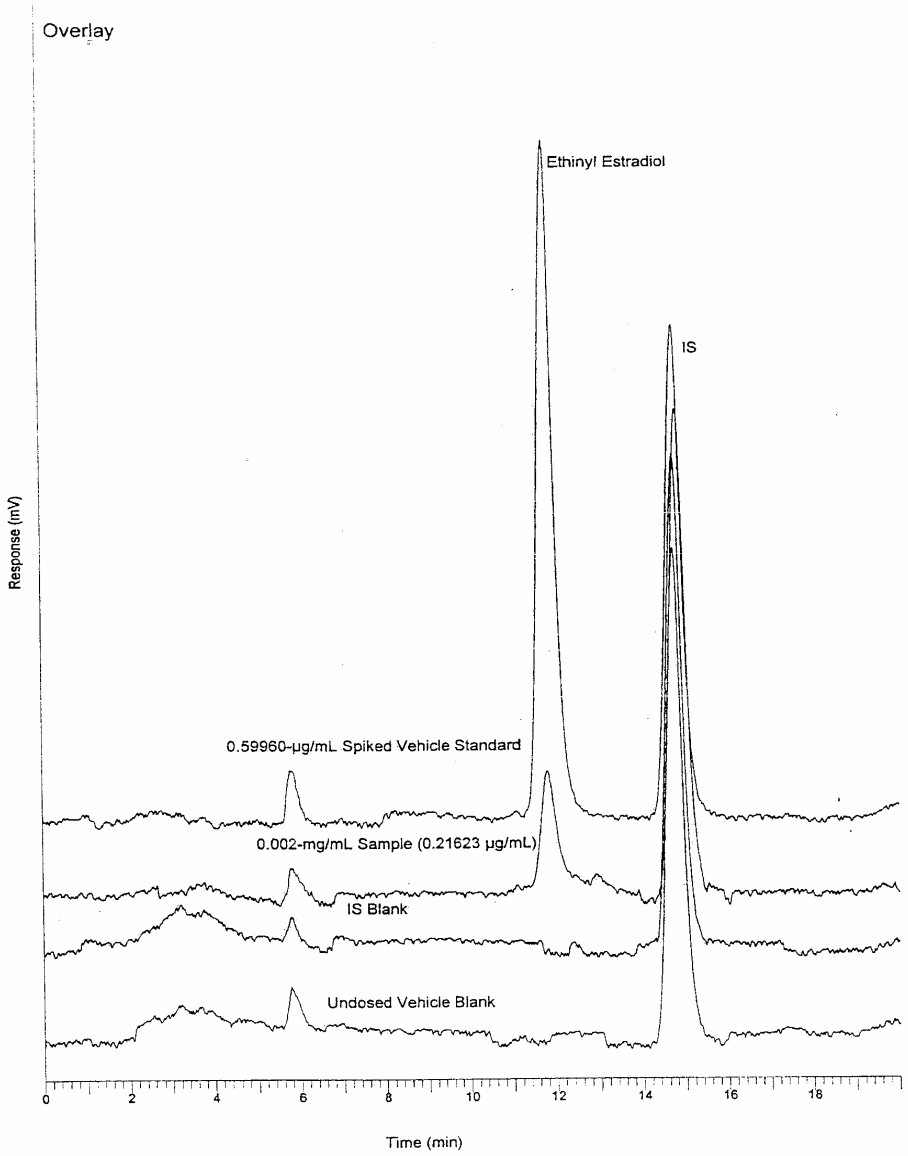

Figure 1. Representative Chromatograms of Ethinyl Estradiol in Corn Oil: 0.59960 µg/mL Spiked Vehicle Standard, 0.002 mg/mL (0.21623 µg/mL) Sample, IS Blank, and Undosed Vehicle Blank

COPY

Formulation Analysis of Ethinyl Estradiol in Corn Oil– Formulation Date: December 13, 2002

NIEHS Contract No. N01-ES-05457
ETP Task No. CHEM07226
MRI Project No. 110100
MRI Task No. 603

Midwest Research Institute
425 Volker Boulevard
Kansas City, Missouri 64110

February 4, 2003

7244-600
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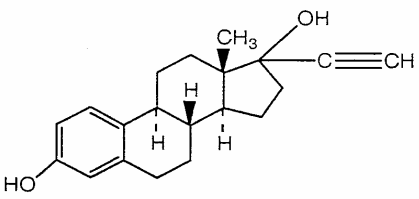
Formulation Analysis (FA)

Chemical Information: Ethinyl Estradiol

CAS No.: 57-63-6 MRI Task No.: 603 ETP Task No.: CHEM07226 Program Supported: RACB Analysis Date: December 18, 2002 Interim Report Date: December 20, 2002	Lot No.: 45H0716 (Supplied by TherImmune Research Corporation) Samples Received: Dose verification samples at 0, 0.001, and 0.002 mg/mL of ethinyl estradiol in corn oil; and neat ethinyl estradiol under MRI Task No. 593 Sample Receipt Dates: December 17, 2002 (dose verification samples) and December 5, 2002 (neat chemical) Submitter: TherImmune Research Corporation Study Lab: TherImmune Research Corporation Formulation Date: December 13, 2002 Receipt Condition: Intact, Ice packs (formulations) Storage Conditions (@ MRI): Dose verification samples, refrigerated (~ 5°C) and neat test article, frozen (~ –20°C)
Structure  	Molecular weight: 296.4 Molecular formula: $C_{20}H_{24}O_2$

Quality Assurance Statement

Formulation Analysis of Ethinyl Estradiol in Corn Oil—Formulation Date: December 13, 2002

ETP Task No. CHEM07226
MRI Project No. 110100
MRI Task No. 603

This study was inspected by the Quality Assurance Unit of MRI (QAU) and the findings reported to the Study Director and Management as follows:

Phase inspected	Date inspected	Date reported
Protocol Audit	12/18/02	12/20/02
Protocol Amendment No.1 Audit	2/3/03	2/4/03
In-Life Audit; formulation analysis	12/18/02	12/20/02
Data Audit	2/3/03	2/4/03
Report Audit	2/3/03	2/4/03

The raw data and report will be stored in the MRI Archives.

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

Facility/equipment	Date inspected	Date reported
HPLC facility	11/18/02	11/19/02
PCA laboratory complex	9/13/02	9/16/02

MIDWEST RESEARCH INSTITUTE

Esther McClellan
Senior Quality Assurance Auditor

Approved:

James Dworak, Ph.D.
Manager, Quality Assurance

February 4, 2003

Good Laboratory Practice Compliance Statement

Formulation Analysis of Ethinyl Estradiol in Corn Oil—Formulation Date: December 13, 2002

ETP Task No. CHEM07226
MRI Project No. 110100
MRI Task No. 603

This formulation analysis study of ethinyl estradiol in corn oil was conducted in compliance with the Good Laboratory Practice Regulations of the U.S. Food and Drug Administration (21 *CFR* 58).

Amir Kazerooni
Study Director

2/4/03
Date

Executive Summary

The purpose of this study was to perform a formulation analysis of ethinyl estradiol in corn oil received from TherImmune Research Corporation. Samples included dose verification samples (one vial each) at concentrations of 0 (control), 0.001, and 0.002 mg/mL ethinyl estradiol in corn oil. The concentrations of the dose formulations were determined with TherImmune-supplied test article received on December 5, 2002, for MRI Task No. 593 (ETP Task No. CHEM07194) as an analytical standard.

The average concentrations of the dose verification samples (Mix No. 2) were determined to be 3.514 and 6.654 µg/mL ethinyl estradiol in corn oil. All of the dose verification samples were not within the respective target concentrations. The 0.001 mg/mL formulation was 351.4% of the target concentration. The 0.002 mg/mL formulation was 332.8% of the target concentration. No test article was detected in the 0 mg/mL control sample.

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Formulation Analysis of Ethinyl Estradiol in Corn Oil—Formulation Date: December 13, 2002

1. Introduction

The purpose of this study was to perform a formulation analysis of ethinyl estradiol in corn oil received from TherImmune Research Corporation. Samples included dose verification samples (one vial each) at concentrations of 0 (control), 0.001, and 0.002 mg/mL ethinyl estradiol in corn oil. The concentrations of the dose formulations were determined with TherImmune-supplied test article received on December 5, 2002, for MRI Task No. 593 (ETP Task No. CHEM07194) as an analytical standard. This study was initiated on December 17, 2002.

The formulation analysis was based on the validated method as described in MRI amended report.[1]

2. Chemical and Sample Information

Test Article:	Ethinyl Estradiol
Lot No.:	45H0716
Supplier:	TherImmune Research Corporation
Molecular Formula:	$C_{20}H_{24}O_2$
Molecular Weight:	296.4
CAS No.:	57-63-6

The formulations submitted for formulation analysis from TherImmune Research Corporation were received at MRI on December 17, 2002, in amber glass bottles and are identified in Table 1 using TherImmune Research Corporation designations. Neat ethinyl estradiol was not received; therefore, the neat chemical received from TherImmune Research Corporation on December 5, 2002, stored at frozen conditions (~ –20°C) was used in this study.

Table 1. Formulations From TherImmune Research Corporation—Formulation Date: December 13, 2002

Sample identification	Concentration (mg/mL)	Volume (~ mL)
Group No. 1, Mix No. 2	0	35
Group No. 2, Mix No. 2	0.001	35
Group No. 3, Mix No. 2	0.002	35

[1] MRI Report, "Dose Formulation Development Study for Ethinyl Estradiol in Corn Oil," NIEHS Contract No. N01-ES-05457, ETP Task No. CHEM05958, MRI Project No. 110100, MRI Task No. 197, amended report issued on January 14, 2002.

3. Materials and Equipment

HPLC system equipped with a Waters Alliance 2695 Separations Module, with a Waters 474 scanning fluorescence detector, and Turbochrom data system, version 6.1.1
Column, Inertsil ODS-2, 5 µm, 250 mm x 2.0 mm ID (Metachem)
Guard column, Inertsil ODS-2, 5 µm, 25 mm x 2.0 mm ID (Metachem)
2-Phenylphenol, > 99% purity, Aldrich Chemical Company, used as internal standard
Acetonitrile (ACN), Burdick and Jackson, Inc., High Purity
ASTM Type I reagent grade water, 18.2 MΩ-cm, from in-house purification system
Sonicator, Branson, Model 5510
Vortex mixer, SP, speed control on 10
Centrifuge, Damon IEC Division CU-5000
Syringes, 5-mL disposable
Vials, 2-mL amber autosampler vials
Filters, Gelman, Acrodisc, 0.2-µm, PVDF, syringe type
Centrifuge tubes, 15-mL (plastic)
Vehicle, Corn Oil, Spectrum Quality Products, Inc.
Glacial acetic acid, Mallinckrodt, 99.5% purity
Volumetric glassware, Class A, as needed

4. Formulation Analysis

A formulation analysis was performed to determine the concentration of ethinyl estradiol in corn oil formulations received from TherImmune Research Corporation. The concentrations of the formulations were determined using neat ethinyl estradiol standard supplied by TherImmune Research Corporation. All formulations were analyzed in duplicate and quantitation was performed using a six-point spiked vehicle standard curve.

4.1 Preparation of Extraction Solution

A 200-µL aliquot of glacial acetic acid was transferred into a 200-mL volumetric flask, diluted to volume with acetonitrile, and mixed by inversion.

4.2 Determination of Corn Oil Density

The density of corn oil (vehicle) was determined by transferring triplicate aliquots of corn oil into individual 5-mL volumetric flasks and obtaining the weight. The density and average density were calculated using commonly accepted techniques.

4.3 Preparation of Standard Solutions

4.3.1 Internal Standard Solution

An internal standard (IS) stock solution was prepared by accurately weighing ~ 69 mg of 2-phenylphenol into a 50-mL volumetric flask, diluting the contents to volume with acetonitrile, and mixing by inversion. The IS solution was prepared by transferring 1 mL of IS stock solution into a 100-mL volumetric flask, diluting to volume with acetonitrile, and mixing by inversion.

4.3.2 Stock Solutions

Two stock solutions of ethinyl estradiol were prepared by accurately weighing and transferring 63.32 mg (Stock B) and 60.586 mg (Stock A) into 200-mL and 100-mL volumetric flasks, respectively, diluting the contents to volume with acetonitrile, and mixing by inversion. The expected concentrations: Stock A, 605.86 µg/mL; Stock B, 316.6 µg/mL.

4.3.3 Intermediate Solutions

Six intermediate solutions (1 to 6) were prepared by transferring alternate aliquots from stock solution A or B by volumetric pipette into individual 100-mL volumetric flasks, diluting to volume with acetonitrile, and mixing by inversion (see Table 2).

Table 2. Preparation of Intermediate Solutions

Intermediate solution	Aliquot of Stock A or B (mL)	Final volume (mL)	Intermediate concentration (µg/mL)
1	0.5, B	100	1.583
2	0.5, A	100	3.0293
3	2, B	100	6.332
4	2, A	100	12.117
5	6, B	100	19.00
6	4, A	100	24.234

4.3.4 Spiked Vehicle Standards

Six spiked vehicle standards (B11 to A61) were prepared by transferring a 1-mL aliquot of each intermediate solution into individual 15-mL centrifuge tubes containing ~ 2 g of undosed corn oil. Each centrifuge tube was capped and vortexed for ~ 1 min. An aliquot (7 mL) of extraction solution was added to each tube, and the extraction procedure listed in Table 3 was followed.

NOTE: Due to lack of availability of recently purchased corn oil, expired corn oil was used for the preparation of the spiked vehicle standards. However, analysis of this corn oil (Dose vehicle analysis, MRI Task No. 595[2]) determined that the oil was below (2.44 meq/Kg) the ETP criteria for maximum peroxide content (< 3.0 meq/Kg). Inspection of the chromatography indicated that the corn oil contributed no interferences to the analysis, and the use of this corn oil did not affect the integrity of the study.

Table 3. Extraction Procedure

1.	Vortex for 1 min
2.	Sonicate at 45°C for 2 min
3.	Vortex for 30 seconds
4.	Sonicate at 45°C for 2 min
5.	Vortex for 30 seconds
6.	Sonicate at 45°C for 2 min
7.	Vortex for 1 min
8.	Centrifuge for 5 min @ 2200 rpm

A 2-mL aliquot of each clarified extract was transferred into individual 5-mL volumetric flasks containing 0.5 mL of IS solution, diluted to volume with water, and mixed by inversion (see Table 4). NOTE: The dilution scheme was adjusted to compensate for low extract volume.

Table 4. Preparation of Spiked Vehicle Standards

Spiked vehicle standard	Intermediate solution	Corn oil aliquot (~ g)	Intermediate solution (mL) + extraction solvent (mL)	Extract dilution in water[a] (mL)	Expected conc. in corn oil (~ µg/mL)	Expected analytical conc. in diluent (µg/mL)
B_{11}	1	2	1 + 7	2/5	0.72	0.07915
A_{21}	2	2	1 + 7	2/5	1.4	0.15147
B_{31}	3	2	1 + 7	2/5	2.9	0.3166
A_{41}	4	2	1 + 7	2/5	5.5	0.60586
B_{51}	5	2	1 + 7	2/5	8.7	0.9498
A_{61}	6	2	1 + 7	2/5	11.1	1.2117

[a] Final dilution contained 0.5-mL IS solution.

4.3.5 Blanks

4.3.5.1 IS Blank

An IS blank was prepared by pipetting 0.5 mL of IS solution into a 5-mL volumetric flask containing 2 mL of acetonitrile, diluting the contents to volume with water, and mixing by inversion.

[2] MRI Report, "Dose Vehicle Analysis of Corn Oil," NIEHS Contract No. N01-ES-05457, ETP Task No. CHEM07197, MRI Project No. 110100, MRI Task No. 595, to be submitted.

4.3.5.2 Reagent Blank (D0)

A reagent blank (D0) was prepared by transferring 2.5 mL of acetonitrile into a 5-mL volumetric flask, diluting to volume with water, and mixing by inversion.

4.3.5.3 Undosed Vehicle Blanks (C01 and C02)

An undosed vehicle blank (C01) was prepared by transferring a 1-mL aliquot of acetonitrile into a 15-mL centrifuge tube containing ~ 2 g of undosed corn oil. The centrifuge tube was capped and vortexed for ~ 1 min. An aliquot (7 mL) of extraction solution was added to the tube, and the extraction procedure listed in Section 4.3.4, Table 3, was followed. After the extraction procedure, a 2-mL aliquot of the clarified extract was transferred into a 5-mL volumetric flask containing 0.5 mL of acetonitrile, diluted to volume with water, and mixed by inversion.

A second undosed vehicle blank (C02) was prepared by transferring a 1-mL aliquot of acetonitrile into a 15-mL centrifuge tube containing ~ 2 g of undosed corn oil. The centrifuge tube was capped and vortexed for ~ 1 min. An aliquot (7 mL) of extraction solution was added to the tube, and the extraction procedure listed in Section 4.3.4, Table 3, was followed. After the extraction procedure, a 2-mL aliquot of the clarified extract was transferred into a 5-mL volumetric flask containing 0.5 mL of IS solution, diluted to volume with water, and mixed by inversion.

4.4 Determination of Formulation Density

The density of each formulation was determined by transferring triplicate aliquots of formulation into individual 5-mL volumetric flasks and obtaining the weight. The density and average density were calculated using commonly accepted techniques.

4.5 Preparation of Formulation Samples

The validated formulation range for the method of analysis of ethinyl estradiol in corn oil was from 0.69 to 11.2 µg/mL.[1] Each formulation was prepared in duplicate by transferring a 1-mL aliquot of acetonitrile into individual 15-mL centrifuge tubes containing ~ 2 g of formulation. Each centrifuge tube was capped and vortexed for ~ 1 min. An aliquot (7 mL) of extraction solution was added to each tube, and the extraction procedure listed in Section 4.3.4, Table 3, was followed. After the extraction procedure, a 2-mL aliquot of each clarified extract was transferred into individual 5-mL volumetric flasks containing 0.5 mL of IS solution, diluted to volume with water, and mixed by inversion (see Table 5).

Table 5. Preparation of Formulation Samples

Formulation	Formulation aliquot (~ g)	Acetonitrile (mL) + extraction solvent (mL)	Extract dilution in water[a] (mL)	Expected analytical conc. in diluent (µg/mL)
Control	2	1 + 7	2/5	0
0.001 mg/mL	2	1 + 7	2/5	0.10912
0.002 mg/mL	2	1 + 7	2/5	0.21608

[a] Final dilution contained 0.5 mL IS solution.

4.6 Preparation of Mobile Phase

Equal volumes of water and acetonitrile were transferred into an appropriate container and mixed by inversion. Before use, the mobile phase was degassed using sonication under vacuum pressure.

4.7 Instrumental System and Parameters

Aliquots of the spiked vehicle standards, samples, and blanks were filtered through 0.2-µm Acrodisc filters into autosampler vials and analyzed using the HPLC system and parameters described below in Table 6. Representative chromatograms for the formulation analysis of ethinyl estradiol in corn oil are presented in Figure 1.

Table 6. HPLC Conditions

Liquid Chromatograph:	Waters Alliance 2695 Separations Module
HPLC Column:	Metachem Inertsil ODS-2, 5 µm, 250 mm x 2.0 mm ID
Guard Column:	Metachem MetaGuard, Inertsil ODS-2, 5 µm, 25 mm x 2.0 mm ID
Detector:	Waters 474 Scanning Fluorescence (210 nm for excitation & 310 nm for emission), Gain = 100
Mobile Phase:	ACN:H_2O (50:50, v/v)
Flow Rate:	0.2 mL/min
Injection Volume:	10 µL
Data System:	Turbochrom, Version: 6.1.1
Run Time:	20 min
Retention Time:	Ethinyl Estradiol: ~ 12.5 min 2-Phenylphenol (IS): ~ 15.6 min

4.8 Calculations

1. The density of each formulation was calculated by dividing the weight of each formulation aliquot by the aliquot volume. The mean density of each formulation was calculated.
2. The density of corn oil was calculated by dividing the weight of each corn oil aliquot by the aliquot volume. The mean density of corn oil was calculated.
3. A peak height ratio (PHR) was calculated for ethinyl estradiol as follows:

$$PHR = \frac{\text{Peak Height}_{\text{Ethinyl Estradiol}}}{\text{Peak Height}_{\text{Internal Standard}}}$$

4. The slope, y-intercept, and correlation coefficient were calculated using a linear regression analysis of the spiked vehicle standard curve by relating the PHR of each spiked vehicle standard to its corresponding expected analytical concentration (µg/mL).
5. The determined concentration of each sample was calculated using the regression equation obtained for the spiked vehicle standard curve and the PHR for each sample by using the following equation:

$$\text{Determined conc. } (\mu g/mL) = \frac{\text{PHR - intercept}}{\text{slope}} \times \frac{\text{Dilution Factor}}{\text{Weight of Sample}} \times \frac{\text{Density}}{}$$

where: Dilution factor = 20 mL
Weight of sample = ~2 g
Density = ~0.92 g/mL

6. The sample average, average standard deviation, and percent relative average deviation (% RAD) were calculated using commonly accepted techniques.
7. The percent target concentration was calculated as follows:

$$\%\text{Target} = \frac{D}{T} \times 100$$

where: D = determined concentration (µg/mL in corn oil)
T = target concentration (µg/mL in corn oil)

8. The average percent target concentration with the corresponding statistical data was calculated for each concentration level.
9. The system suitability parameters for instrumental precision, peak tailing (T), theoretical plates (N), and peak resolution (R) were calculated for ethinyl

estradiol and the internal standard peaks according to USP guidelines.[3] The instrumental precision (% RSD) for system suitability was calculated from six replicate injections of a midrange spiked vehicle standard.

10. Sample mean ($\bar{x}$), standard deviation (s), and percent relative standard deviation (% RSD) were calculated using commonly accepted techniques.
11. The response ratio (smallest value/largest value) of the duplicate analysis was calculated for each formulation.

4.9 Results

The density of corn oil was 0.91585 g/mL. The density of the 0 (control), 0.001, and 0.002 mg/mL formulations were 0.91561, 0.9164, and 0.9256 g/mL, respectively. The data for the determined concentrations of the formulations of ethinyl estradiol in corn oil are presented in Table 7.

The linear regression equation derived from the data for the spiked vehicle standard curve used for quantitation of the formulation concentrations had a linear correlation of ≥ 0.9999. The y-intercept and slope values calculated from linear regression equation were 0.0145546 and 2.22033, respectively. The data for system suitability are presented in Table 8.

Table 7. Results of the Formulation Analysis of Ethinyl Estradiol in Corn Oil for TherImmune Research Corporation—Formulation Date: December 13, 2002

Formulation sample	Target concentration (µg/mL)	Determined concentration (µg/mL)	Average determined concentration (µg/mL)	% Target	Average % Target
Group No. 1	0	0	0	NA	NA
Corn oil		0			
Group No. 2,	1.000	3.519	3.514 ± 0.006	351.9	351.4 ± 0.6
Mix No. 2		3.508	0.2% RAD	350.8	0.2% RAD
Group No. 3,	2.000	6.741	6.654 ± 0.087	337.1	332.8 ± 4.4
Mix No. 2		6.567	1.3% RSD	328.4	1.3% RSD

[3] *United States Pharmacopeia*, 25th Edition, p. 1990-1991.

Table 8. System Suitability Results

System precision	Theoretical plates	Tailing factor	Resolution[a]
Analytical results:			
% RSD = 0.9 (n = 6)	Ethinyl Estradiol = 4912	1.4	4.4
	IS = 7452	1.2	
Method criteria[b]			
% RSD ≤ 4.5	Ethinyl Estradiol ≥ 900 IS ≥ 1600	1.0 ≤ T ≤ 1.6	≥ 1.8

[a] Between ethinyl estradiol and IS peaks.
[b] Acceptable system suitability criteria was established for this method.

5. Conclusions

The purpose of this study was to perform a formulation analysis of ethinyl estradiol in corn oil received from TherImmune Research Corporation. Samples included dose verification samples (one vial each) at concentrations of 0 (control), 0.001, and 0.002 mg/mL ethinyl estradiol in corn oil. The concentrations of the dose formulations were determined with TherImmune-supplied test article received on December 5, 2002, for MRI Task No. 593 (ETP Task No. CHEM07194) as an analytical standard.

The average concentrations of the dose verification samples (Mix No. 2) were determined to be 3.514 and 6.654 µg/mL ethinyl estradiol in corn oil. All of the dose verification samples were not within the respective target concentrations. The 0.001 mg/mL formulation was 351.4% of the target concentration. The 0.002 mg/mL formulation was 332.8% of the target concentration. No test article was detected in the 0 mg/mL control sample.

6. Contributors

Personnel contributing to this study were Sherry Ferguson, Sarah Barnes, and Kathryn Brackman.

MIDWEST RESEARCH INSTITUTE

Amir Kazerooni
Study Director

Dale Messer, Ph.D.
Group Leader

Approved:

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Principal Investigator
Environmental Toxicology Program

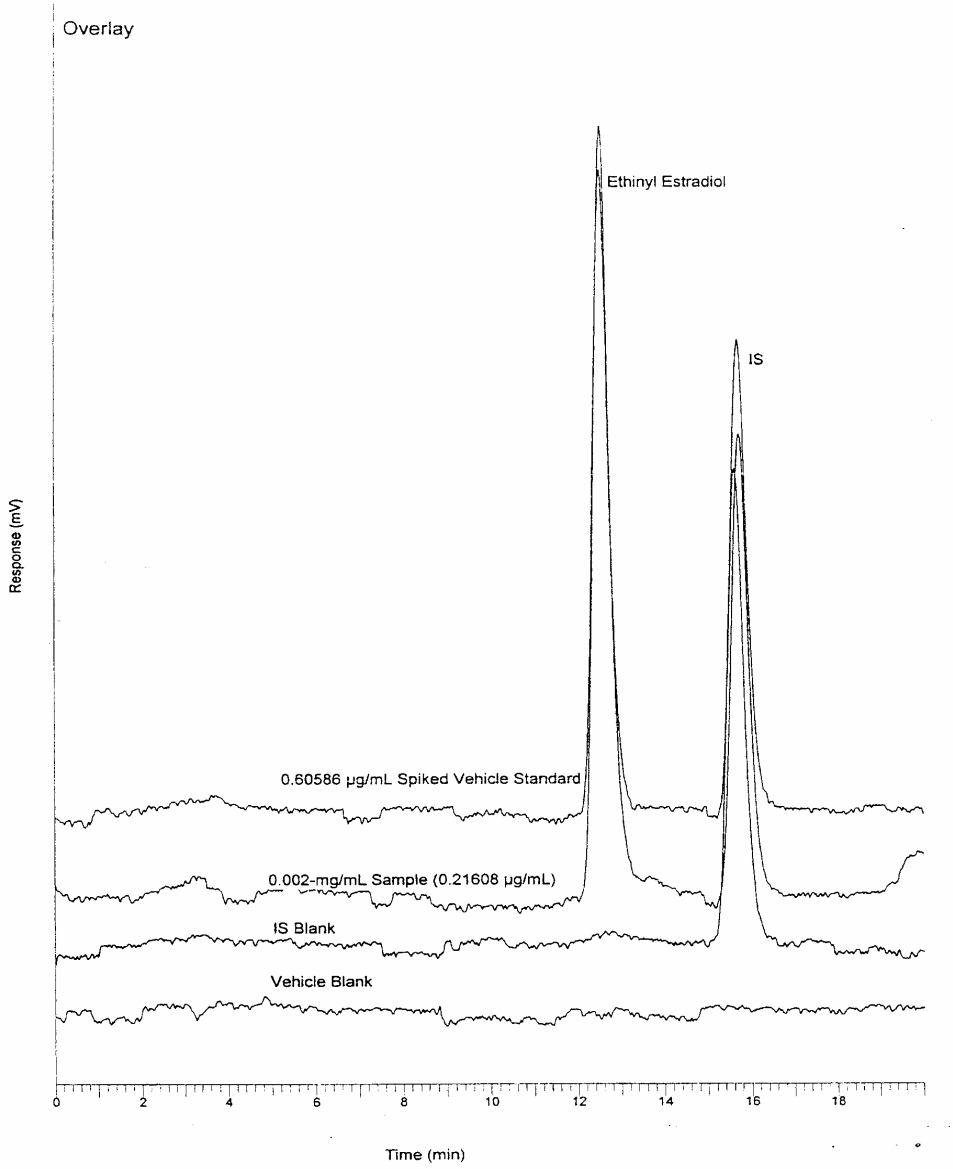


Figure 1. Representative Chromatograms of Ethinyl Estradiol in Corn Oil: 0.60586 µg/mL Spiked Vehicle Standard, 0.002 mg/mL Sample (0.21608 µg/mL), IS Blank, and Vehicle Blank

COPY

Formulation Analysis of Ethinyl Estradiol in Corn Oil– Formulation Date: December 21, 2002

NIEHS Contract No. N01-ES-05457
ETP Task No. CHEM07235
MRI Project No. 110100
MRI Task No. 609

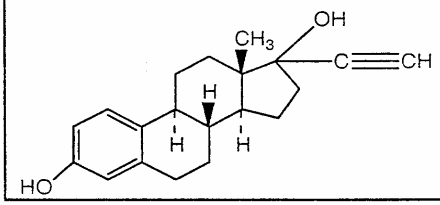
Midwest Research Institute
425 Volker Boulevard
Kansas City, Missouri 64110

7244-600
Rocca
Wolfe
Holley

February 3, 2003

Formulation Analysis (FA)

Chemical Information: Ethinyl Estradiol

CAS No.: 57-63-6 MRI Task No.: 609 ETP Task No.: CHEM07235 Program Supported: RACB Analysis Dates: December 24, 2002, and January 3, 2003 Interim Report Date: January 7, 2003	Lot No.: 45H0716 (supplied by TherImmune Research Corporation, 98.84% per BCR at MRI) Samples Received: Dose verification samples at 0, 0.001, and 0.002 mg/mL of ethinyl estradiol in corn oil; and neat ethinyl estradiol under MRI Task No. 593 Sample Receipt Dates: December 24, 2002 (dose verification samples) and December 5, 2002 (neat chemical) Submitter: TherImmune Research Corporation Study Lab: TherImmune Research Corporation Formulation Date: December 21, 2002 Receipt Condition: Intact, Ice packs (formulations) Storage Conditions (@ MRI): Dose verification samples, refrigerated (~ 5°C) and neat test article, frozen (~ –20°C)
Structure  	Molecular Weight: 296.4 Molecular Formula: $C_{20}H_{24}O_2$

Quality Assurance Statement

Formulation Analysis of Ethinyl Estradiol in Corn Oil—Formulation Date: December 21, 2002

ETP Task No. CHEM07235
MRI Project No. 110100
MRI Task No. 609

This study was inspected by the Quality Assurance Unit of MRI (QAU) and the findings reported to the Study Director and Management as follows:

Phase inspected	Date inspected	Date reported
Protocol Audit	12/24/02	1/2/03
Protocol Amendment No.1 Audit	1/31/03	1/31/03
In-Life Audit; formulation analysis	12/24/02	1/2/03
Data Audit	1/31/03	1/31/03
Report Audit	1/31/03	1/31/03

The raw data and report will be stored in the MRI Archives.

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

Facility/equipment	Date inspected	Date reported
HPLC facility	11/18/02	11/19/02
PCA laboratory complex	9/13/02	9/16/02

MIDWEST RESEARCH INSTITUTE

Esther McClellan
Senior Quality Assurance Auditor

Approved:

James Dworak, Ph.D.
Manager, Quality Assurance

February 3, 2003

Good Laboratory Practice Compliance Statement

Formulation Analysis of Ethinyl Estradiol in Corn Oil—Formulation Date: December 21, 2002

ETP Task No. CHEM07235
MRI Project No. 110100
MRI Task No. 609

This formulation analysis study (MRI Task No. 609) of ethinyl estradiol in corn oil was conducted in compliance with the Good Laboratory Practice Regulations of the U.S. Food and Drug Administration (21 *CFR* 58).

Amir Kazerooni
Study Director

2/3/03
Date

Executive Summary

The purpose of this study was to perform a formulation analysis of ethinyl estradiol in corn oil received from TherImmune Research Corporation. Samples included dose verification samples (one vial each) at concentrations of 0 (control), 0.001, and 0.002 mg/mL ethinyl estradiol in corn oil. The concentrations of the dose formulations were determined with TherImmune-supplied test article received on December 5, 2002, for MRI Task No. 593 (ETP Task No. CHEM07194) as an analytical standard.

The average concentrations of the dose verification samples (Mix No. 3) were determined to be 1.020 and 2.224 µg/mL ethinyl estradiol in corn oil. The 0.001 mg/mL formulation was determined to be within 2.0% of target concentration and the 0.002 mg/mL formulation was determined to be within 11.2% of target. No test article was detected in the 0 mg/mL control sample.

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Figure 1. Representative Chromatograms of Ethinyl Estradiol in Corn Oil: 0.59114 µg/mL Spiked Vehicle Standard, 0.002 mg/mL Sample (0.21708 µg/mL), IS Blank, and Vehicle Blank 11

Formulation Analysis of Ethinyl Estradiol in Corn Oil—Formulation Date: December 21, 2002

1. Introduction

The purpose of this study was to perform a formulation analysis of ethinyl estradiol in corn oil received from TherImmune Research Corporation. Samples included dose verification samples (one vial each) at concentrations of 0 (control), 0.001, and 0.002 mg/mL ethinyl estradiol in corn oil. The concentrations of the dose formulations were determined with TherImmune-supplied test article received on December 5, 2002, for MRI Task No. 593 (ETP Task No. CHEM07194) as an analytical standard. This study was initiated on December 24, 2002.

The formulation analysis was based on the validated method as described in the MRI amended report for "Dose Formulation Development Study for Ethinyl Estradiol in Corn Oil, "NIEHS Contract No. N01-ES-05457, ETP Task No. CHEM05958, MRI Project No. 110100, MRI Task No. 197, amended report issued on January 14, 2002.

2. Chemical and Sample Information

Test Article:	Ethinyl Estradiol
Lot No.:	45H0716
Supplier:	TherImmune Research Corporation
Molecular Formula:	$C_{20}H_{24}O_2$
Molecular Weight:	296.4
CAS No.:	57-63-6

The formulations submitted for formulation analysis from TherImmune Research Corporation were received at MRI on December 24, 2002, in amber glass bottles and are identified in Table 1 using TherImmune Research Corporation designations. Neat ethinyl estradiol was not received; therefore, the neat chemical received from TherImmune Research Corporation on December 5, 2002, stored at frozen conditions (~ –20°C) was used in this study. The formulation samples were stored under refrigerated conditions (~ 5°C) prior to analysis.

Table 1. Formulations From TherImmune Research Corporation–Formulation Date: December 21, 2002

Sample identification	Concentration (mg/mL)	Volume (~ mL)
Group No. 1, Corn Oil	0	50
Group No. 2, Mix No. 3	0.001	35
Group No. 3, Mix No. 3	0.002	35

3. Materials and Equipment

HPLC system equipped with Waters Alliance 2695 Separations Module, a Waters 474 scanning fluorescence detector, and Turbochrom data system, version 6.1.1
Column, Inertsil ODS-2, 5 µm, 250 x 2.0 mm ID (Metachem)
Guard column, Inertsil ODS-2, 5 µm, 25 x 2.0 mm ID (Metachem)
2-Phenylphenol, > 99% purity, Aldrich, used as internal standard
Acetonitrile (ACN), Burdick and Jackson, Inc., High Purity
ASTM Type I reagent grade water, 18.2 MΩ-cm, from in-house purification system
Sonicator, Branson, model 5510
Vortex mixer, SP, speed control on 10
Centrifuge, Damon IEC Division CU-5000
Syringes, 5-mL disposable
Vials, 2-mL amber autosampler vials
Filters, Gelman, Acrodisc, 0.2-µm, PVDF, syringe type
Centrifuge tubes, 15-mL (plastic)
Vehicle, Corn Oil, Spectrum Quality Products, Inc.
Glacial acetic acid, Mallinckrodt, 99.5% purity
Volumetric glassware, Class A, as needed

4. Formulation Analysis

A formulation analysis was performed to determine the concentration of ethinyl estradiol in corn oil formulations received from TherImmune Research Corporation. The concentrations of the formulations were determined using neat ethinyl estradiol standard supplied by TherImmune Research Corporation. All formulations were analyzed in duplicate and quantitation was performed using a six-point spiked vehicle standard curve.

4.1 Preparation of Extraction Solution

A 200-µL aliquot of glacial acetic acid was transferred into a 200-mL volumetric flask, diluted to volume with acetonitrile, and mixed by inversion.

4.2 Determination of Corn Oil Density

The density of corn oil was determined by transferring triplicate aliquots of corn oil into appropriate individual volumetric flasks and obtaining the weight. The density and average density were calculated using commonly accepted techniques.

4.3 Preparation of Standard Solutions

4.3.1 Internal Standard Solution

An internal standard (IS) stock solution was prepared by accurately weighing ~ 65 mg of 2-phenylphenol into a 50-mL volumetric flask, diluting the contents to volume with acetonitrile, and mixing by inversion. The IS solution was prepared by transferring 1 mL of IS stock solution into a 100-mL volumetric flask, diluting to volume with acetonitrile, and mixing by inversion.

4.3.2 Stock Solutions

Two stock solutions of ethinyl estradiol were prepared by accurately weighing and transferring 60.958 mg (Stock B) and 62.179 mg (Stock A) into 200-mL and 100-mL volumetric flasks, respectively, diluting the contents to volume with acetonitrile, and mixing by inversion. The expected concentrations: Stock A, 621.79 µg/mL; Stock B, 304.79 µg/mL.

4.3.3 Intermediate Solutions

Six intermediate solutions (1 to 6) were prepared by transferring alternate aliquots from stock solution A or B by volumetric pipette into individual 100-mL volumetric flasks, diluting to volume with acetonitrile, and mixing by inversion (see Table 2).

Table 2. Preparation of Intermediate Solutions

Intermediate solution	Aliquot of Stock A or B (mL)	Final volume (mL)	Intermediate concentration (µg/mL)
1	0.5, B	100	1.5240
2	0.5, A	100	3.1090
3	2, B	100	6.0958
4	2, A	100	12.436
5	6, B	100	18.287
6	4, A	100	24.872

4.3.4 Spiked Vehicle Standards

Six spiked vehicle standards (B11 to A61) were prepared by transferring a 1-mL aliquot of each intermediate solution into individual 15-mL centrifuge tubes containing ~ 2 g of undosed corn oil. Each centrifuge tube was capped and vortexed for ~ 1 min. An aliquot (7 mL) of extraction solution was added to each tube, and the extraction procedure listed in Table 3 was followed.

NOTE: Due to lack of availability of currently purchased corn oil, expired corn oil had to be used for the preparation of the spiked vehicle standards. However, analysis of this corn oil (Dose vehicle analysis, MRI Task No. 595)[1] determined that the oil was below (2.44 meq/Kg) the ETP criteria for maximum peroxide content (< 3.0 meq/Kg). Inspection of the chromatography indicated that the corn oil contributed no interferences to the analysis, and the use of this corn oil did not affect the integrity of the study.

Table 3. Extraction Procedure

1.	Vortex for 1 min
2.	Sonicate at 45°C for 2 min
3.	Vortex for 30 sec
4.	Sonicate at 45°C for 2 min
5.	Vortex for 30 sec
6.	Sonicate at 45°C for 2 min
7.	Vortex for 1 min
8.	Centrifuge for 5 min @ 2200 rpm

A 4-mL aliquot of each clarified extract was transferred into individual 10-mL volumetric flasks containing 1 mL of IS solution, diluted to volume with water, and mixed by inversion (see Table 4).

Table 4. Preparation of Spiked Vehicle Standards

Spiked vehicle standard	Intermediate solution	Corn oil aliquot (~ g)	Intermediate solution (mL) + extraction solvent (mL)	Extract dilution in water[a] (mL)	Expected conc. in corn oil (~ µg/mL)	Expected analytical conc. in diluent (µg/mL)
B_{11}	1	2	1 + 7	4/10	0.70054	0.076200
A_{21}	2	2	1 + 7	4/10	1.4273	0.15545
B_{31}	3	2	1 + 7	4/10	2.8000	0.30479
A_{41}	4	2	1 + 7	4/10	5.7128	0.62180
B_{51}	5	2	1 + 7	4/10	8.3847	0.91435
A_{61}	6	2	1 + 7	4/10	11.410	1.2436

[a] Final dilution contained 1-mL IS solution.

4.3.5 Blanks

4.3.5.1 IS Blank

An IS blank was prepared by pipetting 1 mL of IS solution into a 10-mL volumetric flask containing 4 mL of acetonitrile, diluting the contents to volume with water, and mixing by inversion.

[1] MRI Report, "Dose Vehicle Analysis of Corn Oil," NIEHS Contract No. N01-ES-05457, ETP Task No. CHEM07197, MRI Project No. 110100, MRI Task No. 595, to be submitted.

4.3.5.2 Undosed Vehicle Blanks (C01 and C02)

An undosed vehicle blank (C02) was prepared by transferring a 1-mL aliquot of acetonitrile into a 15-mL centrifuge tube containing ~ 2 g of undosed corn oil. The centrifuge tube was capped and vortexed for ~ 1 min. An aliquot (7 mL) of extraction solution was added to the tube, and the extraction procedure listed in Section 4.3.4, Table 3, was followed. After the extraction procedure, a 4-mL aliquot of the clarified extract was transferred into a 10-mL volumetric flask containing 1 mL of acetonitrile, diluted to volume with water, and mixed by inversion.

A second undosed vehicle blank (C01) was prepared by transferring a 1-mL aliquot of acetonitrile into a 15-mL centrifuge tube containing ~ 2 g of undosed corn oil. The centrifuge tube was capped and vortexed for ~ 1 min. An aliquot (7 mL) of extraction solution was added to the tube, and the extraction procedure listed in Section 4.3.4, Table 3, was followed. After the extraction procedure, a 4-mL aliquot of the clarified extract was transferred into a 10-mL volumetric flask containing 1 mL of IS solution, diluted to volume with water, and mixed by inversion.

4.4 Determination of Formulation Density

The density of each formulation was determined by transferring triplicate aliquots of formulation into individual 5-mL volumetric flasks and obtaining the weight. The density and average density were calculated using commonly accepted techniques.

4.5 Preparation of Formulation Samples

The validated formulation range for the method of analysis of ethinyl estradiol in corn oil was from 0.69 to 11.2 µg/mL. Each formulation was prepared in duplicate by transferring a 1-mL aliquot of acetonitrile into individual 15-mL centrifuge tubes containing ~ 2 g of formulation. Each centrifuge tube was capped and vortexed for ~ 1 min. An aliquot (7 mL) of extraction solution was added to each tube, and the extraction procedure listed in Section 4.3.4, Table 3, was followed. After the extraction procedure, a 4-mL aliquot of each clarified extract was transferred into individual 10-mL volumetric flask containing 1 mL of IS solution, diluted to volume with water, and mixed by inversion (see Table 5).

Table 5. Preparation of Formulation Samples

Formulation	Formulation aliquot (~ g)	Acetonitrile (mL) + extraction solvent (mL)	Extract dilution in water [a] (mL)	Expected analytical conc. in diluent (µg/mL)
Control	2	1 + 7	4/10	0.0
0.001 mg/mL	2	1 + 7	4/10	0.1088
0.002 mg/mL	2	1 + 7	4/10	0.2171

[a] Final dilution contained 1-mL IS solution.

4.6 Preparation of Mobile Phase

Equal volumes of water and acetonitrile were transferred into an appropriate container and mixed by inversion. Before use, the mobile phase was degassed using sonication under vacuum pressure.

4.7 Instrumental System and Parameters

Aliquots of the spiked vehicle standards, samples, and blanks were filtered through 0.2-µm Acrodisc filters into autosampler vials and analyzed using the HPLC system and parameters described below in Table 6. Representative chromatograms for the formulation analysis of ethinyl estradiol in corn oil are presented in Figure 1.

Table 6. HPLC Conditions

Liquid Chromatograph:	Waters Alliance 2695 Separations Module
HPLC Column:	Metachem, Inertsil ODS-2, 5 µm, 250 mm x 2.0 mm ID
Guard Column:	Metachem, Inertsil ODS-2, 5 µm, 25 mm x 2.0 mm ID
Detector:	Waters 474 Scanning Fluorescence (210 nm for excitation & 310 nm for emission), Gain = 100
Mobile Phase:	ACN:H_2O (50:50, v/v)
Flow Rate:	0.2 mL/min
Injection Volume:	10 µL
Data System:	Turbochrom, Version 6.1.1
Run Time:	20 min
Retention Time:	Ethinyl Estradiol: ~ 11.4 min 2-Phenylphenol (IS): ~ 14.3 min

4.8 Calculations

1. The density of each formulation was calculated by dividing the weight of each formulation aliquot by the aliquot volume. The mean density of each formulation was calculated.

2. The density of corn oil was calculated by dividing the weight of each corn oil aliquot by the aliquot volume. The mean density of corn oil was calculated.

3. A peak height ratio (PHR) was calculated for ethinyl estradiol as follows:

$$PHR = \frac{\text{Peak Height}_{\text{Ethinyl Estradiol}}}{\text{Peak Height}_{\text{Internal Standard}}}$$

4. The slope, y-intercept, and correlation coefficient were calculated using a linear regression analysis of the spiked vehicle standard curve by relating the PHR of each spiked vehicle standard to its corresponding expected analytical concentration (µg/mL).

5. The determined concentration of each sample was calculated using the regression equation obtained for the spiked vehicle standard curve and the PHR for each sample by using the following equation:

$$\text{Determined conc. } (\mu g/mL) = \frac{\text{PHR - intercept}}{\text{slope}} \times \frac{\text{Dilution Factor}}{\text{Weight of Sample}} \times \text{Density}$$

where: Dilution factor = 20 mL
Weight of sample = ~ 2 g
Density = 0.92 g/mL

6. The sample average, average standard deviation, and percent relative average deviation (% RAD) were calculated using commonly accepted techniques.

7. The percent target concentration was calculated as follows:

$$\%\,\text{Target} = \frac{D}{T} \times 100$$

where: D = determined concentration (µg/mL corn oil)
T = target concentration (µg/mL corn oil)

8. The average percent target concentration with the corresponding statistical data was calculated for each concentration level.

9. The system suitability parameters for instrumental precision, peak tailing (T), theoretical plates (N), and peak resolution (R) were calculated for ethinyl estradiol and the internal standard peaks according to USP guidelines.[2] The instrumental precision (% RSD) for system suitability was calculated from six replicate injections of a mid-range spiked vehicle standard.

10. Sample mean ($\bar{x}$), standard deviation (s), and percent relative standard deviation (% RSD) were calculated using commonly accepted techniques.

11. The response ratio (smallest value/largest value) of the duplicate analysis was calculated for each formulation.

4.9 Reanalysis

Since the response ratio criteria of > 0.90 was not met for the 0.002 mg/mL formulation, a third sample was prepared and analyzed against another spiked vehicle standard curve on January 3, 2003, according to Sections 4.3 and 4.5. The expected

[2] *United States Pharmacopeia*, 25th edition, p. 1990-1991.

concentrations: Stock A, 591.14 µg/mL; Stock B, 315.94 µg/mL. The spiked vehicle standard curve was within the validated range and all system suitability criteria were met.

5. Results

The density of corn oil was 0.9204 g/mL. The density of the 0 (control), 0.001, and 0.002 mg/mL formulations were 0.9156, 0.9195, and 0.9213 g/mL, respectively. The determined concentration data of the formulations of ethinyl estradiol in corn oil are presented in Table 7. Since response ratio criteria of > 0.90 was not met for the 0.002 mg/mL formulation, a third sample was prepared and analyzed against another spiked vehicle standard curve on January 3, 2003.

The linear regression equation derived from the data on December 24, 2002, for the spiked vehicle standard curve used for quantitation of the formulation concentrations had a linear correlation of ≥ 0.999. The y-intercept and slope values calculated from the linear regression equation were 0.01433725 and 2.269031, respectively. The data for system suitability are presented in Table 8. **NOTE:** The upper spiked vehicle standard was higher very slightly. This difference did not affect the integrity of the study.

The linear regression equation derived from the data on January 3, 2003, for the spiked vehicle standard curve used for quantitation of the formulation concentrations had a linear correlation of ≥ 0.999. The y-intercept and slope values calculated from the linear regression equation were 0.0290875 and 2.34561, respectively. The data for system suitability are presented in Table 8.

Table 7. Results of the Formulation Analysis of Ethinyl Estradiol in Corn Oil for TherImmune Research Corporation—Formulation Date: December 21, 2002

Formulation sample	Target[a] concentration (µg/mL)	Determined concentration (µg/mL)	Average determined concentration (µg/mL)	% Target	Average % Target
Group No. 1, Corn Oil	0	0 0	0	NA	NA
Group No. 2, Mix No. 3	1.000	1.017 1.023	1.020 ± 0.003 (d) 0.3% RAD	101.7 102.3	102.0 ± 0.3 0.3% RAD
Group No. 3, Mix No. 3	2.000	2.505 2.186 1.980	2.224 ± 0.265 (s) 11.9% RSD	125.3 109.3 99.0	111.2 ± 13.3 12.0% RSD

[a] 0.001 mg/mL = 1 µg/mL, 0.002 mg/mL = 2 µg/mL.

Table 8. System Suitability Results

System precision	Theoretical plates	Tailing factor	Resolution [a]
Analytical results (December 24, 2002):			
	Ethinyl Estradiol = 4953	1.4	4.3
%RSD = 1.5			
(n = 6)[a]	IS = 6932	1.3	
Analytical results (January 3, 2003):			
	Ethinyl Estradiol = 4311	1.4	4.1
%RSD = 1.7			
(n = 6)[a]	IS = 6585	1.3	
Method criteria [b]			
% RSD ≤ 4.5	Ethinyl Estradiol ≥ 900	1.0 ≤ T ≤ 1.6	≥ 1.8
	IS ≥ 1600		

[a] Between ethinyl estradiol and IS peaks.
[b] Acceptable system suitability criteria was established for this method.

6. Conclusions

The purpose of this study was to perform a formulation analysis of ethinyl estradiol in corn oil received from TherImmune Research Corporation. Samples included dose verification samples (one vial each) at concentrations of 0 (control), 0.001, and 0.002 mg/mL ethinyl estradiol in corn oil. The concentrations of the dose formulations were determined with TherImmune-supplied test article received on December 5, 2002, for MRI Task No. 593 (ETP Task No. CHEM07194) as an analytical standard.

The average concentrations of the dose verification samples (Mix No. 3) were determined to be 1.020 and 2.224 µg/mL ethinyl estradiol in corn oil. The 0.001 mg/mL formulation was determined to be within 2.0% of target concentration, and the 0.002 mg/mL formulation was determined to be within 11.2% of target. No test article was detected in the 0 mg/mL control sample.

7. Contributors

Personnel contributing to this study were Andy Hodges, Karl Decker, Sherry Ferguson, and Sarah Barnes.

MIDWEST RESEARCH INSTITUTE

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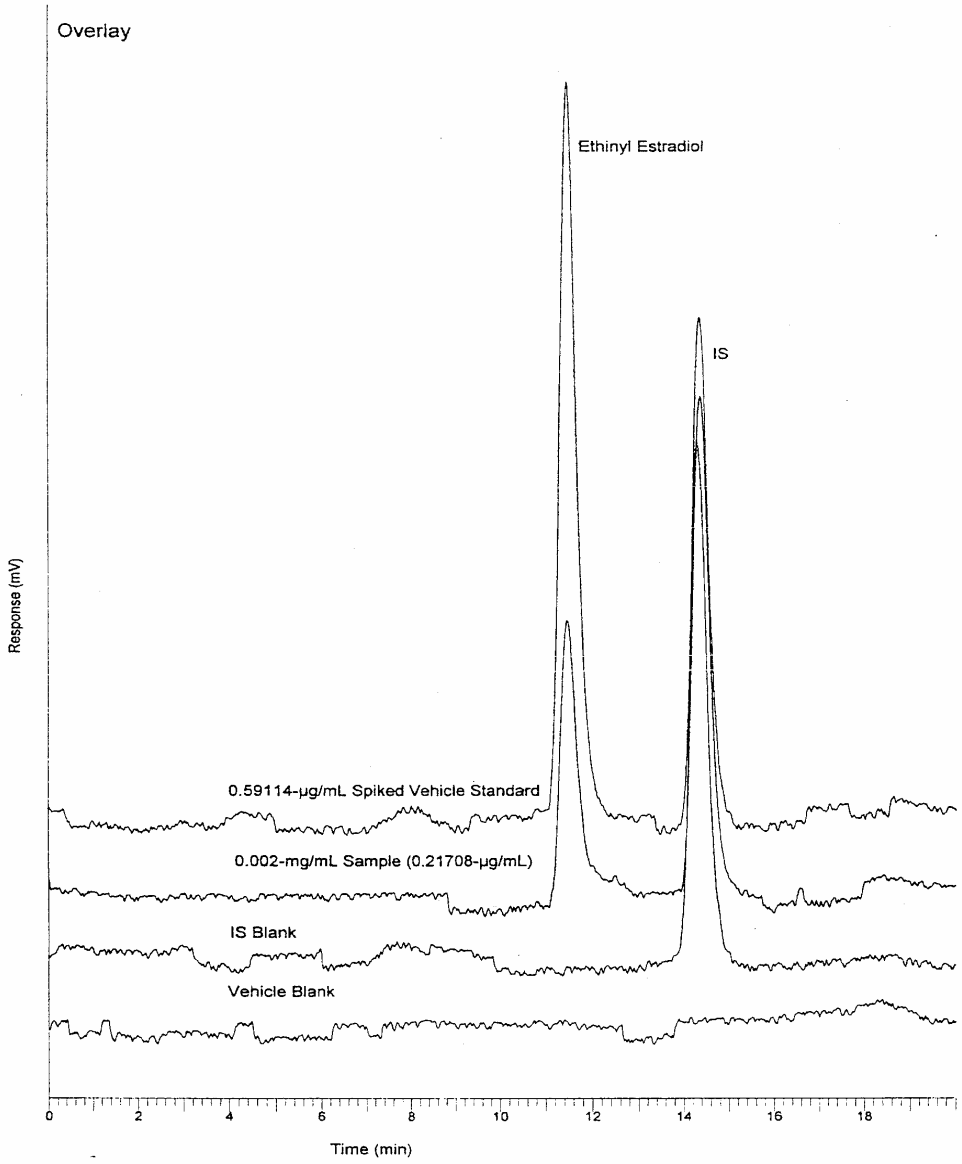


Figure 1. Representative Chromatograms of Ethinyl Estradiol in Corn Oil: 0.59114 µg/mL Spiked Vehicle Standard, 0.002 mg/mL Sample (0.21708 µg/mL), IS Blank, and Vehicle Blank

COPY

Formulation Analysis of Ethinyl Estradiol in Corn Oil– Formulation Date: December 23, 2002

NIEHS Contract No. N01-ES-05457
ETP Task No. CHEM07228
MRI Project No. 110100
MRI Task No. 605

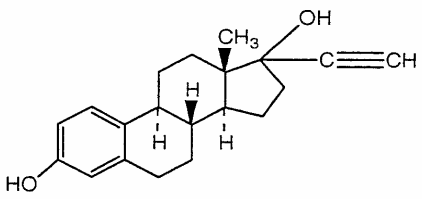
Midwest Research Institute
425 Volker Boulevard
Kansas City, Missouri 64110

February 13, 2003

7244-600
Rocca
Wolfe
Holley

Formulation Analysis (FA)

Chemical Information: Ethinyl Estradiol

CAS No.: 57-63-6 MRI Task No.: 605 ETP Task No.: CHEM07228 Program Supported: RACB Analysis Date: January 13, 2003 Interim Report Date: January 16, 2003	Lot No.: 45H0716 (Supplied by TherImmune Research Corporation, 98.84% per BCR at MRI) Samples Received: dose verification samples at 0.001 and 0.002 mg/mL of ethinyl estradiol in corn oil; and neat ethinyl estradiol under MRI Task No. 593 Sample Receipt Dates: January 10, 2003 (dose verification samples) and December 5, 2002 (neat chemical) Submitter: TherImmune Research Corporation Study Lab: TherImmune Research Corporation Formulation Date: December 23, 2002 Receipt Condition: Intact, ice packs (Formulations) Storage Conditions (@ MRI): Dose verification samples, refrigerated (~ 5°C) and neat test article, frozen (~ –20°C)
Structure  	Molecular Weight: 296.4 Molecular Formula: $C_{20}H_{24}O_2$

Quality Assurance Statement

Formulation Analysis of Ethinyl Estradiol in Corn Oil—Formulation Date: December 23, 2002

ETP Task No. CHEM07228
MRI Project No. 110100
MRI Task No. 605

This study was inspected by the Quality Assurance Unit of MRI (QAU) and the findings reported to the Study Director and Management as follows:

Phase inspected	Date inspected	Date reported
Protocol Audit	01/13/03	01/13/03
In-Life Audit; Formulation Analysis	01/13/03	01/13/03
Protocol Amendment No. 1 Audit	2/11/03	2/11/03
Data Audit	2/11/03	2/11/03
Report Audit	2/11/03	2/11/03

The raw data and report will be stored in the MRI Archives.

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

Facility/equipment	Date inspected	Date reported
HPLC facility	11/18/02	11/19/02
PCA laboratory complex	9/13/02	9/16/02

MIDWEST RESEARCH INSTITUTE

Rosemary Moran

Rosemary Moran
Quality Assurance Officer

Approved:

James Dworak

James Dworak, Ph.D.
Manager, Quality Assurance

February 13, 2003

Good Laboratory Practice Compliance Statement

Formulation Analysis of Ethinyl Estradiol in Corn Oil—Formulation Date: December 23, 2002

ETP Task No. CHEM07228
MRI Project No. 110100
MRI Task No. 605

This formulation analysis study of ethinyl estradiol in corn oil was conducted in compliance with the Good Laboratory Practice Regulations of the U.S. Food and Drug Administration (21 *CFR* 58).

Amir Kazerooni
Study Director

2/13/03
Date

Executive Summary

The purpose of this study was to perform a formulation analysis of ethinyl estradiol in corn oil received from TherImmune Research Corporation. Samples included dose verification samples (one vial each) at concentrations of 0.001 and 0.002 mg/mL Ethinyl estradiol in corn oil. The concentration of the dose formulations was determined with TherImmune-supplied test article received on December 5, 2002, for MRI Task No. 593 (ETP Task No. CHEM07194) as an analytical standard.

The average concentrations of the dose verification samples (Mix No.: 3) were determined to be 1.838 and 2.241 µg/mL ethinyl estradiol in corn oil. The dose verification samples were not within ± 10% of the respective target concentrations. The 0.001 mg/mL formulation was 183.8% of the target concentration. The 0.002 mg/mL formulation was 112.1% of the target concentration.

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Figure 1. Representative Chromatograms of Ethinyl Estradiol in Corn Oil: 0.60138 µg/mL Spiked Vehicle Standard, 0.002 mg/mL Sample (0.21680 µg/mL), IS Blank, and Undosed Vehicle Blank 10

Formulation Analysis of Ethinyl Estradiol in Corn Oil— Formulation Date: December 23, 2002

1. Introduction

The purpose of this study was to perform a formulation analysis of ethinyl estradiol in corn oil received from TherImmune Research Corporation. Samples included dose verification samples (one vial each) at concentrations of 0.001 and 0.002 mg/mL Ethinyl estradiol in corn oil. The concentration of the dose formulations was determined with TherImmune-supplied test article received on December 5, 2002, for MRI Task No. 593 (ETP Task No. CHEM07194) as an analytical standard. This study was initiated on January 10, 2003.

The formulation analysis was based on the validated method as described in the MRI dose formulation development report.[1]

2. Chemical and Sample Information

Test Article:	Ethinyl Estradiol
Lot No.:	45H0716
Supplier:	TherImmune Research Corporation
Molecular Formula:	$C_{20}H_{24}O_2$
Molecular Weight:	296.4
CAS No.:	57-63-6

The formulations submitted for formulation analysis from TherImmune Research Corporation were received at MRI on January 10, 2003, in amber glass bottles and are identified in Table 1 using TherImmune Research Corporation designations. Neat ethinyl estradiol was also received from TherImmune Research Corporation on December 5, 2002. The neat chemical was expired, but the purity was verified to be 98.8%[2] and therefore suitable for use. The formulation samples were stored under refrigerated (~ 5°C) conditions, and neat test article was stored under frozen (~ –20°C) conditions prior to analysis.

[1] MRI Report, "Dose Formulation Development Study for Ethinyl Estradiol in Corn Oil," NIEHS Contract No. N01-ES-05457, ETP Task No. CHEM05958, MRI Project No. 110100, MRI Task No. 197, amended report issued on January 14, 2002.

[2] MRI Report, "Bulk Chemical Reanalysis for Ethinyl Estradiol," NIEHS Contract No. N01-ES-05457, ETP Task No. CHEM07193, MRI Project No. 110100, MRI Task No. 592, report issued on January 31, 2002.

Table 1. Formulations From TherImmune Research Corporation—Formulation Date: December 23, 2002

Sample identification	Concentration (mg/mL)	Volume (~ mL)
Group No. 2, Mix No. 3	0.001	35
Group No. 3, Mix No. 3	0.002	35

3. Materials and Equipment

HPLC system equipped with a Waters Alliance 2695 Separations Module, with a Waters 474 scanning fluorescence detector, and Turbochrom data system, Version 6.1.1
Column, Inertsil ODS-2, 5 µm, 250 mm x 2.0 mm ID (Metachem)
Guard column, Inertsil ODS-2, 5 µm, 25 mm x 2.0 mm ID (Metachem)
2-Phenylphenol, > 99% purity, Aldrich Chemical Company, used as internal standard
Acetonitrile (ACN), Burdick and Jackson, Inc., and Fisher Scientific, High Purity
ASTM Type I reagent grade water, 18.2 MΩ-cm, from in-house purification system
Sonicator, Branson, Model 5510
Vortex mixer, SP, speed control on 10
Centrifuge, Damon IEC Division CU-5000
Syringes, 5-mL disposable
Vials, 2-mL amber autosampler vials
Filters, Gelman, Acrodisc, 0.2-µm, PVDF, syringe type
Centrifuge tubes, 15-mL (plastic)
Vehicle, Corn Oil, Spectrum Quality Products, Inc.
Glacial acetic acid, Mallinckrodt, 99.5% purity
Volumetric glassware, Class A, as needed

4. Formulation Analysis

A formulation analysis was performed to determine the concentration of ethinyl estradiol in corn oil formulations received from TherImmune Research Corporation. The concentrations of the formulations were determined using neat ethinyl estradiol standard supplied by TherImmune Research Corporation. All formulations were analyzed in duplicate and quantitation was performed using a six-point spiked vehicle standard curve.

4.1 Preparation of Extraction Solution

A 200-µL aliquot of glacial acetic acid was transferred into a 200-mL volumetric flask, diluted to volume with acetonitrile, and mixed by inversion.

4.2 Determination of Corn Oil Density

The density of corn oil was determined by transferring triplicate aliquots of corn oil into individual 5-mL volumetric flasks and obtaining the weight. The density and mean density were calculated using commonly accepted techniques.

4.3 Preparation of Standard Solutions

4.3.1 Internal Standard Solution

An internal standard (IS) stock solution was prepared by accurately weighing ~ 63 mg of 2-phenylphenol into a 50-mL volumetric flask, diluting the contents to volume with acetonitrile, and mixing by inversion. The IS solution was prepared by transferring 1 mL of IS stock solution into a 100-mL volumetric flask, diluting to volume with acetonitrile, and mixing by inversion.

4.3.2 Stock Solutions

Two stock solutions of ethinyl estradiol were prepared by accurately weighing and transferring 62.029 mg (Stock B) and 60.138 mg (Stock A) into 200-mL and 100-mL volumetric flasks, respectively, diluting the contents to volume with acetonitrile, and mixing by inversion. The expected concentrations: Stock A, 601.38 µg/mL; Stock B, 310.15 µg/mL.

4.3.3 Intermediate Solutions

Six intermediate solutions (1 to 6) were prepared by transferring alternate aliquots from stock solution A or B by volumetric pipette into individual 100-mL volumetric flasks, diluting to volume with acetonitrile, and mixing by inversion (see Table 2).

Table 2. Preparation of Intermediate Solutions

Intermediate solution	Aliquot of stock A or B (mL)	Final volume (mL)	Intermediate concentration (µg/mL)
1	0.5, B	100	1.5507
2	0.5, A	100	3.0069
3	2, B	100	6.2029
4	2, A	100	12.028
5	6, B	100	18.609
6	4, A	100	24.055

4.3.4 Spiked Vehicle Standards

Six spiked vehicle standards (B11 to A61) were prepared by transferring a 1-mL aliquot of each intermediate solution into individual 15-mL centrifuge tubes containing ~ 2 g of undosed corn oil. Each centrifuge tube was capped and vortexed for ~ 1 min. An aliquot (7-mL) of extraction solution was added to each tube and the extraction procedure listed in Table 3 was followed.

Table 3. Extraction Procedure

1.	Vortex for 1 min
2.	Sonicate at 45°C for 2 min
3.	Vortex for 30 sec
4.	Sonicate at 45°C for 2 min
5.	Vortex for 30 sec
6.	Sonicate at 45°C for 2 min
7.	Vortex for 1 min
8.	Centrifuge for 5 min @ 2200 rpm

A 4-mL aliquot of each clarified extract was transferred into individual 10-mL volumetric flasks containing 1 mL of IS solution, diluted to volume with water, and mixed by inversion (see Table 4).

Table 4. Preparation of Spiked Vehicle Standards

Spiked vehicle standard	Intermediate solution	Corn oil aliquot (~ g)	Intermediate solution (mL) + extraction solvent (mL)	Extract dilution in water[a] (mL)	Expected conc. in corn oil (~ µg/mL)	Expected analytical conc. in diluent (µg/mL)
B_{11}	1	2	1 + 7	4/10	0.72	0.077536
A_{21}	2	2	1 + 7	4/10	1.4	0.15035
B_{31}	3	2	1 + 7	4/10	2.9	0.31015
A_{41}	4	2	1 + 7	4/10	5.6	0.60138
B_{51}	5	2	1 + 7	4/10	8.6	0.93044
A_{61}	6	2	1 + 7	4/10	11.2	1.2028

[a] Final dilution contained 1-mL IS solution.

4.3.5 Blanks

4.3.5.1 IS Blank

An IS blank was prepared by transferring 1 mL of IS solution into a 10-mL volumetric flask containing 4 mL of acetonitrile, diluting the contents to volume with water, and mixing by inversion.

4.3.5.2 Reagent Blank (D0)

A reagent blank (D0) was prepared by filtering ~ 1 mL of acetonitrile into an autosampler vial.

4.3.5.3 Undosed Vehicle Blanks (C01 and C02)

An undosed vehicle blank (C01) was prepared by transferring a 1-mL aliquot of acetonitrile into a 15-mL centrifuge tube containing ~ 2 g of undosed corn oil. The centrifuge tube was capped and vortexed for ~ 1 min. An aliquot (7 mL) of extraction solution was added to the tube, and the extraction procedure described in Section 4.3.4, Table 3, was followed. After the extraction procedure, a 4-mL aliquot of the clarified extract was transferred into a 10-mL volumetric flask containing 1 mL of IS solution, diluted to volume with water, and mixed by inversion.

A second undosed vehicle blank (C02) was prepared by transferring a 1-mL aliquot of acetonitrile into a 15-mL centrifuge tube containing ~ 2 g of undosed corn oil. The centrifuge tube was capped and vortexed for ~ 1 min. An aliquot (7 mL) of extraction solution was added to the tube, and the extraction procedure described in Section 4.3.4, Table 3, was followed. After the extraction procedure, a 4-mL aliquot of the clarified extract was transferred into a 10-mL volumetric flask containing 1 mL of acetonitrile, diluted to volume with water, and mixed by inversion.

4.4 Determination of Formulation Density

The density of each formulation was determined by transferring triplicate aliquots of formulation into individual 5-mL volumetric flasks and obtaining the weight. The density and mean density were calculated using commonly accepted techniques.

4.5 Preparation of Formulation Samples

The validated formulation range for the method of analysis of ethinyl estradiol in corn oil was from 0.69 to 11.2 µg/mL.[1] Each formulation was prepared in duplicate by transferring a 1-mL aliquot of acetonitrile into individual 15-mL centrifuge tubes containing ~ 2 g of formulation. Each centrifuge tube was capped and vortexed for ~ 1 min. An aliquot (7 mL) of extraction solution was added to each tube, and the extraction procedure described in Section 4.3.4, Table 3, was followed. After the extraction procedure, a 4-mL aliquot of each clarified extract was transferred into an individual 10-mL volumetric flask containing 1 mL of IS solution, diluted to volume with water, and mixed by inversion (see Table 5).

Table 5. Preparation of Formulation Samples

Formulation	Formulation aliquot (~ g)	Acetonitrile (mL) + extraction solvent (mL)	Extract dilution in water [a] (mL)	Expected analytical conc. in diluent (µg/mL)
0.001 mg/mL	2	1 + 7	4/10	0.10830
0.002 mg/mL	2	1 + 7	4/10	0.21680

[a] Final dilution contained 1-mL IS solution.

4.6 Preparation of Mobile Phase

Equal volumes of water and acetonitrile were transferred into an appropriate container and mixed by inversion. Before use, the mobile phase was degassed using sonication under vacuum pressure.

4.7 Instrumental System and Parameters

Aliquots of the spiked vehicle standards, samples, and blanks were filtered through 0.2-µm Acrodisc filters into autosampler vials and analyzed using the HPLC system and parameters described below in Table 6. Representative chromatograms for the formulation analysis of ethinyl estradiol in corn oil are presented in Figure 1.

Table 6. HPLC Conditions

Liquid Chromatograph:	Waters Alliance 2695 Separations Module
HPLC Column:	Metachem Inertsil ODS-2, 5 µm, 250 mm x 2.0 mm ID
Guard Column:	Metachem MetaGuard, Inertsil ODS-2, 5 µm, 25 mm x 2.0 mm ID
Detector:	Waters 474 Scanning Fluorescence (210 nm for excitation and 310 nm for emission), Gain = 100
Mobile Phase:	ACN:H_2O (50:50, v/v)
Flow Rate:	0.2 mL/min
Injection Volume:	10 µL
Data System:	Turbochrom, Version: 6.1.1
Run Time:	20 min
Retention Time:	Ethinyl Estradiol: ~ 11.1 min 2-Phenylphenol (IS): ~ 13.9 min

4.8 Calculations

1. The density of each formulation was calculated by dividing the weight of each formulation aliquot by the aliquot volume. The mean density of each formulation was calculated.
2. The density of corn oil was calculated by dividing the weight of each corn oil aliquot by the aliquot volume. The mean density of corn oil was calculated.
3. A peak height ratio (PHR) was calculated for ethinyl estradiol as follows:

$$PHR = \frac{\text{Peak Height}_{\text{Ethinyl Estradiol}}}{\text{Peak Height}_{\text{Internal Standard}}}$$

4. The slope, y-intercept, and correlation coefficient were calculated using a linear regression analysis of the spiked vehicle standard curve by relating the PHR of each spiked vehicle standard to its corresponding expected analytical concentration (µg/mL).
5. The determined concentration of each sample was calculated using the regression equation obtained for the spiked vehicle standard curve and the PHR for each sample by using the following equation:

$$\text{Determined conc. } (\mu g/mL) = \frac{PHR - (y\text{-intercept})}{\text{slope}} \times \frac{\text{Dilution Factor}}{\text{Weight of Sample}} \times \text{Density}$$

where:
Dilution factor = 20 mL
Weight of sample = ~2 g
Density = ~0.92 g/mL

6. The sample average, average standard deviation, and percent relative average deviation (% RAD) were calculated using commonly accepted techniques.
7. The percent target concentration was calculated as follows:

$$\%\text{Target} = \frac{D}{T} \times 100$$

where: D = determined concentration (µg/mL in corn oil)
T = target concentration (µg/mL in corn oil)

8. The average percent target concentration with the corresponding statistical data was calculated for each concentration level.
9. The system suitability parameters for instrumental precision, peak tailing (T), theoretical plates (N), and peak resolution (R) were calculated for ethinyl

estradiol and the internal standard peaks according to USP guidelines.[3] The instrumental precision (% RSD) for system suitability was calculated from six replicate injections of a mid-range spiked vehicle standard.

10. Sample mean ($\bar{x}$), standard deviation (s), and percent relative standard deviation (% RSD) were calculated using commonly accepted techniques.
11. The response ratio (smallest value/largest value) of the duplicate analysis was calculated for each formulation.

4.9 Results

The density of corn oil was 0.92857 g/mL. The density of the 0.001 and 0.002 mg/mL formulations were 0.92333, and 0.92253 g/mL, respectively. The data for the determined concentrations of the formulations of ethinyl estradiol in corn oil are presented in Table 7.

The linear regression equation derived from the data on January 13, 2003, for the spiked vehicle standard curve used for quantitation of the formulation concentrations had a linear correlation of ≥ 0.999. The y-intercept and slope values calculated from the linear regression equation were –0.00284900 and 2.45349, respectively. The data for system suitability are presented in Table 8.

Table 7. Results of the Formulation Analysis of Ethinyl Estradiol in Corn Oil for TherImmune Research Corporation—Formulation Date: December 23, 2002

Formulation sample	Target concentration (µg/mL)	Determined concentration (µg/mL)	Average determined concentration (µg/mL)	% Target	Average % Target
Group No. 2, Mix No. 3	1.000	1.834 1.842	1.838 ± 0.004 (d) 0.2% RAD	183.4 184.2	183.8 ± 0.4 (d) 0.2% RAD
Group No. 3, Mix No. 3	2.000	2.245 2.236	2.241 ± 0.005 (d) 0.2% RAD	112.3 111.8	112.1 ± 0.3 (d) 0.3% RAD

Table 8. System Suitability Results

System precision	Theoretical plates	Tailing factor	Resolution [a]
Analytical results (January 13, 2003):			
% RSD = 1.6 (n = 6)[a]	Ethinyl Estradiol = 4796	1.4	4.2
	IS = 6601	1.2	
Method criteria [b]			
% RSD ≤ 4.5	Ethinyl Estradiol ≥ 900 IS ≥ 1600	1.0 ≤ T ≤ 1.6	≥ 1.8

[a] Between ethinyl estradiol and IS peaks.
[b] All system suitability criteria were met.

[3] *United States Pharmacopeia*, 25th Edition, pp. 1990-1991.

5. Conclusions

The purpose of this study was to perform a formulation analysis of ethinyl estradiol in corn oil received from TherImmune Research Corporation. Samples included dose verification samples (one vial each) at concentrations of 0.001 and 0.002 mg/mL ethinyl estradiol in corn oil. The concentration of the dose formulations was determined with TherImmune-supplied test article received on December 5, 2002, for MRI Task No. 593 (ETP Task No. CHEM07194) as an analytical standard.

The average concentrations of the dose verification samples (Mix No.: 3) were determined to be 1.838 and 2.241 µg/mL ethinyl estradiol in corn oil. The dose verification samples were not within ± 10% of the respective target concentrations. The 0.001 mg/mL formulation was 183.8% of the target concentration. The 0.002 mg/mL formulation was 112.1% of the target concentration.

6. Contributors

Personnel contributing to this study were Sherry Ferguson and Kathryn Brackman.

MIDWEST RESEARCH INSTITUTE

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Study Director

Dale Messer, Ph.D.
Group Leader

Approved:

for Roger K. Harris, Ph.D.
Principal Investigator
Environmental Toxicology Program

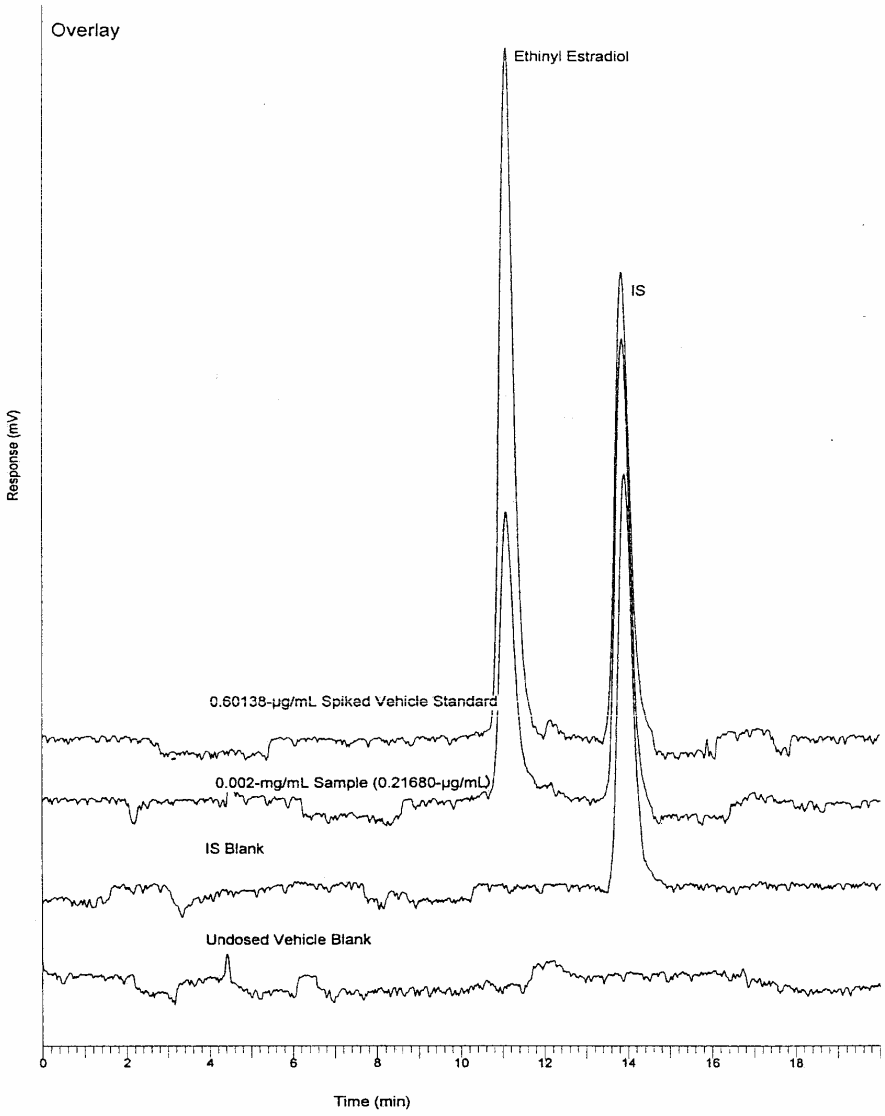

Figure 1. Representative Chromatograms of Ethinyl Estradiol in Corn Oil: 0.60138 µg/mL Spiked Vehicle Standard, 0.002 mg/mL Sample (0.21680 µg/mL), IS Blank, and Undosed Vehicle Blank

RTI INTERNATIONAL

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Chemistry Support Services for the ETP
NIH Contract No. N01-ES-05455
RTI Project 07939.004.003.102
ChemTask No. CHEM07223

METHOXYCHLOR (95%)

IN CORN OIL

FORMULATION ANALYSIS REPORT

Mix Date: December 3, 2002

March 7, 2003

Prepared by:

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Approved by:

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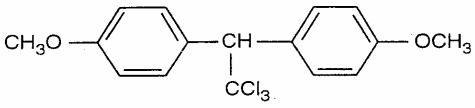
7244-600
Rocca
Nehrebecky
Patel

METHOXYCHLOR (95%)

CAS No.: 72-43-5	Lot No.: 124F0575 (Sigma)
ChemTask No.: CHEM07223	Samples Received (RTI Log Nos.): 9 x 35 mL dosed (10011-44-02 to 10)
RTI Chemical ID Code: J93	1 x 35 mL control (10011-44-01)
Program Supported: RTX	Sample Receipt Date: Dec 12, 2002
Analysis Dates: Dec 19-30, 2002	Submitter: G. Holley
Interim Results Date: Dec 31, 2002	Study Lab: Therimmune Research Corp
Mix Date: Dec 3, 2002	Shipping Container: Amber glass septum vials in polybags, on cold packs, in metal can,
Vehicle: Corn Oil	in fibreboard box
Storage Condition: Refrigeration	Receipt Condition: Good

STRUCTURE	MOL. WT.	FORMULA
	345.65	$C_{16}H_{15}Cl_3O_2$

EXECUTIVE SUMMARY

In support of the Reproductive Toxicology Program, a formulation analysis was performed to determine the methoxychlor (95%) content of nine dose formulations and one control prepared in corn oil. Analytical results generated by a gas chromatography method indicated the analytical samples of the formulation samples submitted for analysis ranged from 98% to 106% of the target concentrations. The homogeneity samples collected at the low dose level (5 mg/mL) were found to contain 107% (n=6; 1.8% RSD) of their nominal concentration while the homogeneity samples collected at the high dose level (20 mg/mL) contained 101% (n=6; 3.4%RSD) of their nominal concentration. No test chemical was detected in the control sample (estimated detection limit was 0.001 mg/mL).

RTI INTERNATIONAL

Quality Assurance Statement

Chemical Name: Methoxychlor (95%)

Task Type: Formulation Analysis

RTI Task Number: 07939.004.003.102

Chem Task Number: CHEM07223

This task was audited by the Chemistry and Life Sciences Quality Assurance Unit and the results of the inspections and audits were reported to the task leader and management as identified below. To the best of our knowledge, the reported results accurately describe the study methods and procedures used, and the reported results accurately reflect the raw data.

Inspections and Audits	Inspection and Audit Date(s)	Date Inspection/Audit Report Sent to Task Leader and Management
Sample Preparation Inspection	12/19/2002	12/19/2002
Data/Report Audit	2/11-19/2003	2/24/2003

Tiffany M. Kenney
Quality Assurance Specialist

3-7-2003
Date

Approval:

Doris J. Smith
Quality Assurance Manager

3-7-2003
Date

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METHOXYCHLOR (95%)

1.0 INTRODUCTION

The purpose of this work was determine the methoxychlor (95%) content in corn oil for samples submitted by Therimmune Research Corp. To accomplish this objective, a formulation analysis (SOP CSC-1030.8) was performed.

2.0 SAMPLE IDENTIFICATION

The following dosage samples were received and analyzed for methoxychlor (95%) content.

RTI Log No.	Study Lab ID	Target Conc. (mg/mL)
10011-44-01	White	0
10011-44-02	Pink FA	5
10011-44-03	Pink Top	5
10011-44-04	Pink Middle	5
10011-44-05	Pink Bottom	5
10011-44-06	Yellow FA	10
10011-44-07	Purple FA	20
10011-44-08	Purple Top	20
10011-44-09	Purple Middle	20
10011-44-10	Purple Bottom	20

3.0 SAMPLE ANALYSIS

Procedures: SOP CSC-1321.13
SOP CSC-1030.8
AM CSC- 3406.0

The AM cited in this report contained the following revisions:

1) The estimated LOQ was corrected to state 0.003 mg/mL.
2) A GC column with a film thickness of 0.5 µm was used for this effort.
3) Section numbering was revised where required.

4.0 SAMPLE RESULTS

Notebook No.: 10589 *pp.*: 27-29

The concentrations of methoxychlor (95%) found in the dose formulations are tabulated below. Typical chromatograms are shown in Figure 1. A representative vehicle standards plot is illustrated in Figure 2. The weighted ($1/x^2$) linear regression equations: y = 1.178x + 0.1225; r = 0.9989 and y=1.329x - 0.7579; r=0.9936 were used for instrument calibration and sample quantitation.

Study Lab ID	RTI Log No.	Nominal Conc. (mg/mL)	Found Conc. (mg/mL)	Replicate Ratio[a]	% of Nominal
White	10011-44-01	0	ND[b]	-	-
Pink Group 4/ Mix 1	10011-44-02	5	5.28	0.97	106
Pink Top	10011-44-03	5	5.25 5.36 5.45	0.98	
Pink Middle	10011-44-04	5	5.37 5.19 5.38	0.99	$\bar{x}$=107[d]
Pink Bottom	10011-44-05	5	$\bar{x}$=5.33 %RSD = 1.8 n = 6	0.96	
Yellow Group 5/Mix 1	10011-44-06	10	9.78	0.98	97.8
Purple Group 6/Mix 1	10011-44-07	20	19.6	0.97	98.0
Purple Top[c]	10011-44-08	20	19.4 19.3 21.0	0.99	
Purple Middle	10011-44-09	20	20.6 20.3 20.4	0.98	$\bar{x}$=101[d]
Purple Bottom	10011-44-10	20	$\bar{x}$=20.2 %RSD = 3.4 n = 6	1.0	

[a] Y_l/Y_h (low/high found concentrations from duplicate analysis).
[b] Not Detected. The estimated limit of detection = 0.001 mg/mL.
[c] The weighted ($1/x^2$) linear regression equation; y=1.329x - 0.7579; r=0.9936 was used to quantitate this sample. All other samples used the following: y = 1.178x + 0.1225; r = 0.9989.
[d] % Nominal value based on $\bar{x}$ found concentration.

5.0 ACKNOWLEDGMENT

Personnel contributing to the performance of this task included Gwen McNeill and James Blake.

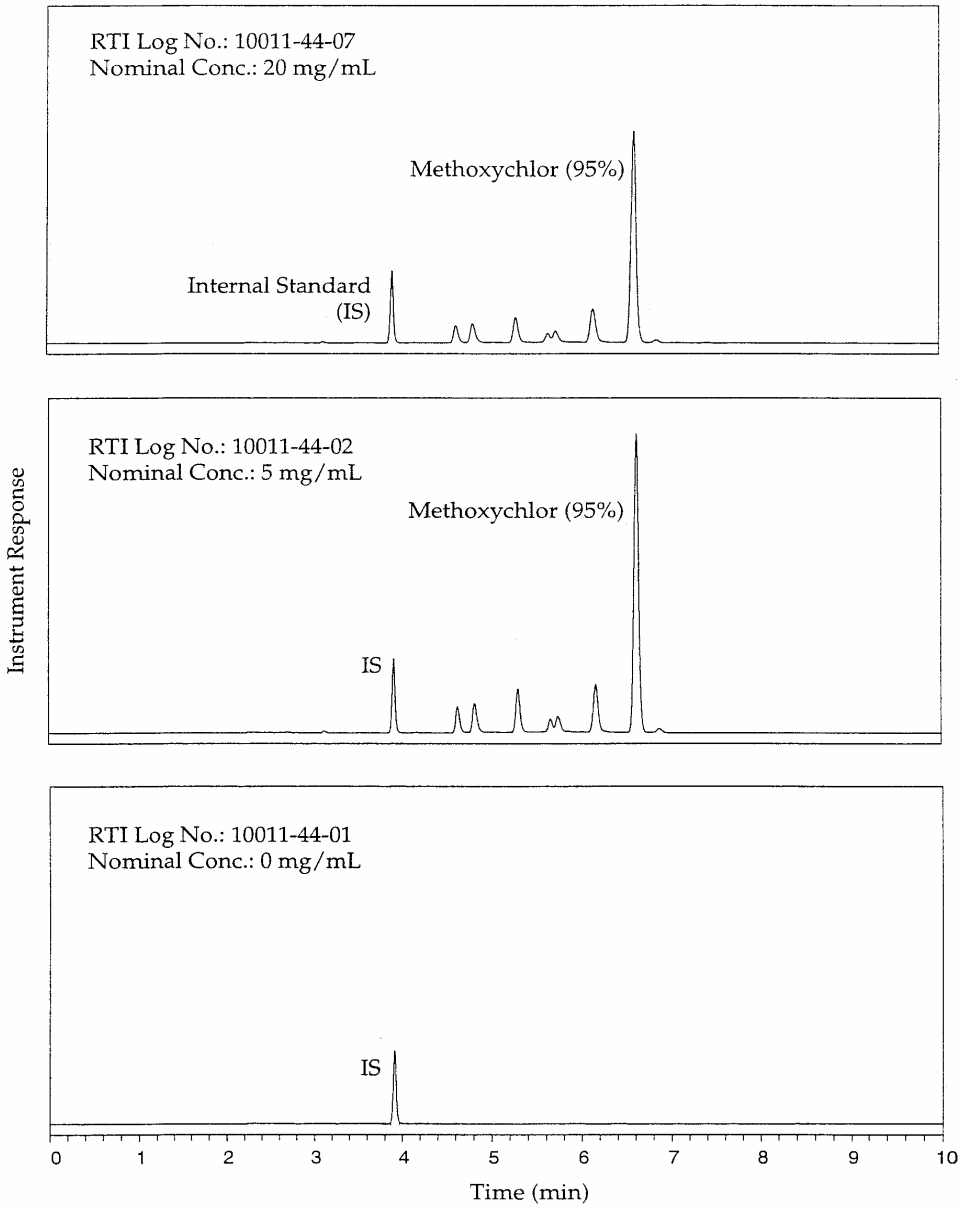


Figure 1. Representative Gas Chromatograms of Methoxychlor (95%) in Corn Oil

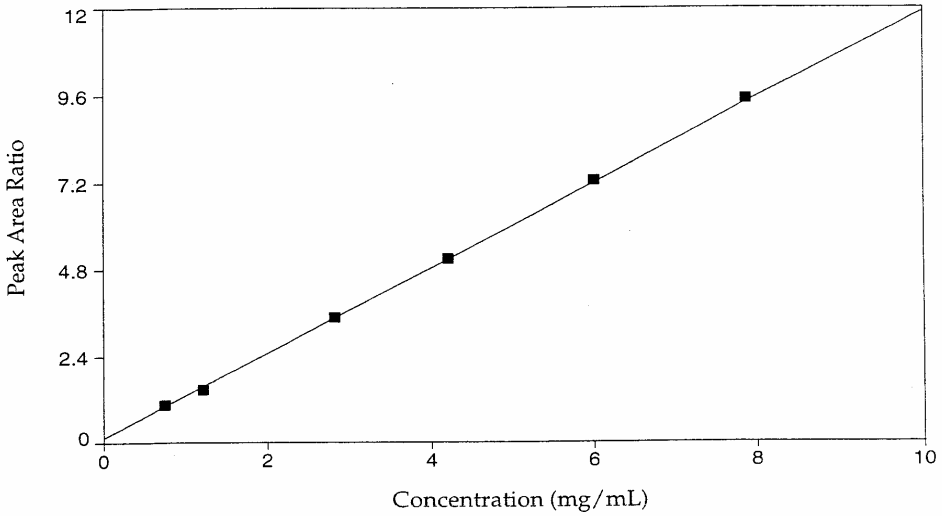


Figure 2. Representative Plot of Vehicle Standards Data - Methoxychlor (95%) in Corn Oil

APPENDIX

Analytical Method CSC - 3406.0

Formulation Analysis of Methoxychlor (95%) in Corn Oil

ANALYTICAL METHOD	RTI INTERNATIONAL 3040 CORNWALLIS ROAD RESEARCH TRIANGLE PARK, NC 27709-2194	AM CSC-3406.0 Page 1 of 12

TITLE: FORMULATION ANALYSIS OF METHOXYCHLOR (95%) IN CORN OIL

AUTHOR: Signed _____
Analytical Chemist

Date 12-19-02

APPROVED BY: Signed _____
Principal Investigator

Date 12-19-02

ANALYTICAL METHOD	RTI INTE ATIONAL 3040 CORNWALLIS ROAD RESEARCH TRIANGLE PARK, NC 27709-2194	AM CSC-3406.0 Page 2 of 12

1.0 METHOD SUMMARY

The methoxychlor (95%) content in corn oil formulations submitted from various study laboratories is determined using a capillary gas chromatographic method coupled with electron capture detection (GC/ECD). The analytical procedure for which this AM is based was validated from 0.008 to 8 mg/mL with a verification to 30 mg/mL. The experimental limit of quantitation (ELOQ) is 0.008 mg/mL, with an estimated limit of detection (LOD) of 0.001 mg/mL, and an estimated limit of quantitation (LOQ) of 0.004 mg/mL. Formulation sample sets consisting of more than one dose level should be analyzed using a 6-point curve. Sets with only one dose level may be analyzed with a 3-point curve which brackets the dose sample by approximately +/-20%.

AM is used for ETP Task No.:_____

2.0 SAMPLE RECEIPT, STORAGE, AND HANDLING

The formulation samples should be stored at refrigerated temperatures. Samples throughout the dose range can be handled as solutions.

> NOTE: Due to the toxicity of the test compound, all transferring of the neat material should be performed in a sealed glove box as a measure of safety. Formulations should be mixed by a combination of sonication and magnetic stirring overnight and prior to sampling to insure a uniform solution. Formulations at the upper end of the dose range (30 mg/mL) may require additional mixing time.

3.0 REAGENTS

Refer to Table 1 for all required chemicals. Use this table to document the supplier, the lot number and expiration date for each reagent, making changes and/or additions where necessary. Any critical requirements for reagents are noted.

Table 1. Reagents

Reagent	Supplier	Lot Number	Exp. Date
Methoxychlor			
Aldrin- Internal Standard(IS)			
Hexane			
Corn Oil (vehicle)			

Analyst: _____ Date: _____

ANALYTICAL METHOD	RTI INTE ATIONAL 3040 CORNWALLIS ROAD RESEARCH TRIANGLE PARK, NC 27709-2194	AM CSC-3406.0 Page 3 of 12

4.0 EQUIPMENT

Refer to Table 2 for a list of the major equipment required. Equivalent systems are acceptable. Use this table to document the specific information (where applicable) for each piece of equipment. Check the maintenance and calibration records for each piece of equipment (where applicable) to be sure they are current.

Table 2. Equipment

Equipment	Manufacturer/Model No.	Other Info.
500-mL bottle (or size as required) for IS		
Mohr pipettes, large graduated cylinder(s) 5,25-mL, 1-L volumetric flasks, 20-mL scintillation vials , beakers, Pasteur pipets		
Analytical balance		
Vortex mixer		
Stirrer		
Autosampler vials (amber, glass)		
GC column	SEE ATTACHMENT	
GC system with ECD	SEE ATTACHMENT	

Analyst: _____ Date: _____

ANALYTICAL METHOD	RTI INTE TIONAL 3040 CORNWALLIS ROAD RESEARCH TRIANGLE PARK, NC 27709-2194	AM CSC-3406.0 Page 4 of 12

5.0 LABWARE PREPARATION

Be sure all labware is properly cleaned per normal procedures prior to use. No additional precautions are required.

6.0 PROCEDURE

Note: At each critical step, the analyst should provide a check mark to signify that step was completed.

6.1 General Instructions

Check or calibrate equipment according to relevant SOPs. ☐

It is also important to record scientific observations and deviations from the SOP in the notebook. ☐

List of relevant SOPs: ☐

1.
2.
3.
4.
5.
6.

6.2 Preparation of Internal Standard Solution (IS) and Methoxychlor Standards

6.2.7. Preparation of IS ☐

NOTE: This procedure describes the preparation of 1 L of working IS. If more or less volume is required, then adjust weights and volumes accordingly.

Prepare a stock solution (1000 µg/mL) of Aldrin by transferring 5 mg of Aldrin into a 5-mL volumetric flask and dilute to volume with hexane. Sonicate to mix.

Prepare a working solution of IS at ~ 1 µg/mL by transferring 1 mL of the above stock solution into a 1-liter volumetric flask and dilute to volume with hexane. Mix by inversion and transfer to an appropriately sized bottle and cap. The working IS should have a concentration of approximately 1 µg of Aldrin/mL.

Analyst: _____ Date: _____

ANALYTICAL METHOD	RTI INTE ATIONAL 3040 CORNWALLIS ROAD RESEARCH TRIANGLE PARK, NC 27709-2194	AM CSC-3406.0 Page 5 of 12

Actual aliquot wt. of Aldrin_____

Actual Dilution Volume (mL):_____

Working IS concentration: _____ µg/mL ☐

6.2.2. Preparation of Vehicle Standards

NOTE: Procedure describes a 6-pt calibration scheme. If only a 3-pt calibration is required, a single stock may be prepared with three subsequent dilutions at the appropriate concentrations for each calibration standard required.

Prepare an ~12 mg/mL stock (A) by transferring ~240 mg of methoxychlor to a tared pre-calibrated (to 20 mL) scintillation vial. Dilute to the mark with corn oil (vehicle), and mix by sonication for 45 minutes followed by stirring for an additional 30 minutes.

Actual aliquot wt. of Test Chemical: _____

Final total dilution volume (mL): _____

Final Concentration: _____ mg/mL ☐

Prepare an ~8 mg/mL stock (B) by transferring ~160 mg of methoxychlor to a tared pre-calibrated (to 20 mL) scintillation vial. Dilute to the mark with corn oil (vehicle), and mix by sonication for 45 minutes followed by stirring for and additional 30 minutes.

Actual aliquot wt. of Test Chemical: _____

Final total dilution volume (mL): _____

Final Concentration: _____ mg/mL ☐

Six dilutions (or three as required) from the stock solutions (A and B) may be prepared as shown in the table below. For a three-point calibration, only three dilutions from one of the above stocks is required. All calibration standards are prepared in corn oil on a weight/weight basis. Transfers from the two stocks should be conducted with disposable pipets into volumetric flasks and/or scintillation vials. The stock(s) should be re-mixed prior to their use.

Analyst: _____ Date: _____

ANALYTICAL METHOD	RTI INTE[\TIONAL 3040 CORNWALLIS ROAD RESEARCH TRIANGLE PARK, NC 27709-2194	AM CSC-3406.0 Page 6 of 12

Table 3. Preparation of Calibration Standards

Vehicle Standard	Vehicle Stock	Target Dil. (g)/ Actual Dil. (g)	Final Dil. (g)/ Actual Dil. (g)		Target Concentration (mg/mL)	✔ = Prep.
VA_1	A	~0.667/	~1.0/	=	~8.0 Actual Conc.	
VB_1	B	~0.75/	~1.0/	=	~6.0 Actual Conc.	
VA_2	A	~0.35/	~1.0/	=	~4.20 Actual Conc.	
VB_2	B	~0.35/	~1.0/	=	~2.8 Actual Conc.	
VA_3[c]	A	~1.0/	~10 g with blank corn oil/ ~1.0 g aliquot for analysis/	=	~1.2 Actual Conc.	
VB_3[c]	B	~1.0/	~10 g with blank corn oil/ ~1.0 g aliquot for analysis/	=	~0.80 Actual Conc.	
Vblank	Blank Corn Oil	~1.0/	-	=	0/0 Actual Conc.	

[a]Dilutions conducted with blank corn oil. Final dilution should be +/-1% of target.
[b]If the final dilution wt. is ≤1% of target than use target wt. to express concentration; If >1% use actual dilution wt. to express concentration.
[c]Standard requires subsequent removal of 1.0-g aliquot prior to its use.

Analyst: _____ Date: _____

ANALYTICAL METHOD	RTI INTE ATIONAL 3040 COHNWALLIS ROAD RESEARCH TRIANGLE PARK, NC 27709-2194	AM CSC-3406.0 Page 7 of 12

6.2.3 Preparation of Vehicle Calibration Standards ☐

Standards B2 through A1 and the vehicle blank are prepared directly in 25-mL volumetric flask while standards B3 and A3 are prepared initially in 20-mL scintillation vials from which 1.0-g aliquots are transferred into 25-mL volumetric flasks. Dilute each to volume with hexane, mix by inversion, and then transfer 4-mL aliquots from each to individual 25-mL volumetric flasks. Dilute to volume with IS (1 µg of Aldrin/mL hexane) and mix by inversion. Transfer an aliquot into autosampler vials for GC/ECD analysis.

6.3 Preparation of Formulation Samples for Analysis ☐

[Note: All samples should be stirred prior to and during their analytical preparation.]

Remove from storage and mix each dose formulation by a combination of vortex action and stirring. Transfer duplicate 1-g aliquots from each dose (≤6.4 mg/mL) and control sample to individual 25-mL volumetric flasks, dilute to volume with hexane, and mix each by inversion. ☐

Remove duplicate 1-g aliquots from any formulations with nominal concentrations greater than 6.4 mg/mL but not exceeding 30 mg/mL to scintillation vials and dilute with blank vehicle to within the calibration range, mix, and then remove single ~1-g aliquots from these dilutions and proceed as stated above. ☐

Transfer 4-mL aliquots from all dilutions to individual 25-mL volumetric flasks and dilute to volume with IS (1 µg of aldrin/mL of hexane) and mix by inversion. Transfer an aliquot from each into autosampler vials for GC/ECD analysis. ☐

Analyst: _____ Date: _____

ANALYTICAL METHOD	RTI INTE \TIONAL 3040 CORNWALLIS ROAD RESEARCH TRIANGLE PARK, NC 27709-2194	AM CSC-3406.0 Page 8 of 12

Table 4. Formulation Aliquot Transfers (≤ 6.4 mg/mL)

Sample ID/Color Code	Aliquot No.	Target Sample Aliquot Wt. (g) +/-1%	Actual Sample Aliquot Wt.[a](g) +/-1%
	1	1	
	2	1	
	1	1	
	2	1	
	1	1	
	2	1	
	1	1	
	2	1	
	1	1	
	2	1	
	1	1	
	2	1	
	1	1	
	2	1	

[a]Actual weights should be recorded and used in determining the final calculations for that sample.

Analyst: _____ Date:_____

ANALYTICAL METHOD	RTI INTE ATIONAL 3040 CORNWALLIS ROAD RESEARCH TRIANGLE PARK, NC 27709-2194	AM CSC-3406.0 Page 9 of 12

Table 5. Dilutions of Methoxychlor Formulations
(Nominal Concs.: 6.4 mg/mL ≤ 30 mg/mL)

Sample ID/Color Code	Aliquot No.	Target Sample Aliquot Wt. (g)/ Actual Aliquot Wt. (g)	Target Dilution Wt.[a] (g) Actual Dilution Wt. (g)
	1	~1/	~5/
	2		
	1	~1/	~5/
	2		
	1	~1/	~5/
	2		
	1	~1/	~5/
	2		
	1	~1/	~5/
	2		
	1	~1/	~5/
	2		

[a]Target wts. and dilution wts. are given as estimated values for AM. Actual volumes may differ based on sample amounts and concs. received under a given task but the dilution scheme should achieve a conc. not less than 0.96 mg/mL and not greater than 6.4 mg/mL for this AM.
[b]If the final dilution wt. is ≤1% of target than use target wt. to determine dilution factor; If >1% use actual dilution wt. to determine dilution factor.

Analyst: _____ Date: _____

ANALYTICAL METHOD	RTI INTE ATIONAL 3040 CORNWALLIS ROAD RESEARCH TRIANGLE PARK, NC 27709-2194	AM CSC-3406.0 Page 10 of 12

Table 6. Subsequent Transfers for Formulations (> 6.4 mg/mL)

Sample ID/Color Code	Aliquot No.	Target Sample Aliquot Wt. (g) +/-1%	Actual Sample Aliquot Wt.[a](g) +/-1%
	1	1	
	2	1	
	1	1	
	2	1	
	1	1	
	2	1	
	1	1	
	2	1	
	1	1	
	2	1	
	1	1	
	2	1	
	1	1	
	2	1	

[a]Actual weights should be recorded and used in determining the final calculations for that sample.

Analyst: _____ Date:_____

ANALYTICAL METHOD	RTI INTERNATIONAL 3040 CORNWALLIS ROAD RESEARCH TRIANGLE PARK, NC 27709-2194	AM CSC-3406.0 Page 11 of 12

6.4 Analytical Method

The following instrumentation and parameters may be used for the analysis of methoxychlor formulations. Equivalent instrumentation and parameters are acceptable, if they give equivalent results to the method. Single injections of each standard, blank, and sample are sufficient unless otherwise warranted.

GC Parameters

System	HP 5890 GC-ECD with HP 6890 Autosampler, or equivalent
Software	LabSystems Atlas 2000 rev. 2 or equiv.
Column[a]	J & W DB-5 (30m x 0.32mm ID); 1 um film, or equivalent
Carrier Gas	Helium
Column Temperature[a]	Isothermal @ 290°C
Flow Rate	2.0 mL/min
Injection Temperature-Type	250°C- Split (~20/1)
Inj. Solvent-Volume	Hexane - 1 µL
Retention Times (min)	Aldrin (IS) - ~3.7 min, Methoxychlor - ~6.1 min
Detector	Electron Capture (ECD) at 300°C

[a]A GC column with a differing film thickness or diameter may be substituted but may require an alteration of the column temperature to achieve similar retention times listed. Any such change should be noted in the appropriate notebook by the analyst.

6.5 Calculations

Methoxychlor is quantitated based on the observed peak area of its major component. For each injection, the peak area ratio for test chemical to the area of the internal standard is calculated as:

$$Peak\ Area\ Ratio = \frac{Peak\ Area\ for\ Methoxychlor}{Area\ of\ Internal\ Standard}$$

Analyst: _____ Date: _____

ANALYTICAL METHOD	RTI INTERNATIONAL 3040 CORNWALLIS ROAD RESEARCH TRIANGLE PARK, NC 27709-2194	AM CSC-3406.0 Page 12 of 12

A weighted ($1/x^2$) or a non-weighted linear regression fit may be selected based on the calibration range required. The estimated LOD is 0.001 mg/mL; the estimated LOQ is 0.004 mg/mL. The ELOQ is defined as 0.008 mg/mL.

For each dose formulation, calculate the peak area ratio of each aliquot for the test chemical as above. Use these peak area ratios and the linear regression equation to determine the methoxychlor concentration of each formulation aliquot. Also, account for any samples that required dilution prior to analysis in order to determine their final found concentrations. Based on final found concentration values, a replicate ratio (Y_l/Y_h) of ≥ 0.9 must be achieved between duplicate grabs of a given dose sample. If not, a third aliquot is prepared and a mean value is reported (n=3) with a percent relative standard deviation (%RSD). The Q-test (95% confidence interval) may be used to define an extreme outlier with its result recorded in the appropriate notebook.

Analyst: _____ Date: _____

APPENDIX

(See Attached)

HEWLETT-PACKARD _____ GC-_____
S/N: _____
in Lab 135

Data System:
LabSystems Atlas 2000 Revision 2
Chromserver: _____
Channel: _____
System Maintenance: Last: _____ Next: _____

Column:
Manufacturer: _____
Stationary Phase: _____
Dimensions: _____
S/N: _____

Injection:
6890 Autosampler - S/N_____/_____ (√) Manual _____ (√)
Split: _____ (√) Splitless: _____ (√)
Splitless/Split: _____ (√)
Time Splitless: _____ (min) Volume: _____ (µL)
Split Ratio: _____ : _____ Injector Temperature: _____ (°C)

Conditions:
Carrier: _____ Flow Rate: _____ (mL/min) Head Pressure: _____ (psi)
Temperature Program: _____
_____
Flows: Make-up gas: _____ (mL/min) Air: _____ (mL/min)
Septum purge: _____ (mL/min) Anode purge gas: _____
Hydrogen: _____ (mL/min) Anode purge gas: _____ (mL/min)

Detector:
Type: FID _____ (√) ECD _____ (√)
Temperature: _____ (°C)
Range: _____
Attenuation: _____

Analyst: _____ Date: _____

REVIEW AND REVISION LOG	RTI INTERNATIONAL 3040 CORNWALLIS ROAD RESEARCH TRIANGLE PARK, NC 27709-2194	AM CSC-3406 Page 1 of 1

FORMULATION ANALYSIS OF METHOXYCHLOR (95%) IN CORN OIL

REVIEW/REVISION LOG

Rev. #	Date	Description
0	12/19/02	Original version.

RTI INTERNATIONAL
NTP Chemistry Support Services
3040 Cornwallis Road ▪ PO Box 12194 ▪ Research Triangle Park, NC 27709-2194 ▪ USA
Telephone 919-541-6581 or 919-541-5975 ▪ Fax 919-541-7208 ▪ www.rti.org

Chemistry Support Services for the ETP
NIH Contract No. N01-ES-05455
RTI Project 07939.004.003.103
ChemTask No. CHEM07231

METHOXYCHLOR (95%)

IN CORN OIL

FORMULATION ANALYSIS REPORT

Mix Date: December 13, 2002

March 4, 2003

Prepared by:
Gwen McNeill
Analytical Chemist

Approved by:
James C. Blake
Task Leader

Charles M. Sparacino, Ph.D.
Principal Investigator

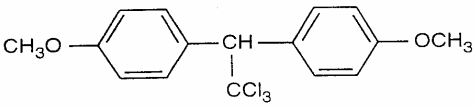
Submitted to:

Mr. Bradley J. Collins
National Institute of Environmental Health Sciences
P.O. Box 12233
111 T. W. Alexander Drive
Research Triangle Park, NC 27709-2233

7244-600
WOLFE
ROCCA
NEHRFBECKYJ

METHOXYCHLOR (95%)

CAS No.: 72-43-5	Lot No.: 124F0575 (Sigma)
ChemTask No.: CHEM07231	Samples Received (RTI Log Nos.): 3 x 35 mL dosed (10011-52-02 to 04)
RTI Chemical ID Code: J93	1 x 35 mL control (10011-52-01)
Program Supported: RTX	Sample Receipt Date: Dec 17, 2002
Analysis Dates: Dec 19-30, 2002	Submitter: G. Holley
Interim Results Date: Dec 31, 2002	Study Lab: Therimmune Research Corp
Mix Date: Dec 13, 2002	Shipping Container: Amber glass septum vials wrapped in bubblewrap, on cold packs, in in metal can, in fiberboard box
Vehicle: Corn Oil	
Storage Condition: Refrigeration	Receipt Condition: Good

STRUCTURE	MOL. WT.	FORMULA
	345.65	$C_{16}H_{15}Cl_3O_2$

EXECUTIVE SUMMARY

In support of the Reproductive Toxicology Program, a formulation analysis was performed to determine the methoxychlor (95%) content of three dose formulations and one control prepared in corn oil. Analytical results generated by a gas chromatography method indicated the samples submitted for analysis ranged from 91.4% to 98.2% of the target concentrations. No test chemical was detected in the control sample (estimated detection limit was 0.001 mg/mL).

RTI INTERNATIONAL

Quality Assurance Statement

Chemical Name: Methoxychlor (95%)

Task Type: Formulation Analysis

RTI Task Number: 07939.004.003.103

Chem Task Number: CHEM07231

This task was audited by the Chemistry and Life Sciences Quality Assurance Unit and the results of the inspections and audits were reported to the task leader and management as identified below. To the best of our knowledge, the reported results accurately describe the study methods and procedures used, and the reported results accurately reflect the raw data.

Inspections and Audits	Inspection and Audit Date(s)	Date Inspection/Audit Report Sent to Task Leader and Management
Sample Preparation	12/19/02	12/19/02
Data and Report Audit	2/13,14,17-19,21,24,25/03	2/25/03

Jennifer Jones
Quality Assurance Specialist

3/4/03
Date

Approval:

Doris J. Smith
Quality Assurance Manager

3-4-2003
Date

turning knowledge into practice

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APPENDIX, Analytical Method CSC-3406.0, Formulation Analysis of Methoxychlor (95%) in Corn Oil

METHOXYCHLOR (95%)

1.0 INTRODUCTION

The purpose of this work was determine the methoxychlor (95%) content in corn oil for samples submitted by Therimmune Research Co. To accomplish this objective, a formulation analysis (SOP CSC-1030.8) was performed.

2.0 SAMPLE IDENTIFICATION

The following dosage samples were received and analyzed for Methoxychlor (95%) content.

RTI Log No.	Study Lab ID	Target Conc. (mg/mL)
10011-52-01	White	0
10011-52-02	Pink	5
10011-52-03	Yellow	10
10011-52-04	Purple	20

3.0 SAMPLE ANALYSIS

Procedures: SOP CSC-1321.13
SOP CSC-1030.8
AM CSC- 3406.0

The AM cited in this report contained the following amendments:

1) The LOQ stated as 0.004 mg/mL was corrected to state 0.003 mg/mL.
2) A GC column with a film thickness of 0.5 µm was used for this effort.
3) Incorrect section numbering was revised where required.

4.0 SAMPLE RESULTS

Notebook No.: 10589 *pp.*: 27-28

The concentrations of Methoxychlor (95%) found in the dose formulations are tabulated below. Representative chromatograms are shown in Figure 1. The vehicle standards plot is illustrated in Figure 2 for the weighted ($1/x^2$) linear regression equation: $y = 1.178x + 0.1225$; $r = 0.9989$.

Study Lab ID	RTI Log No.	Nominal Conc. (mg/mL)	Found Conc. (mg/mL)	Replicate Ratio[a]	% of Nominal
White	10011-52-01	0	ND[b]	-	-
Pink	10011-52-02	5	4.91	0.95	98.2
Yellow	10011-52-03	10	9.14	0.96	91.4
Purple	10011-52-04	20	18.5	0.94	92.5

[a] Y_l/Y_h (low/high found concentrations from duplicate analysis).
[b] Not Detected. The estimated limit of detection = 0.001 mg/mL.

5.0 ACKNOWLEDGMENT

Personnel contributing to the performance of this task included Gwen McNeill.

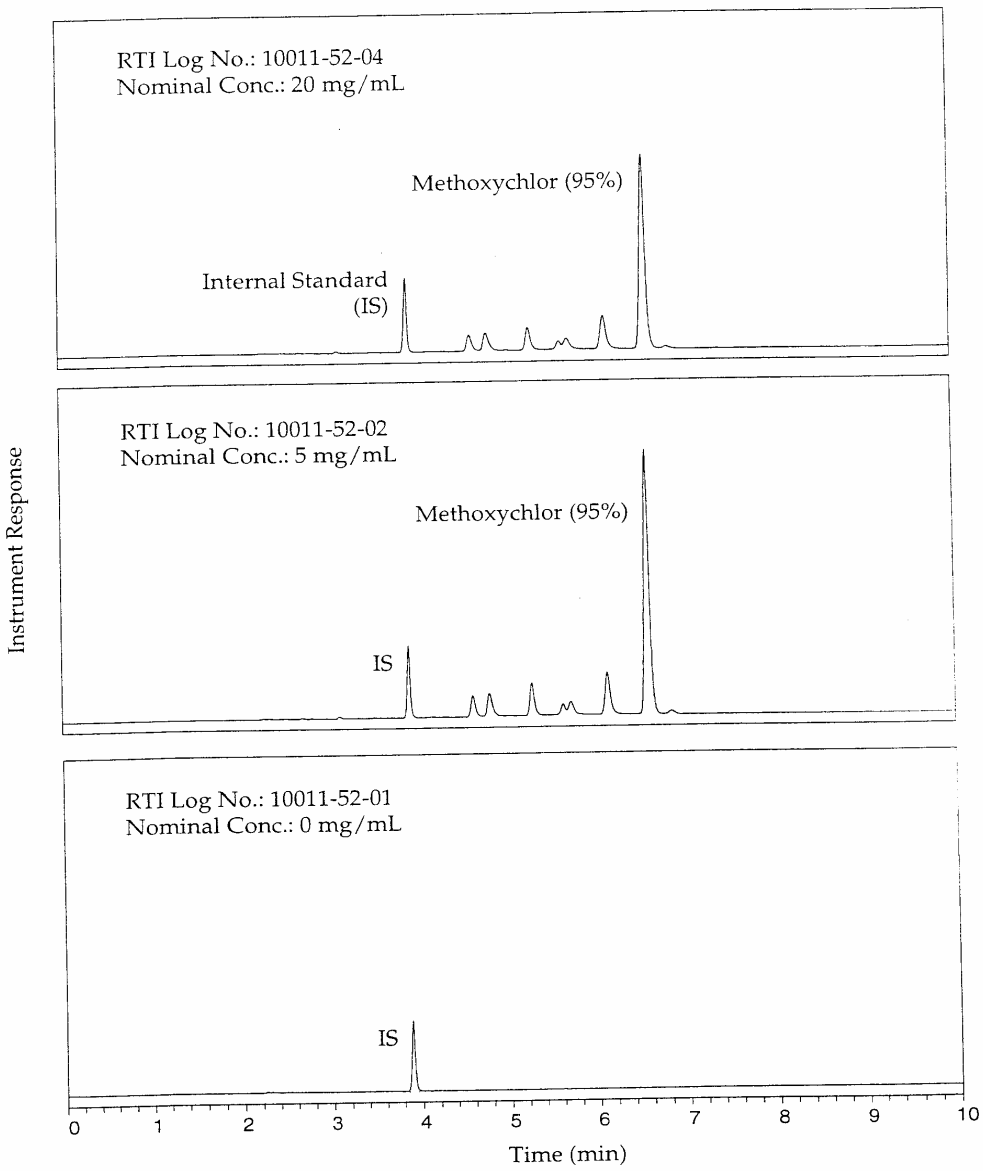

Figure 1. Representative Gas Chromatograms of Methoxychlor (95%) in Corn Oil

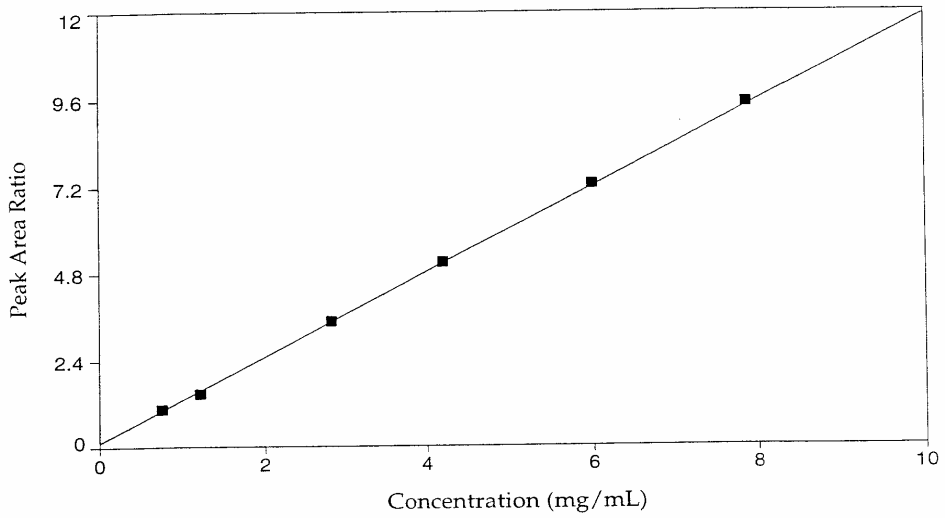

Figure 2. Plot of Vehicle Standards Data - Methoxychlor (95%) in Corn Oil

APPENDIX

Analytical Method CSC - 3406.0

Formulation Analysis of Methoxychlor (95%) in Corn Oil

ANALYTICAL METHOD	RTI INTERNATIONAL 3040 CORNWALLIS ROAD RESEARCH TRIANGLE PARK, NC 27709-2194	AM CSC-3406.0 Page 1 of 12

TITLE: FORMULATION ANALYSIS OF METHOXYCHLOR (95%) IN CORN OIL

AUTHOR: Signed _____
Analytical Chemist

Date 12-19-02

APPROVED BY: Signed _____
Principal Investigator

Date 12-19-02

ANALYTICAL METHOD	RTI INTERNATIONAL 3040 CORNWALLIS ROAD RESEARCH TRIANGLE PARK, NC 27709-2194	AM CSC-3406.0 Page 2 of 12

1.0 METHOD SUMMARY

The methoxychlor (95%) content in corn oil formulations submitted from various study laboratories is determined using a capillary gas chromatographic method coupled with electron capture detection (GC/ECD). The analytical procedure for which this AM is based was validated from 0.008 to 8 mg/mL with a verification to 30 mg/mL. The experimental limit of quantitation (ELOQ) is 0.008 mg/mL, with an estimated limit of detection (LOD) of 0.001 mg/mL, and an estimated limit of quantitation (LOQ) of 0.004 mg/mL. Formulation sample sets consisting of more than one dose level should be analyzed using a 6-point curve. Sets with only one dose level may be analyzed with a 3-point curve which brackets the dose sample by approximately +/-20%.

AM is used for ETP Task No.:_____

2.0 SAMPLE RECEIPT, STORAGE, AND HANDLING

The formulation samples should be stored at refrigerated temperatures. Samples throughout the dose range can be handled as solutions.

NOTE: Due to the toxicity of the test compound, all transferring of the neat material should be performed in a sealed glove box as a measure of safety. Formulations should be mixed by a combination of sonication and magnetic stirring overnight and prior to sampling to insure a uniform solution. Formulations at the upper end of the dose range (30 mg/mL) may require additional mixing time.

3.0 REAGENTS

Refer to Table 1 for all required chemicals. Use this table to document the supplier, the lot number and expiration date for each reagent, making changes and/or additions where necessary. Any critical requirements for reagents are noted.

Table 1. Reagents

Reagent	Supplier	Lot Number	Exp. Date
Methoxychlor			
Aldrin- Internal Standard(IS)			
Hexane			
Corn Oil (vehicle)			

Analyst: _____ Date: _____

ANALYTICAL METHOD	RTI INTERNATIONAL 3040 CORNWALLIS ROAD RESEARCH TRIANGLE PARK, NC 27709-2194	AM CSC-3406.0 Page 3 of 12

4.0 EQUIPMENT

Refer to Table 2 for a list of the major equipment required. Equivalent systems are acceptable. Use this table to document the specific information (where applicable) for each piece of equipment. Check the maintenance and calibration records for each piece of equipment (where applicable) to be sure they are current.

Table 2. Equipment

Equipment	Manufacturer/Model No.	Other Info.
500-mL bottle (or size as required) for IS		
Mohr pipettes, large graduated cylinder(s) 5,25-mL, 1-L volumetric flasks, 20-mL scintillation vials , beakers, Pasteur pipets		
Analytical balance		
Vortex mixer		
Stirrer		
Autosampler vials (amber, glass)		
GC column	SEE ATTACHMENT	
GC system with ECD	SEE ATTACHMENT	

Analyst: _____ Date: _____

ANALYTICAL METHOD	RTI INTERNATIONAL 3040 CORNWALLIS ROAD RESEARCH TRIANGLE PARK, NC 27709-2194	AM CSC-3406.0 Page 4 of 12

5.0 LABWARE PREPARATION

Be sure all labware is properly cleaned per normal procedures prior to use. No additional precautions are required.

6.0 PROCEDURE

Note: At each critical step, the analyst should provide a check mark to signify that step was completed.

6.1 General Instructions

Check or calibrate equipment according to relevant SOPs. ☐

It is also important to record scientific observations and deviations from the SOP in the notebook. ☐

List of relevant SOPs: ☐

1.
2.
3.
4.
5.
6.

6.2 Preparation of Internal Standard Solution (IS) and Methoxychlor Standards

6.2.7. Preparation of IS ☐

NOTE: This procedure describes the preparation of 1 L of working IS. If more or less volume is required, then adjust weights and volumes accordingly.

Prepare a stock solution (1000 µg/mL) of Aldrin by transferring 5 mg of Aldrin into a 5-mL volumetric flask and dilute to volume with hexane. Sonicate to mix.

Prepare a working solution of IS at ~ 1 µg/mL by transferring 1 mL of the above stock solution into a 1-liter volumetric flask and dilute to volume with hexane. Mix by inversion and transfer to an appropriately sized bottle and cap. The working IS should have a concentration of approximately 1 µg of Aldrin/mL.

Analyst: _____ Date: _____

ANALYTICAL METHOD	RTI INTERNATIONAL 3040 CORNWALLIS ROAD RESEARCH TRIANGLE PARK, NC 27709-2194	AM CSC-3406.0 Page 5 of 12

Actual aliquot wt. of Aldrin_____

Actual Dilution Volume (mL):_____

Working IS concentration: _____ µg/mL ☐

6.2.2. Preparation of Vehicle Standards

NOTE: Procedure describes a 6-pt calibration scheme. If only a 3-pt calibration is required, a single stock may be prepared with three subsequent dilutions at the appropriate concentrations for each calibration standard required.

Prepare an ~12 mg/mL stock (A) by transferring ~240 mg of methoxychlor to a tared pre-calibrated (to 20 mL) scintillation vial. Dilute to the mark with corn oil (vehicle), and mix by sonication for 45 minutes followed by stirring for an additional 30 minutes.

Actual aliquot wt. of Test Chemical: _____

Final total dilution volume (mL): _____

Final Concentration: _____ mg/mL ☐

Prepare an ~8 mg/mL stock (B) by transferring ~160 mg of methoxychlor to a tared pre-calibrated (to 20 mL) scintillation vial. Dilute to the mark with corn oil (vehicle), and mix by sonication for 45 minutes followed by stirring for and additional 30 minutes.

Actual aliquot wt. of Test Chemical: _____

Final total dilution volume (mL): _____

Final Concentration: _____ mg/mL ☐

Six dilutions (or three as required) from the stock solutions (A and B) may be prepared as shown in the table below. For a three-point calibration, only three dilutions from one of the above stocks is required. All calibration standards are prepared in corn oil on a weight/weight basis. Transfers from the two stocks should be conducted with disposable pipets into volumetric flasks and/or scintillation vials. The stock(s) should be re-mixed prior to their use.

Analyst: _____ Date: _____

ANALYTICAL METHOD	RTI INTERNATIONAL 3040 CORNWALLIS ROAD RESEARCH TRIANGLE PARK, NC 27709-2194	AM CSC-3406.0 Page 6 of 12

Table 3. Preparation of Calibration Standards

Vehicle Standard	Vehicle Stock	Target Dil. (g)/ Actual Dil. (g)	Final Dil. (g)/ Actual Dil. (g)		Target Concentration (mg/mL)	✔ = Prep.
VA_1	A	~0.667/	~1.0/	=	~8.0 Actual Conc.	
VB_1	B	~0.75/	~1.0/	=	~6.0 Actual Conc.	
VA_2	A	~0.35/	~1.0/	=	~4.20 Actual Conc.	
VB_2	B	~0.35/	~1.0/	=	~2.8 Actual Conc.	
VA_3[c]	A	~1.0/	~10 g with blank corn oil/ ~1.0 g aliquot for analysis/	=	~1.2 Actual Conc.	
VB_3[c]	B	~1.0/	~10 g with blank corn oil/ ~1.0 g aliquot for analysis/	=	~0.80 Actual Conc.	
Vblank	Blank Corn Oil	~1.0/	-	=	0/0 Actual Conc.	

[a]Dilutions conducted with blank corn oil. Final dilution should be +/-1% of target.
[b]If the final dilution wt. is ≤1% of target than use target wt. to express concentration; If >1% use actual dilution wt. to express concentration.
[c]Standard requires subsequent removal of 1.0-g aliquot prior to its use.

Analyst: _____ Date: _____

ANALYTICAL METHOD	RTI INTERNATIONAL 3040 CORNWALLIS ROAD RESEARCH TRIANGLE PARK, NC 27709-2194	AM CSC-3406.0 Page 7 of 12

6.2.3 Preparation of Vehicle Calibration Standards ☐

Standards B2 through A1 and the vehicle blank are prepared directly in 25-mL volumetric flask while standards B3 and A3 are prepared initially in 20-mL scintillation vials from which 1.0-g aliquots are transferred into 25-mL volumetric flasks. Dilute each to volume with hexane, mix by inversion, and then transfer 4-mL aliquots from each to individual 25-mL volumetric flasks. Dilute to volume with IS (1 µg of Aldrin/mL hexane) and mix by inversion. Transfer an aliquot into autosampler vials for GC/ECD analysis.

6.3 Preparation of Formulation Samples for Analysis ☐

[Note: All samples should be stirred prior to and during their analytical preparation.]

Remove from storage and mix each dose formulation by a combination of vortex action and stirring. Transfer duplicate 1-g aliquots from each dose (≤6.4 mg/mL) and control sample to individual 25-mL volumetric flasks, dilute to volume with hexane, and mix each by inversion. ☐

Remove duplicate 1-g aliquots from any formulations with nominal concentrations greater than 6.4 mg/mL but not exceeding 30 mg/mL to scintillation vials and dilute with blank vehicle to within the calibration range, mix, and then remove single ~1-g aliquots from these dilutions and proceed as stated above. ☐

Transfer 4-mL aliquots from all dilutions to individual 25-mL volumetric flasks and dilute to volume with IS (1 µg of aldrin/mL of hexane) and mix by inversion. Transfer an aliquot from each into autosampler vials for GC/ECD analysis. ☐

Analyst: _____ Date: _____

ANALYTICAL METHOD	RTI INTERNATIONAL 3040 CORNWALLIS ROAD RESEARCH TRIANGLE PARK, NC 27709-2194	AM CSC-3406.0 Page 8 of 12

Table 4. Formulation Aliquot Transfers (≤ 6.4 mg/mL)

Sample ID/Color Code	Aliquot No.	Target Sample Aliquot Wt. (g) +/-1%	Actual Sample Aliquot Wt.[a](g) +/-1%
	1	1	
	2	1	
	1	1	
	2	1	
	1	1	
	2	1	
	1	1	
	2	1	
	1	1	
	2	1	
	1	1	
	2	1	
	1	1	
	2	1	

[a]Actual weights should be recorded and used in determining the final calculations for that sample.

Analyst: _____ Date: _____

ANALYTICAL METHOD	RTI INTE..NATIONAL 3040 CORNWALLIS ROAD RESEARCH TRIANGLE PARK, NC 27709-2194	AM CSC-3406.0 Page 9 of 12

Table 5. Dilutions of Methoxychlor Formulations
(Nominal Concs.: 6.4 mg/mL ≤ 30 mg/mL)

Sample ID/Color Code	Aliquot No.	Target Sample Aliquot Wt. (g)/ Actual Aliquot Wt. (g)	Target Dilution Wt.[a] (g) Actual Dilution Wt. (g)
	1	~1/	~5/
	2		
	1	~1/	~5/
	2		
	1	~1/	~5/
	2		
	1	~1/	~5/
	2		
	1	~1/	~5/
	2		
	1	~1/	~5/
	2		

[a]Target wts. and dilution wts. are given as estimated values for AM. Actual volumes may differ based on sample amounts and concs. received under a given task but the dilution scheme should achieve a conc. not less than 0.96 mg/mL and not greater than 6.4 mg/mL for this AM.
[b]If the final dilution wt. is ≤1% of target than use target wt. to determine dilution factor; If >1% use actual dilution wt. to determine dilution factor.

Analyst: _____ Date: _____

ANALYTICAL METHOD	RTI INTERNATIONAL 3040 CORNWALLIS ROAD RESEARCH TRIANGLE PARK, NC 27709-2194	AM CSC-3406.0 Page 10 of 12

Table 6. Subsequent Transfers for Formulations (> 6.4 mg/mL)

Sample ID/Color Code	Aliquot No.	Target Sample Aliquot Wt. (g) +/-1%	Actual Sample Aliquot Wt.[a](g) +/-1%
	1	1	
	2	1	
	1	1	
	2	1	
	1	1	
	2	1	
	1	1	
	2	1	
	1	1	
	2	1	
	1	1	
	2	1	
	1	1	
	2	1	

[a]Actual weights should be recorded and used in determining the final calculations for that sample.

Analyst: _____ Date:_____

ANALYTICAL METHOD	RTI INTE. .IATIONAL 3040 CORNWALLIS ROAD RESEARCH TRIANGLE PARK, NC 27709-2194	AM CSC-3406.0 Page 11 of 12

6.4 Analytical Method

The following instrumentation and parameters may be used for the analysis of methoxychlor formulations. Equivalent instrumentation and parameters are acceptable, if they give equivalent results to the method. Single injections of each standard, blank, and sample are sufficient unless otherwise warranted.

GC Parameters

System	HP 5890 GC-ECD with HP 6890 Autosampler, or equivalent
Software	LabSystems Atlas 2000 rev. 2 or equiv.
Column[a]	J & W DB-5 (30m x 0.32mm ID); 1 um film, or equivalent
Carrier Gas	Helium
Column Temperature[a]	Isothermal @ 290°C
Flow Rate	2.0 mL/min
Injection Temperature-Type	250°C- Split (~20/1)
Inj. Solvent-Volume	Hexane - 1 µL
Retention Times (min)	Aldrin (IS) - ~3.7 min, Methoxychlor - ~6.1 min
Detector	Electron Capture (ECD) at 300°C

[a]A GC column with a differing film thickness or diameter may be substituted but may require an alteration of the column temperature to achieve similar retention times listed. Any such change should be noted in the appropriate notebook by the analyst.

6.5 Calculations

Methoxychlor is quantitated based on the observed peak area of its major component. For each injection, the peak area ratio for test chemical to the area of the internal standard is calculated as:

$$Peak\ Area\ Ratio = \frac{Peak\ Area\ for\ Methoxychlor}{Area\ of\ Internal\ Standard}$$

Analyst: _____ Date: _____

ANALYTICAL METHOD	RTI INTERNATIONAL 3040 CORNWALLIS ROAD RESEARCH TRIANGLE PARK, NC 27709-2194	AM CSC-3406.0 Page 12 of 12

A weighted ($1/x^2$) or a non-weighted linear regression fit may be selected based on the calibration range required. The estimated LOD is 0.001 mg/mL; the estimated LOQ is 0.004 mg/mL. The ELOQ is defined as 0.008 mg/mL.

For each dose formulation, calculate the peak area ratio of each aliquot for the test chemical as above. Use these peak area ratios and the linear regression equation to determine the methoxychlor concentration of each formulation aliquot. Also, account for any samples that required dilution prior to analysis in order to determine their final found concentrations. Based on final found concentration values, a replicate ratio (Y_l/Y_h) of ≥ 0.9 must be achieved between duplicate grabs of a given dose sample. If not, a third aliquot is prepared and a mean value is reported (n=3) with a percent relative standard deviation (%RSD). The Q-test (95% confidence interval) may be used to define an extreme outlier with its result recorded in the appropriate notebook.

Analyst: _____ Date: _____

APPENDIX

(See Attached)

HEWLETT ACKARD _____ °C-_____
S/N: _____
in Lab 135

Data System:
LabSystems Atlas 2000 Revision 2
Chromserver: _____
Channel: _____
System Maintenance: Last: _____ Next: _____

Column:
Manufacturer: _____
Stationary Phase: _____
Dimensions: _____
S/N: _____

Injection:
6890 Autosampler - S/N_____/_____ (√) Manual _____ (√)
Split: _____ (√) Splitless: _____ (√)
Splitless/Split: _____ (√)
Time Splitless: _____ (min) Volume: _____ (µL)
Split Ratio: _____ : _____ Injector Temperature: _____ (°C)

Conditions:
Carrier: _____ Flow Rate: _____ (mL/min) Head Pressure: _____ (psi)
Temperature Program: _____
_____
Flows: Make-up gas: _____ (mL/min) Air: _____ (mL/min)
Septum purge: _____ (mL/min) Anode purge gas: _____
Hydrogen: _____ (mL/min) Anode purge gas: _____ (mL/min)

Detector:
Type: FID _____ (√) ECD _____ (√)
Temperature: _____ (°C)
Range: _____
Attenuation: _____

Analyst: _____ Date: _____

REVIEW AND REVISION LOG	RTI INTERNATIONAL 3040 CORNWALLIS ROAD RESEARCH TRIANGLE PARK, NC 27709-2194	AM CSC-3406 Page 1 of 1

FORMULATION ANALYSIS OF METHOXYCHLOR (95%) IN CORN OIL

REVIEW/REVISION LOG

Rev. #	Date	Description
0	12/19/02	Original version.

RTI INTERNATIONAL

NTP Chemistry Support Services

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Chemistry Support Services for the ETP
NIH Contract No. N01-ES-05455
RTI Project 07939.004.003.105
ChemTask No. CHEM07253

METHOXYCHLOR (95%)

IN CORN OIL

FORMULATION ANALYSIS REPORT

Mix Date: December 23, 2002

March 4, 2003

Prepared by:
Gwen McNeill
Analytical Chemist

Approved by:
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Task Leader

Charles M. Sparacino, Ph.D.
Principal Investigator

Submitted to:

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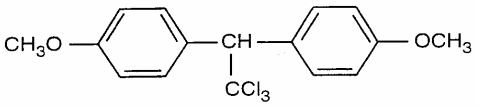
7244-600
WOLFE
RUCCA
NEHRBECKY J

METHOXYCHLOR (95%)

CAS No.: 72-43-5	Lot No.: 124F0575 (Sigma-Aldrich)
ChemTask No.: CHEM07253	Samples Received (RTI Log Nos.): 3 x 35 mL dosed (10011-62-02 to 04)
RTI Chemical ID Code: J93	1 x 35 mL control (10011-62-01)
Program Supported: RTX	Sample Receipt Date: Jan 10, 2003
Analysis Dates: Jan 16-20, 2003	Submitter: G. Holley
Interim Results Date: Jan 20, 2003	Study Lab: Therimmune Research Corp
Mix Date: Dec 23, 2002	Shipping Container: Amber glass septum vials inside a poly bag, with cold packs, inside a metal can, inside a fiberboard box
Vehicle: Corn Oil	
Storage Condition: Refrigeration	Receipt Condition: Good

STRUCTURE	MOL. WT.	FORMULA
	345.65	$C_{16}H_{15}Cl_3O_2$

EXECUTIVE SUMMARY

In support of the Reproductive Toxicology Program, a formulation analysis was performed to determine the methoxychlor (95%) content of three dose formulations and one control prepared in corn oil. Analytical results generated by a gas chromatography method indicated the samples submitted for analysis ranged from 95.8% to 103% of the target concentrations. No test chemical was detected in the control sample (estimated detection limit was 0.001 mg/mL).

RTI INTERNATIONAL

Quality Assurance Statement

Chemical Name: Methoxychlor (95%)

Task Type: Formulation Analysis

RTI Task Number: 07939.004.003.105

Chem Task Number: CHEM07253

This task was audited by the Chemistry and Life Sciences Quality Assurance Unit and the results of the inspections and audits were reported to the task leader and management as identified below. To the best of our knowledge, the reported results accurately describe the study methods and procedures used, and the reported results accurately reflect the raw data.

Inspections and Audits	Inspection and Audit Date(s)	Date Inspection/Audit Report Sent to Task Leader and Management
Sample Preparation	12/19/02	12/19/02
Data and Report Audit	2/27/03	2/27/03

Jennifer Jones
Quality Assurance Specialist

3/4/03
Date

Approval:

Doris J. Smith
Quality Assurance Manager

3-4-2003
Date

turning knowledge into practice

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METHOXYCHLOR (95%)

1.0 INTRODUCTION

The purpose of this work was determine the methoxychlor (95%) content in corn oil for samples submitted by Therimmune Research Co. To accomplish this objective, a formulation analysis (SOP CSC-1030.8) was performed.

2.0 SAMPLE IDENTIFICATION

The following dosage samples were received and analyzed for Methoxychlor (95%) content.

RTI Log No.	Study Lab ID	Target Conc. (mg/mL)
10011-62-01	White	0
10011-62-02	Pink	5
10011-62-03	Yellow	10
10011-62-04	Purple	20

3.0 SAMPLE ANALYSIS

Procedures: SOP CSC-1321.13
SOP CSC-1030.8
AM CSC- 3406.1

The AM cited in this report contained the following amendments:

1) The LOQ stated as 0.004 mg/mL was corrected to state 0.003 mg/mL.
2) A GC column with a film thickness of 0.5 µm was used for this effort.
3) Incorrect section numbering was revised where required.

4.0 SAMPLE RESULTS

Notebook No.: 9804 *pp.*: 14-15

The concentrations of Methoxychlor (95%) found in the dose formulations are tabulated below. Representative chromatograms are shown in Figure 1. The vehicle standards plot is illustrated in Figure 2 for the weighted ($1/x^2$) linear regression equation: $y = 2.636x + 0.08853$; $r = 0.9970$.

Study Lab ID	RTI Log No.	Nominal Conc. (mg/mL)	Found Conc. (mg/mL)	Replicate Ratio[a]	% of Nominal
White	10011-62-01	0	ND[b]	-	-
Pink	10011-62-02	5	4.85	0.99	97.0
Yellow	10011-62-03	10	9.58	0.99	95.8
Purple	10011-62-04	20	20.5	0.99	103

[a]Y_l/Y_h (low /high found concentrations from duplicate analysis).
[b]Not Detected. The estimated limit of detection = 0.001 mg/mL.

5.0 ACKNOWLEDGMENT

Personnel contributing to the performance of this task included Gwen McNeill.

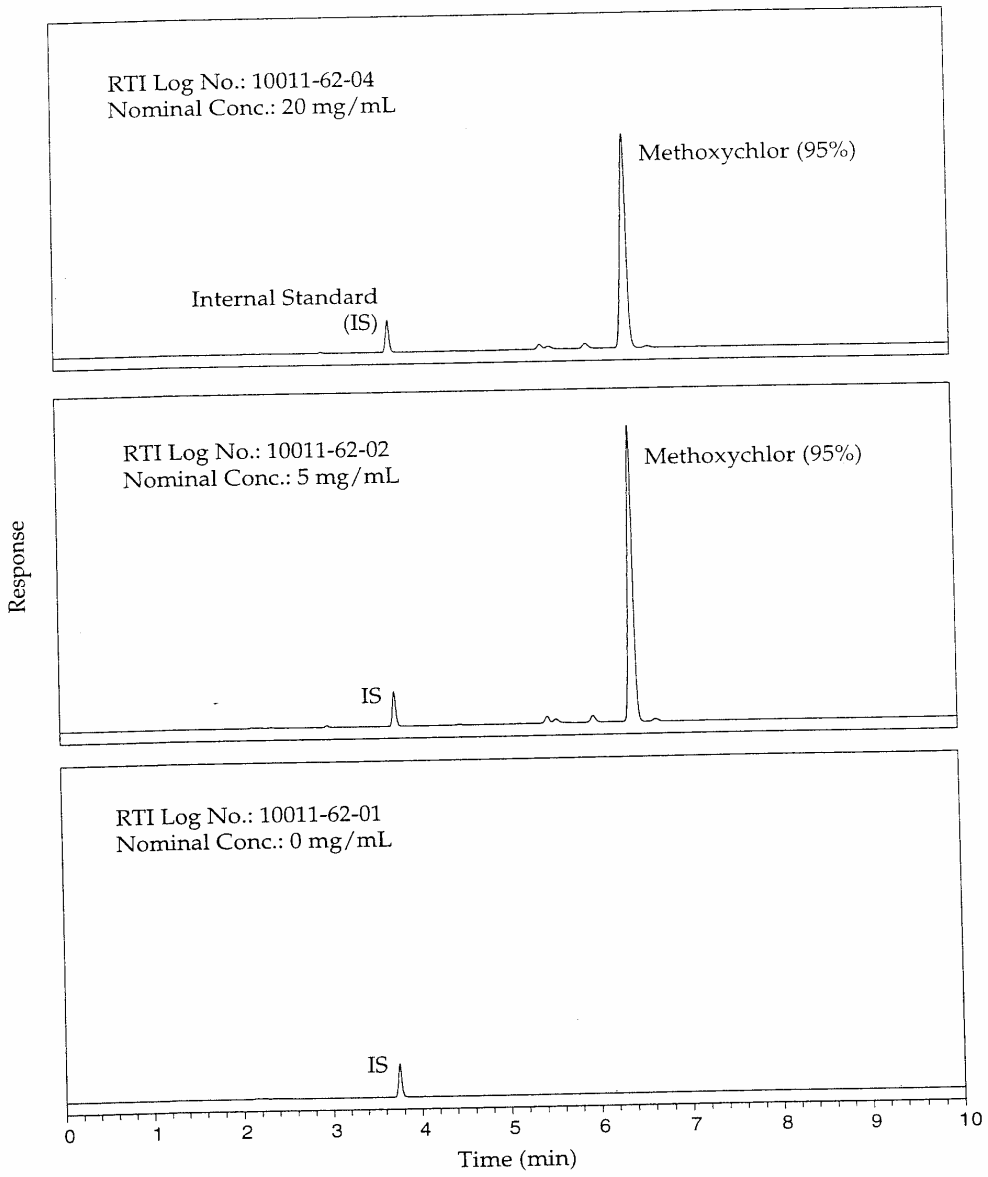

Figure 1. Representative Gas Chromatograms of Methoxychlor (95%) in Corn Oil

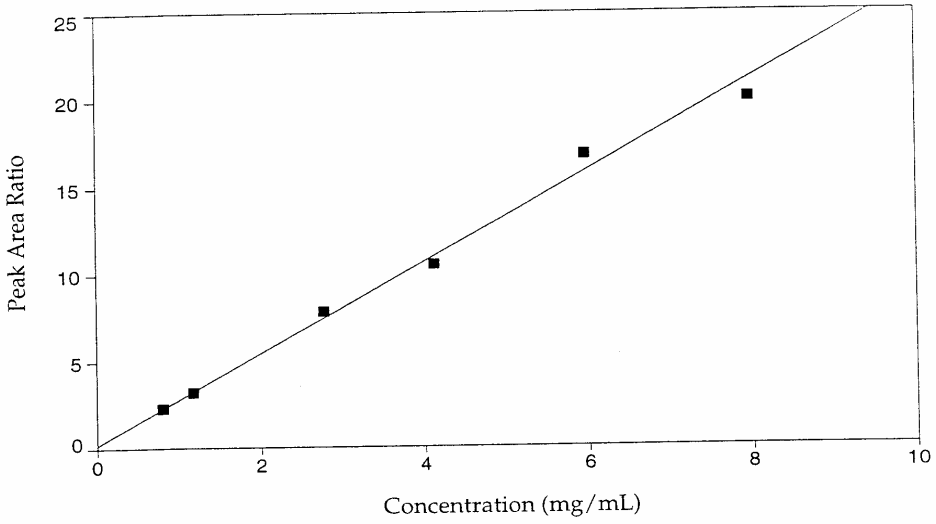

Figure 2. Plot of Vehicle Standards Data - Methoxychlor (95%) in Corn Oil

APPENDIX

Analytical Method CSC - 3406.1

Formulation Analysis of Methoxychlor (95%) in Corn Oil

ANALYTICAL METHOD	RTI INTERNATIONAL 3040 CORNWALLIS ROAD RESEARCH TRIANGLE PARK, NC 27709-2194	AM CSC-3406.1 Page 1 of 12

TITLE: FORMULATION ANALYSIS OF METHOXYCHLOR (95%) IN CORN OIL

AUTHOR: Signed _____ Analytical Chemist

Date 1-15-03

APPROVED BY: Signed _____ Principal Investigator

Date 1-15-03

ANALYTICAL METHOD	RTI INTERNATIONAL 3040 CORNWALLIS ROAD RESEARCH TRIANGLE PARK, NC 27709-2194	AM CSC-3406.1 Page 2 of 12

1.0 METHOD SUMMARY

The methoxychlor (95%) content in corn oil formulations submitted from various study laboratories is determined using a capillary gas chromatographic method coupled with electron capture detection (GC/ECD). The analytical procedure for which this AM is based was validated from 0.008 to 8 mg/mL with a verification to 30 mg/mL. The experimental limit of quantitation (ELOQ) is 0.008 mg/mL, with an estimated limit of detection (LOD) of 0.001 mg/mL, and an estimated limit of quantitation (LOQ) of 0.004 mg/mL. Formulation sample sets consisting of more than one dose level should be analyzed using a 6-point curve. Sets with only one dose level may be analyzed with a 3-point curve which brackets the dose sample by approximately +/-20%.

AM is used for ETP Task No.:_____

2.0 SAMPLE RECEIPT, STORAGE, AND HANDLING

The formulation samples should be stored at refrigerated temperatures. Samples throughout the dose range can be handled as solutions.

> NOTE: Due to the toxicity of the test compound, all transferring of the neat material should be performed in a sealed glove box as a measure of safety. Formulations should be mixed by a combination of sonication and magnetic stirring overnight and prior to sampling to insure a uniform solution. Formulations at the upper end of the dose range (30 mg/mL) may require additional mixing time.

3.0 REAGENTS

Refer to Table 1 for all required chemicals. Use this table to document the supplier, the lot number and expiration date for each reagent, making changes and/or additions where necessary. Any critical requirements for reagents are noted.

Table 1. Reagents

Reagent	Supplier	Lot Number	Exp. Date
Methoxychlor			
Aldrin- Internal Standard(IS)			
Hexane			
Corn Oil (vehicle)			

Analyst: _____ Date: _____

ANALYTICAL METHOD	RTI INTERNATIONAL 3040 CORNWALLIS ROAD RESEARCH TRIANGLE PARK, NC 27709-2194	AM CSC-3406.1 Page 3 of 12

4.0 EQUIPMENT

Refer to Table 2 for a list of the major equipment required. Equivalent systems are acceptable. Use this table to document the specific information (where applicable) for each piece of equipment. Check the maintenance and calibration records for each piece of equipment (where applicable) to be sure they are current.

Table 2. Equipment

Equipment	Manufacturer/Model No.	Other Info.
500-mL bottle (or size as required) for IS		
Mohr pipettes, large graduated cylinder(s) 5,25-mL, 1-L volumetric flasks, 20-mL scintillation vials , beakers, Pasteur pipets		
Analytical balance		
Vortex mixer		
Stirrer		
Autosampler vials (amber, glass)		
GC column	SEE ATTACHMENT	
GC system with ECD	SEE ATTACHMENT	

Analyst: _____ Date: _____

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5.0 LABWARE PREPARATION

Be sure all labware is properly cleaned per normal procedures prior to use. No additional precautions are required.

6.0 PROCEDURE

Note: At each critical step, the analyst should provide a check mark to signify that step was completed.

6.1 General Instructions

Check or calibrate equipment according to relevant SOPs. ☐

It is also important to record scientific observations and deviations from the SOP in the notebook. ☐

List of relevant SOPs: ☐

1.
2.
3.
4.
5.
6.

6.2 Preparation of Internal Standard Solution (IS) and Methoxychlor Standards

6.2.1 Preparation of IS ☐

NOTE: This procedure describes the preparation of 1 L of working IS. If more or less volume is required, then adjust weights and volumes accordingly.

Prepare a stock solution (1000 µg/mL) of Aldrin by transferring 5 mg of Aldrin into a 5-mL volumetric flask and dilute to volume with hexane. Sonicate to mix.

Actual aliquot wt. of Aldrin: _____ Actual Dilution Volume (mL): _____

Stock IS Concentration (µg/mL): _____ ☐

Prepare a working solution of IS at ~ 1 µg/mL by transferring 1 mL of the above stock

Analyst: _____ Date: _____

solution into a 1-liter volumetric flask and dilute to volume with hexane. Mix by inversion and transfer to an appropriately sized bottle and cap. The working IS should have a concentration of approximately 1 µg of Aldrin/mL.

Working IS concentration: _____ µg/mL ☐

6.2.2. Preparation of Vehicle Standards

NOTE: Procedure describes a 6-pt calibration scheme. If only a 3-pt calibration is required, a single stock may be prepared with three subsequent dilutions at the appropriate concentrations for each calibration standard required.

Prepare an ~12 mg/mL stock (A) by transferring ~240 mg of methoxychlor to a tared pre-calibrated (to 20 mL) scintillation vial. Dilute to the mark with corn oil (vehicle), and mix by sonication for 45 minutes followed by stirring for an additional 30 minutes.

Actual aliquot wt. of Test Chemical: _____

Final total dilution volume (mL): _____

Final Concentration: _____ mg/mL ☐

Prepare an ~8 mg/mL stock (B) by transferring ~160 mg of methoxychlor to a tared pre-calibrated (to 20 mL) scintillation vial. Dilute to the mark with corn oil (vehicle), and mix by sonication for 45 minutes followed by stirring for an additional 30 minutes.

Actual aliquot wt. of Test Chemical: _____

Final total dilution volume (mL): _____

Final Concentration: _____ mg/mL ☐

Six dilutions (or three as required) from the stock solutions (A and B) may be prepared as shown in the table below. For a three-point calibration, only three dilutions from one of the above stocks is required. All calibration standards are prepared in corn oil on a weight/weight basis. Transfers from the two stocks should be conducted with disposable pipets into volumetric flasks and/or scintillation vials. The stock(s) should be re-mixed prior to their use.

Analyst: _____ Date: _____

ANALYTICAL METHOD	RTI INTERNATIONAL 3040 CORNWALLIS ROAD RESEARCH TRIANGLE PARK, NC 27709-2194	AM CSC-3406.1 Page 6 of 12

Table 3. Preparation of Calibration Standards

Vehicle Standard	Vehicle Stock	Target Dil. (g)/ Actual Dil. (g)	Final Dil. (g)/ Actual Dil. (g)		Target Concentration (mg/mL)	✔ = Prep.
VA_1	A	~0.667/	~1.0/	=	~8.0 Actual Conc.	
VB_1	B	~0.75/	~1.0/	=	~6.0 Actual Conc.	
VA_2	A	~0.35/	~1.0/	=	~4.20 Actual Conc.	
VB_2	B	~0.35/	~1.0/	=	~2.8 Actual Conc.	
VA_3[c]	A	~1.0/	~10 g with blank corn oil/ ~1.0 g aliquot for analysis/	=	~1.2 Actual Conc.	
VB_3[c]	B	~1.0/	~10 g with blank corn oil/ ~1.0 g aliquot for analysis/	=	~0.80 Actual Conc.	
Vblank	Blank Corn Oil	~1.0/	-	=	0/0 Actual Conc.	

[a]Dilutions conducted with blank corn oil. Final dilution should be +/-1% of target.
[b]If the final dilution wt. is ≤1% of target than use target wt. to express concentration; If >1% use actual dilution wt. to express concentration.
[c]Standard requires subsequent removal of 1.0-g aliquot prior to its use.

Analyst: _____ Date: _____

ANALYTICAL METHOD	RTI INTERNATIONAL 3040 CORNWALLIS ROAD RESEARCH TRIANGLE PARK, NC 27709-2194	AM CSC-3406.1 Page 7 of 12

6.2.3 Preparation of Vehicle Calibration Standards ☐

Standards B2 through A1 and the vehicle blank are prepared directly in 25-mL volumetric flask while standards B3 and A3 are prepared initially in 20-mL scintillation vials from which 1.0-g aliquots are transferred into 25-mL volumetric flasks. Dilute each to volume with hexane, mix by inversion, and then transfer 4-mL aliquots from each to individual 25-mL volumetric flasks. Dilute to volume with IS (1 µg of Aldrin/mL hexane) and mix by inversion. Transfer an aliquot into autosampler vials for GC/ECD analysis.

6.3 Preparation of Formulation Samples for Analysis ☐

[Note: All samples should be stirred prior to and during their analytical preparation.]

Remove from storage and mix each dose formulation by a combination of vortex action and stirring. Transfer duplicate 1-g aliquots from each dose (≤6.4 mg/mL) and control sample to individual 25-mL volumetric flasks, dilute to volume with hexane, and mix each by inversion. ☐

Remove duplicate 1-g aliquots from any formulations with nominal concentrations greater than 6.4 mg/mL but not exceeding 30 mg/mL to scintillation vials and dilute with blank vehicle to within the calibration range, mix, and then remove single ~1-g aliquots from these dilutions and proceed as stated above. ☐

Transfer 4-mL aliquots from all dilutions to individual 25-mL volumetric flasks and dilute to volume with IS (1 µg of aldrin/mL of hexane) and mix by inversion. Transfer an aliquot from each into autosampler vials for GC/ECD analysis. ☐

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Table 4. Formulation Aliquot Transfers (≤ 6.4 mg/mL)

Sample ID/Color Code	Aliquot No.	Target Sample Aliquot Wt. (g) +/-1%	Actual Sample Aliquot Wt.[a](g) +/-1%
	1	1	
	2	1	
	1	1	
	2	1	
	1	1	
	2	1	
	1	1	
	2	1	
	1	1	
	2	1	
	1	1	
	2	1	
	1	1	
	2	1	

[a]Actual weights should be recorded and used in determining the final calculations for that sample.

Analyst: _____ Date:_____

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Table 5. Dilutions of Methoxychlor Formulations
(Nominal Concs.: 6.4 mg/mL ≤ 30 mg/mL)

Sample ID/Color Code	Aliquot No.	Target Sample Aliquot Wt. (g)/ Actual Aliquot Wt. (g)	Target Dilution Wt.[a] (g) Actual Dilution Wt. (g)
	1	~1/	~5/
	2		
	1	~1/	~5/
	2		
	1	~1/	~5/
	2		
	1	~1/	~5/
	2		
	1	~1/	~5/
	2		
	1	~1/	~5/
	2		

[a]Target wts. and dilution wts. are given as estimated values for AM. Actual volumes may differ based on sample amounts and concs. received under a given task but the dilution scheme should achieve a conc. not less than 0.96 mg/mL and not greater than 6.4 mg/mL for this AM.
[b]If the final dilution wt. is ≤1% of target than use target wt. to determine dilution factor; If >1% use actual dilution wt. to determine dilution factor.

Analyst: _____ Date: _____

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Table 6. Subsequent Transfers for Formulations (> 6.4 mg/mL)

Sample ID/Color Code	Aliquot No.	Target Sample Aliquot Wt. (g) +/-1%	Actual Sample Aliquot Wt.[a](g) +/-1%
	1	1	
	2	1	
	1	1	
	2	1	
	1	1	
	2	1	
	1	1	
	2	1	
	1	1	
	2	1	
	1	1	
	2	1	
	1	1	
	2	1	

[a]Actual weights should be recorded and used in determining the final calculations for that sample.

Analyst: _____ Date:_____

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6.4 Analytical Method

The following instrumentation and parameters may be used for the analysis of methoxychlor formulations. Equivalent instrumentation and parameters are acceptable, if they give equivalent results to the method. Single injections of each standard, blank, and sample are sufficient unless otherwise warranted.

GC Parameters

System	HP 5890 GC-ECD with HP 6890 Autosampler, or equivalent
Software	LabSystems Atlas 2000 rev. 2 or equiv.
Column[a]	J & W DB-5 (30m x 0.32mm ID); 1 um film, or equivalent
Carrier Gas	Helium
Column Temperature[a]	Isothermal @ 290°C
Flow Rate	2.0 mL/min
Injection Temperature-Type	250°C- Split (~20/1)
Inj. Solvent-Volume	Hexane - 1 µL
Retention Times (min)	Aldrin (IS) - ~3.7 min, Methoxychlor - ~6.1 min
Detector	Electron Capture (ECD) at 300°C

[a]A GC column with a differing film thickness or diameter may be substituted but may require an alteration of the column temperature to achieve similar retention times listed. Any such change should be noted in the appropriate notebook by the analyst.

6.5 Calculations

Methoxychlor is quantitated based on the observed peak area of its major component. For each injection, the peak area ratio for test chemical to the area of the internal standard is calculated as:

$$Peak\ Area\ Ratio = \frac{Peak\ Area\ for\ Methoxychlor}{Area\ of\ Internal\ Standard}$$

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ANALYTICAL METHOD	RTI INTERNATIONAL 3040 CORNWALLIS ROAD RESEARCH TRIANGLE PARK, NC 27709-2194	AM CSC-3406.1 Page 12 of 12

A weighted ($1/x^2$) or a non-weighted linear regression fit may be selected based on the calibration range required. The estimated LOD is 0.001 mg/mL; the estimated LOQ is 0.004 mg/mL. The ELOQ is defined as 0.008 mg/mL.

For each dose formulation, calculate the peak area ratio of each aliquot for the test chemical as above. Use these peak area ratios and the linear regression equation to determine the methoxychlor concentration of each formulation aliquot. Also, account for any samples that required dilution prior to analysis in order to determine their final found concentrations. Based on final found concentration values, a replicate ratio (Y_i/Y_h) of ≥ 0.9 must be achieved between duplicate grabs of a given dose sample. If not, a third aliquot is prepared and a mean value is reported (n=3) with a percent relative standard deviation (%RSD). The Q-test (95% confidence interval) may be used to define an extreme outlier with its result recorded in the appropriate notebook.

Analyst: _____ Date:_____

APPENDIX

(See Attached)

HEWLETT- ACKARD _____ GC-_____
S/N: _____
in Lab 135

Data System:
LabSystems Atlas 2000 Revision 2
Chromserver: _____
Channel: _____
System Maintenance: Last: _____ Next: _____

Column:
Manufacturer: _____
Stationary Phase: _____
Dimensions: _____
S/N: _____

Injection:
6890 Autosampler - S/N_____/_____ (√) Manual _____ (√)
Split: _____ (√) Splitless: _____ (√)
Splitless/Split: _____ (√)
Time Splitless: _____ (min) Volume: _____ (µL)
Split Ratio: _____ : _____ Injector Temperature: _____ (°C)

Conditions:
Carrier: _____ Flow Rate: _____ (mL/min) Head Pressure: _____ (psi)
Temperature Program: _____
_____
Flows: Make-up gas: _____ (mL/min) Air: _____ (mL/min)
Septum purge: _____ (mL/min) Anode purge gas: _____
Hydrogen: _____ (mL/min) Anode purge gas: _____ (mL/min)

Detector:
Type: FID _____ (√) ECD _____ (√)
Temperature: _____ (°C)
Range: _____
Attenuation: _____

Analyst: _____ Date: _____

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FORMULATION ANALYSIS OF METHOXYCHLOR (95%) IN CORN OIL

REVIEW/REVISION LOG

Rev. #	Date	Description
0	12/19/02	Original version.
1	01/15/03	Section 6.2.1 - Added line for Stock IS concentration; Minor editorial correction in Section 6.2.2.

Appendix 3
Dose Formulation Procedure
Pubertal Toxicity Study of Vinclozolin, Flutamide and Phenobarbital in Male Sprague Dawley Rats and Methoxychlor, Ethinyl Estradiol and Phenobarbital in Female Sprague Dawley Rats when Administered in Corn Oil by Oral Gavage

THERIMMUNE RESEARCH CORPORATION
DOSE FORMULATION PROCEDURE FORM

Study No.: 7244-600 (Males)
Task No.: N/A
ID No.: 1317B
Test Article: Vinclozolin
Vehicle: Corn Oil
Route of Administration: Oral Gavage

Procedure:

Refer to S.O.P. 511.0."Preparation of Test Formulations--Solutions/Suspensions, Test Diets, and Dosed Water", for the mixing procedures to be followed.

The amount of test article required to prepare dose levels of 10, 30 and 100 mg/kg/day of Vinclozolin in Corn Oil will be determined based on the following example calculations: The test article is assumed to be 100%.

Group	Letter Code	Dose Level (mg/kg/day)	Dose Volume (ml/kg/day)	Intended Conc. (mg/ml/day)	Vinclozolin (g)	Corn Oil(QS) (ml)
5	E	10	2.5	4	2.000	500
6	F	30	2.5	12	6.000	500
7	G	100	2.5	40	20.000	500

Weigh the appropriate amount of test article into a pre-calibrated beaker using a stainless steel spatula. QS to the final volume with Corn Oil. Mix well using a magnetic stir bar for at least 10 minutes. Sonicate if necessary. Transfer formulation to daily aliquots in amber glass bottles with Teflon®-coated lids. Formulations will be protected from light and stored refrigerated (~2-8°C). Formulations are stable for up to 42 days.

Sampling Procedure:

Two, 5 gram samples of the bulk test article will be collected into glass bottles with Teflon®-coated lids, sealed and stored in the freezer (~-20°C)protected from light for possible future reanalysis. A 5 gram sample of the bulk test article will be collected and sent to a NTP subcontractor for purity and stability analysis.

Collect three (3) 35 mL samples of each batch (per mix) of each dose level of test article formulation and store refrigerated (2-8°C). Homogeneity samples: collect three (3) 35 mL samples from the top, mid, and bottom from the first mix of the high and low dose levels.

Sample Shipment:

For samples submitted to Battelle for analysis: Send one sample from each dose level of the first, second and last mix for dose verification. Send one sample from the low and high dose levels of the first mix for homogeneity analysis. The samples should be sent refrigerated with blue ice packs and shipped according to S.O.P. 506.1 "Shipping of Test Article Samples". Send one 5 gram sample of the bulk test article on dry ice. Sample will be shipped according to S.O.P 506.1 "Shipping of Test Article Samples".

Prepared by: CJ 12/3/02 Initials/date Approved by: MJR 12.3.02 Initials/date

Reviewed by Formulation Technician: AAA 12/3/02; CAJ 12/3/02 Initials/date

THERIMMUNE RESEARCH CORPORATION
DOSE FORMULATION PROCEDURE FORM

Study No.: 7244-600 (Males)
Task No.: N/A
ID No.: 1198E
Test Article: Flutamide
Vehicle: Corn Oil
Route of Administration: Oral Gavage

Procedure:

Refer to S.O.P. 511.0,"Preparation of Test Formulations--Solutions/Suspensions, Test Diets, and Dosed Water", for the mixing procedures to be followed.

The amount of test article required to prepare dose levels of 25 and 50 mg/kg/day of Flutamide in Corn Oil will be determined based on the following example calculations: The test article is assumed to be 100%.

Group	Letter Code	Dose Level (mg/kg/day)	Dose Volume (ml/kg/day)	Intended Conc. (mg/ml/day)	Flutamide (g)	Corn Oil(QS) (ml)
8	H	25	2.5	10	5.000	500
9	I	50	2.5	20	10.000	500

Weigh the appropriate amount of test article into a pre-calibrated beaker using a stainless steel spatula. QS to the final volume with Corn Oil. Mix well using a magnetic stir bar for at least 10 minutes. Sonicate if necessary. Transfer formulation to daily aliquots in amber glass bottles with Teflon®-coated lids. Formulations will be protected from light and stored refrigerated (~2-8°C). Formulations are stable for up to 42 days.

Sampling Procedure:

Two, 5 gram samples of the bulk test article will be collected into glass bottles with Teflon®-coated lids, sealed and stored in the freezer (~-20°C)protected from light for possible future reanalysis. A 5 gram sample of the bulk test article will be collected and sent to a NTP subcontractor for purity and stability analysis.

Collect three (3) 35 mL samples of each batch (per mix) of each dose level of test article formulation and store refrigerated (2-8°C). Homogeneity samples: collect three (3) 35 mL samples from the top, mid, and bottom from the first mix of each dose level.

Sample Shipment:

For samples submitted to Battelle for analysis: Send one sample from each dose level of the first, second and last mix for dose verification. Send one sample from each dose level of the first mix for homogeneity analysis. The samples should be sent refrigerated with blue ice packs and shipped according to S.O.P. 506.1 "Shipping of Test Article Samples". Send one 5 gram sample of the bulk test article on dry ice. Sample will be shipped according to S.O.P 506.1 "Shipping of Test Article Samples".

Prepared by: GR 12/3/02 (Initials/date) Approved by: MJF 12.3.02 (Initials/date)

Reviewed by Formulation Technician: AAA 12/3/02 ; CDJ 12/3/02 (Initials/date)

THERIMMUNE RESEARCH CORPORATION
DOSE FORMULATION PROCEDURE FORM

Study No.: 7244-600 (Males)
Task No.: N/A
ID No.: 1909A
Test Article: Phenobarbital
Vehicle: Corn Oil
Route of Administration: Oral Gavage

Procedure:

Refer to S.O.P. 511.0,"Preparation of Test Formulations--Solutions/Suspensions, Test Diets, and Dosed Water", for the mixing procedures to be followed.

The amount of test article required to prepare dose levels of 0, 25, 50 and 100 mg/kg/day of Phenobarbital in Corn Oil will be determined based on the following example calculations: The test article is assumed to be 100%.

Group	Letter Code	Dose Level (mg/kg/day)	Dose Volume (ml/kg/day)	Intended Conc. (mg/ml/day)	Phenobarbital (g)	Corn Oil(QS) (ml)
1	A	0	2.5	0	0.000	500
2	B	25	2.5	10	5.000	500
3	C	50	2.5	20	10.000	500
4	D	100	2.5	40	20.000	500

Weigh the appropriate amount of test article into a pre-calibrated beaker using a stainless steel spatula. QS to the final volume with Corn Oil. Mix well using a magnetic stir bar for at least 10 minutes. Sonicate if necessary. Transfer formulation to daily aliquots in amber glass bottles with Teflon®-coated lids. Formulations will be protected from light and stored refrigerated (~2-8°C). Formulations are stable for up to 14 days.

Sampling Procedure:

Two, 5 gram samples of the bulk test article will be collected into glass bottles with Teflon®-coated lids, sealed and stored in the freezer (~-20°C)protected from light for possible future reanalysis. A 5 gram sample of the bulk test article will be collected and sent to a NTP subcontractor for purity and stability analysis.

Collect three (3) 35 mL samples of each batch (per mix) of each dose level of test article formulation and store refrigerated (2-8°C). Homogeneity samples: collect three (3) 35 mL samples from the top, mid, and bottom from the first mix of the high and low dose levels.

Sample Shipment:

For samples submitted to Midwest Research for analysis: Send one sample from each dose level of the first, second and last mix for dose verification. Send one sample from the low and high dose levels of the first mix for homogeneity analysis. The samples should be sent refrigerated with blue ice packs and shipped according to S.O.P. 506.1 "Shipping of Test Article Samples". Send one 5 gram sample of the bulk test article on dry ice. Sample will be shipped according to S.O.P 506.1 "Shipping of Test Article Samples".

Prepared by: JJ 12/3/02 (Initials/date) Approved by: MSR 12.3.02 (Initials/date)

Reviewed by Formulation Technician: AHA 12/3/02 ; CDJ 12/3/02 (Initials/date)

THERIMMUNE RESEARCH CORPORATION
DOSE FORMULATION PROCEDURE FORM

Study No.: 7244-600 (Females)
Task No.: N/A
ID No.: 1321B
Test Article: Methoxychlor
Vehicle: Corn Oil
Route of Administration: Oral Gavage

Procedure:

Refer to S.O.P. 511.0,"Preparation of Test Formulations--Solutions/Suspensions, Test Diets, and Dosed Water", for the mixing procedures to be followed.

The amount of test article required to prepare dose levels of 12.5, 25 and 50 mg/kg/day of Methoxychlor in Corn Oil will be determined based on the following example calculations: The test article is assumed to be 100%.

Group	Letter Code	Dose Level (mg/kg/day)	Dose Volume (ml/kg/day)	Intended Conc. (mg/ml/day)	Methoxychlor (g)	Corn Oil(QS) (ml)
4	M	12.5	2.5	5	2.500	500
5	N	25	2.5	10	5.000	500
6	O	50	2.5	20	10.000	500

Weigh the appropriate amount of test article into a pre-calibrated beaker using a stainless steel spatula. QS to the final volume with Corn Oil. Mix well using a magnetic stir bar for at least 10 minutes. Sonicate if necessary. Transfer formulation to daily aliquots in amber glass bottles with Teflon®-coated lids. Formulations will be protected from light and stored refrigerated (~2-8°C). Formulations are stable for up to 30 days.

Sampling Procedure:

Two, 5 gram samples of the bulk test article will be collected into glass bottles with Teflon®-coated lids, sealed and stored in the freezer (~-20°C)protected from light for possible future reanalysis. A 5 gram sample of the bulk test article will be collected and sent to a NTP subcontractor for purity and stability analysis.

Collect three (3) 35 mL samples of each batch (per mix) of each dose level of test article formulation and store refrigerated (2-8°C). Homogeneity samples: collect three (3) 35 mL samples from the top, mid, and bottom from the first mix of the low and high dose levels.

Sample Shipment:

For samples submitted to RTI for analysis: Send one sample from each dose level of the first, second and last mix for dose verification. Send one sample from the high and low dose levels of the first mix for homogeneity analysis. The samples should be sent refrigerated with blue ice packs and shipped according to S.O.P. 506.1 "Shipping of Test Article Samples". Send one 5 gram sample of the bulk test article on dry ice. Sample will be shipped according to S.O.P 506.1 "Shipping of Test Article Samples".

Prepared by: 12/3/02
Initials/date

Approved by: 12.3.02
Initials/date

Reviewed by Formulation Technician: CeCl 12/3/02 ; CDJ 12/3/02
Initials/date

THERIMMUNE RESEARCH CORPORATION
DOSE FORMULATION PROCEDURE FORM

Study No.: 7244-600 (Females)
Task No.: N/A
ID No.: 1318B
Test Article: Ethinyl Estradiol
Vehicle: Corn Oil
Route of Administration: Oral Gavage

Procedure:

Refer to S.O.P. 511.0,"Preparation of Test Formulations--Solutions/Suspensions, Test Diets, and Dosed Water", for the mixing procedures to be followed.

The amount of test article required to prepare dose levels of 0, 0.0025 and 0.005 mg/kg/day of Ethinyl Estradiol in Corn Oil will be determined based on the following example calculations: The test article is assumed to be 100%.

Group	Letter Code	Dose Level (mg/kg/day)	Dose Volume (ml/kg/day)	Intended Conc. (mg/ml/day)	Ethinyl Estradiol (g)	Corn Oil(QS) (ml)
1	J	0	2.5	0	0.0000	1000
2	K	0.0025	2.5	0.001	0.0010	1000
3	L	0.0050	2.5	0.002	0.0020	1000

Weigh the appropriate amount of test article into a pre-calibrated beaker using a stainless steel spatula. QS to the final volume with Corn Oil. Mix well using a magnetic stir bar for at least 10 minutes. Sonicate if necessary. Transfer formulation to daily aliquots in amber glass bottles with Teflon®-coated lids. Formulations will be protected from light and stored refrigerated (~2-8°C). Formulations are stable for up to 14 days.

Sampling Procedure:

Two, 5 gram samples of the bulk test article will be collected into glass bottles with Teflon®-coated lids, sealed and stored in the freezer (~-20°C)protected from light for possible future reanalysis. A 5 gram sample of the bulk test article will be collected and sent to a NTP subcontractor for purity and stability analysis.

Collect three (3) 35 mL samples of each batch (per mix) of each dose level of test article formulation and store refrigerated (2-8°C). Homogeneity samples: collect three (3) 35 mL samples from the top, mid, and bottom from the first mix of each dose level.

Sample Shipment:

For samples submitted to Midwest Research for analysis: Send one sample from each dose level of the first, second and last mix for dose verification. Send one sample from each dose level of the first mix for homogeneity analysis. The samples should be sent refrigerated with blue ice packs and shipped according to S.O.P. 506.1 "Shipping of Test Article Samples". Send one 5 gram sample of the bulk test article on dry ice. Sample will be shipped according to S.O.P 506.1 "Shipping of Test Article Samples".

Prepared by: 12/3/02 Initials/date Approved by: 12.3.02 Initials/date

Reviewed by Formulation Technician: 12/3/02, 12/3/02 Initials/date

THERIMMUNE RESEARCH CORPORATION
DOSE FORMULATION PROCEDURE FORM

Study No.: 7244-600 (Females)
Task No.: N/A
ID No.: 1909A
Test Article: Phenobarbital
Vehicle: Corn Oil
Route of Administration: Oral Gavage

Procedure:

Refer to S.O.P. 511.0,"Preparation of Test Formulations--Solutions/Suspensions, Test Diets, and Dosed Water", for the mixing procedures to be followed.

The amount of test article required to prepare dose levels of 25, 50 and 100 mg/kg/day of Phenobarbital in Corn Oil will be determined based on the following example calculations: The test article is assumed to be 100%.

Group	Letter Code	Dose Level (mg/kg/day)	Dose Volume (ml/kg/day)	Intended Conc. (mg/ml/day)	Phenobarbital (g)	Corn Oil(QS) (ml)
7	P	25	2.5	10	5.000	500
8	Q	50	2.5	20	10.000	500
9	R	100	2.5	40	20.000	500

Weigh the appropriate amount of test article into a pre-calibrated beaker using a stainless steel spatula. QS to the final volume with Corn Oil. Mix well using a magnetic stir bar for at least 10 minutes. Sonicate if necessary. Transfer formulation to daily aliquots in amber glass bottles with Teflon®-coated lids. Formulations will be protected from light and stored refrigerated (~2-8°C). Formulations are stable for up to 14 days.

Sampling Procedure:

Collect three (3) 35 mL samples of each batch (per mix) of each dose level of test article formulation and store refrigerated (2-8°C). Homogeneity samples: collect three (3) 35 mL samples from the top, mid, and bottom from the first mix of the high and low dose levels.

Sample Shipment:

For samples submitted to Midwest Research for analysis: Send one sample from each dose level of the first, second and last mix for dose verification. Send one sample from the high and low dose levels of the first mix for homogeneity analysis. The samples should be sent refrigerated with blue ice packs and shipped according to S.O.P. 506.1 "Shipping of Test Article Samples".

Prepared by: 12/3/02 (Initials/date) Approved by: 12.3.02 (Initials/date)

Reviewed by Formulation Technician: 12/3/02 ; 12/3/02 (Initials/date)

Appendix 4
F_0 Dam Body Weights
Pubertal Toxicity Study of Vinclozolin, Flutamide and Phenobarbital in Male Sprague Dawley Rats and Methoxychlor, Ethinyl Estradiol and Phenobarbital in Female Sprague Dawley Rats when Administered in Corn Oil by Oral Gavage

Appendix 4
F_0 Dam Body Weights (Grams)
Pubertal Toxicity Study of Vinclozolin, Flutamide and Phenobarbital in Male Sprague Dawley Rats and Methoxychlor, Ethinyl Estradiol and Phenobarbital in Female Sprague Dawley Rats When Administered in Corn Oil by Oral Gavage

F_0 Dam Number	Postnatal Day 0	Postnatal Day 21	F_0 Dam Number	Postnatal Day 0	Postnatal Day 21
1	275.4	306.2	31	275.5	306.2
3	259.6	295.5	32	285.6	313.7
4	273.4	289.7	33	302.2	319.0
6	273.4	309.8	34	285.6	339.9
7	298.8	313.1	35	260.8	310.8
8	286.9	334.0	36	281.6	316.3
10	292.6	309.8	37	265.7	286.6
12	255.8	285.6	40	275.8	310.7
13	261.8	300.3	41	271.5	319.3
15	267.8	341.1	42	261.7	293.3
16	265.2	281.8	43	268.0	308.7
18	271.0	293.6	44	277.7	301.5
19	277.2	316.6	47	265.5	313.6
20	234.3	278.9	48	271.9	322.1
25	283.6	327.4	49	284.4	324.2
26	250.4	281.4	50	252.9	301.9
27	265.8	296.6	55	284.4	307.0
28	288.1	314.4			

Appendix 5
F_0 Litter Sex-Size
Pubertal Toxicity Study of Vinclozolin, Flutamide and Phenobarbital in Male Sprague Dawley Rats and Methoxychlor, Ethinyl Estradiol and Phenobarbital in Female Sprague Dawley Rats when Administered in Corn Oil by Oral Gavage

Appendix 5
F_0 Litter Sex-Size
Pubertal Toxicity Study of Vinclozolin, Flutamide and Phenobarbital in Male Sprague Dawley Rats and Methoxychlor, Ethinyl Estradiol and Phenobarbital in Female Sprague Dawley Rats When Administered in Corn Oil by Oral Gavage

F_0 Female	PND 0 Dead Pups	PND 0 Live Pups			PND 4 Live Pups			PND 7 Live Pups			PND 14 Live Pups			PND 21 Live Pups		
Number	Total	M	F	Total	M	F	Total	M	F	Total	M	F	Total	M	F	Total
1	0	9	4	13	9	4	13	6	4	10	6	4	10	6	4	10
3	0	9	4	13	9	4	13	6	4	10	6	4	10	6	4	10
4	0	6	5	11	5	5	10	5	5	10	5	5	10	5	5	10
6	1	8	5	13	8	5	13	5	5	10	5	5	10	5	5	10
7	0	2	8	10	2	8	10	2	8	10	2	8	10	2	8	10
8	0	7	6	13	6	6	12	5	5	10	5	5	10	5	5	10
10	0	6	5	11	6	5	11	5	5	10	5	5	10	5	5	10
12	4	5	6	11	5	6	11	5	5	10	5	5	10	5	5	10
13	0	3	10	13	3	10	13	3	7	10	3	7	10	3	7	10
15	0	7	5	12	7	5	12	5	5	10	5	5	10	5	5	10
16	0	3	3	6	3	3	6	3	3	6	3	3	6	3	3	6
18	0	7	8	15	7	8	15	5	5	10	5	5	10	5	5	10
19	0	4	7	11	4	7	11	4	6	10	4	6	10	4	6	10
20	0	6	6	12	6	6	12	5	5	10	5	5	10	5	5	10
25	0	6	5	11	6	5	11	5	5	10	5	5	10	5	5	10
26	0	7	8	15	7	8	15	5	5	10	5	5	10	5	5	10
27	0	8	6	14	8	6	14	5	5	10	5	5	10	5	5	10

M = Male F = Female
PND = Postnatal day

Appendix 5 (continued)
F_0 Litter Sex-Size
Pubertal Toxicity Study of Vinclozolin, Flutamide and Phenobarbital in Male Sprague Dawley Rats and Methoxychlor, Ethinyl Estradiol and Phenobarbital in Female Sprague Dawley Rats When Administered in Corn Oil by Oral Gavage

F_0 Female	PND 0 Dead Pups	PND 0 Live Pups			PND 4 Live Pups			PND 7 Live Pups			PND 14 Live Pups			PND 21 Live Pups		
Number	Total	M	F	Total	M	F	Total	M	F	Total	M	F	Total	M	F	Total
28	0	7	6	13	7	6	13	5	5	10	5	5	10	5	5	10
31	0	4	9	13	4	9	13	4	6	10	4	6	10	4	6	10
32	0	8	4	12	8	4	12	6	4	10	6	4	10	6	4	10
33	0	8	5	13	8	5	13	5	5	10	5	5	10	5	5	10
34	0	5	7	12	5	7	12	5	5	10	5	5	10	5	5	10
35	0	6	7	13	6	7	13	5	5	10	5	5	10	5	5	10
36	0	7	3	10	7	3	10	7	3	10	7	3	10	7	3	10
37	0	4	7	11	4	7	11	4	6	10	4	6	10	4	6	10
40	0	10	3	13	10	3	13	7	3	10	7	3	10	7	3	10
41	0	5	6	11	5	6	11	5	5	10	5	5	10	5	5	10
42	0	6	7	13	6	6	12	5	5	10	5	5	10	5	5	10
43	0	5	6	11	5	6	11	5	5	10	5	5	10	5	5	10
44	0	6	7	13	6	7	13	5	5	10	5	5	10	5	5	10
47	0	7	4	11	7	4	11	6	4	10	6	4	10	6	4	10
48	0	8	7	15	8	7	15	5	5	10	5	5	10	5	5	10
49	0	8	3	11	8	3	11	7	3	10	7	3	10	7	3	10
50	0	6	7	13	6	7	13	5	5	10	5	5	10	5	5	10
55	0	2	9	11	2	9	11	2	8	10	2	8	10	2	8	10

M = Male F = Female
PND = Postnatal day

Appendix 6
F_0 Litter Weights
Pubertal Toxicity Study of Vinclozolin, Flutamide and Phenobarbital in Male Sprague Dawley Rats and Methoxychlor, Ethinyl Estradiol and Phenobarbital in Female Sprague Dawley Rats when Administered in Corn Oil by Oral Gavage

Appendix 6
F_0 Litter Weights (Grams)
Pubertal Toxicity Study of Vinclozolin, Flutamide and Phenobarbital in Male Sprague Dawley Rats and Methoxychlor, Ethinyl Estradiol and Phenobarbital in Female Sprague Dawley Rats When Administered in Corn Oil by Oral Gavage

F_0 Dam Number	Postnatal Day	Pup Sex	Mean Weight	Individual Pup Weights									
1	4	Males	10.2	9.6	10.4	10.7	10.8	8.9	11.0	9.6	10.9	10.0	
		Females	10.1	9.7	10.4	10.3	10.0						
	7	Males	16.3	15.4	17.2	16.5	15.7	16.6	16.5				
		Females	16.1	16.3	16.0	16.3	15.5						
	14	Males	32.2	32.7	30.7	32.8	32.4	31.5	32.8				
		Females	32.4	32.0	31.6	32.6	33.5						
	21	Males	56.9	58.4	59.0	55.3	57.5	57.2	53.9				
		Females	54.9	52.9	53.2	57.9	55.5						
3	4	Males	11.0	10.5	11.0	11.1	11.2	11.1	11.2	10.8	10.9	11.2	
		Females	10.5	11.0	10.3	9.9	10.7						
	7	Males	17.2	17.2	17.3	16.8	16.7	17.7	17.5				
		Females	16.2	17.2	15.8	16.6	15.2						
	14	Males	26.6	27.7	30.4	24.9	26.3	24.1	26.3				
		Females	27.3	26.0	26.4	27.7	29.1						
	21	Males	48.9	52.8	53.0	42.9	47.1	47.9	49.7				
		Females	48.2	50.6	45.3	45.7	51.1						
4	4	Males	11.9	12.2	11.6	11.9	12.1	11.6					
		Females	11.4	11.5	12.2	11.8	10.0	11.7					
	7	Males	15.2	15.4	14.9	14.6	15.9	15.0					
		Females	14.8	15.8	14.6	15.2	15.3	13.1					
	14	Males	29.9	28.5	30.1	28.8	31.7	30.3					
		Females	29.5	30.0	26.8	30.9	29.7	30.1					
	21	Males	54.6	52.6	54.9	53.1	59.5	52.9					
		Females	52.8	53.5	55.6	53.0	49.2	52.8					

Appendix 6 (continued)
F_0 Litter Weights (Grams)
Pubertal Toxicity Study of Vinclozolin, Flutamide and Phenobarbital in Male Sprague Dawley Rats and Methoxychlor, Ethinyl Estradiol and Phenobarbital in Female Sprague Dawley Rats When Administered in Corn Oil by Oral Gavage

F_0 Dam Number	Postnatal Day	Pup Sex	Mean Weight	Individual Pup Weights								
6	4	Males	10.7	11.6	9.4	10.2	11.1	11.2	11.3	10.2	10.7
		Females	10.7	10.8	9.4	11.0	11.5	11.1			
	7	Males	17.4	16.7	14.9	18.2	18.7	18.6			
		Females	17.6	18.6	18.1	15.4	17.3	18.8			
	14	Males	35.9	34.1	32.1	37.9	37.4	37.8			
		Females	36.2	33.0	37.8	35.7	38.5	36.1			
	21	Males	58.2	59.5	60.2	63.5	55.0	52.7			
		Females	58.0	56.2	63.3	60.9	50.2	59.6			
7	4	Males	13.7	13.6	13.9						
		Females	12.8	13.6	12.3	11.1	13.2	12.4	13.1	13.1	13.9
	7	Males	20.0	19.9	20.1						
		Females	18.6	19.5	18.7	20.4	16.7	18.1	19.0	18.3	18.3
	14	Males	39.3	40.1	38.5						
		Females	36.2	37.2	36.7	35.6	39.8	34.9	36.8	34.8	33.5
	21	Males	63.4	62.9	63.9						
		Females	58.4	55.0	63.1	61.0	57.2	60.4	55.9	57.4	57.0
8	4	Males	12.3	12.4	12.3	12.9	11.6	12.4	12.1		
		Females	11.1	11.4	10.7	10.3	11.0	11.5	11.7		
	7	Males	18.6	19.7	18.2	17.7	18.7	18.7			
		Females	16.4	15.0	17.3	16.9	16.6	16.2			
	14	Males	37.3	37.9	37.1	35.8	37.3	38.4			
		Females	34.5	36.1	35.8	35.1	31.1	34.3			
	21	Males	61.6	61.7	57.9	61.8	63.9	62.6			
		Females	55.9	59.4	50.7	59.1	51.1	59.2			

Appendix 6 (continued)
F_0 Litter Weights (Grams)
Pubertal Toxicity Study of Vinclozolin, Flutamide and Phenobarbital in Male Sprague Dawley Rats and Methoxychlor, Ethinyl Estradiol and Phenobarbital in Female Sprague Dawley Rats When Administered in Corn Oil by Oral Gavage

F_0 Dam Number	Postnatal Day	Pup Sex	Mean Weight	Individual Pup Weights										
10	4	Males	10.9	11.2	9.8	11.0	10.5	11.8	10.8					
		Females	10.0	9.9	10.5	10.3	10.0	9.3						
	7	Males	15.6	18.3	15.2	14.0	15.4	15.4						
		Females	14.4	15.1	14.2	13.0	15.1	14.6						
	14	Males	30.2	29.9	30.4	33.9	26.9	30.1						
		Females	27.5	25.2	27.8	28.3	27.6	28.4						
	21	Males	48.9	50.1	51.9	47.6	44.9	50.0						
		Females	45.8	45.5	47.1	46.4	47.9	42.1						
12	4	Males	11.3	11.4	10.7	10.9	11.8	11.9						
		Females	10.1	9.1	10.1	10.1	10.8	11.0	9.6					
	7	Males	16.7	17.3	16.4	17.0	15.8	17.1						
		Females	15.1	14.1	15.5	16.0	16.3	13.8						
	14	Males	30.9	30.9	29.2	32.6	31.4	30.4						
		Females	29.0	27.6	28.6	29.4	30.8	28.8						
	21	Males	53.8	52.8	54.7	50.0	55.3	56.3						
		Females	50.0	50.2	50.4	50.3	51.7	47.4						
13	4	Males	11.3	11.4	11.2	11.3								
		Females	10.7	10.7	11.3	8.4	10.5	10.9	10.8	11.8	10.6	11.4	10.6	
	7	Males	17.8	17.9	17.4	18.1								
		Females	16.7	17.2	16.8	13.8	17.1	16.6	16.9	18.4				
	14	Males	32.8	32.2	33.5	32.8								
		Females	30.8	31.9	31.4	27.5	29.3	32.5	30.9	31.7				
	21	Males	56.6	57.5	55.4	57.0								
		Females	52.2	51.5	52.9	54.1	54.2	47.2	49.5	55.8				

Appendix 6 (continued)
F_0 Litter Weights (Grams)
Pubertal Toxicity Study of Vinclozolin, Flutamide and Phenobarbital in Male Sprague Dawley Rats and Methoxychlor, Ethinyl Estradiol and Phenobarbital in Female Sprague Dawley Rats When Administered in Corn Oil by Oral Gavage

F_0 Dam Number	Postnatal Day	Pup Sex	Mean Weight	Individual Pup Weights							
15	4	Males	10.6	10.8	11.3	10.4	10.4	10.0	10.3	10.9	
		Females	9.4	9.8	9.8	6.5	10.4	10.7			
	7	Males	16.1	16.4	16.3	17.2	15.6	15.3			
		Females	14.5	15.2	10.3	16.3	15.7	14.8			
	14	Males	30.0	29.8	29.7	31.1	29.7	29.7			
		Females	27.8	28.6	28.4	30.5	28.8	22.4			
	21	Males	49.3	49.3	49.8	49.7	50.1	47.6			
		Females	45.5	51.3	35.1	46.9	48.1	46.3			
16	4	Males	12.9	12.5	12.9	13.1					
		Females	12.7	12.6	12.7	12.9					
	7	Males	19.0	18.7	19.1	19.4					
		Females	18.8	18.4	19.2	19.0					
	14	Males	34.8	33.7	35.1	35.5					
		Females	34.9	34.8	35.6	34.5					
	21	Males	56.7	60.1	56.2	53.9					
		Females	56.9	55.2	56.6	59.0					
18	4	Males	10.7	11.0	11.8	11.4	11.0	11.4	8.3	10.0	
		Females	10.5	10.4	11.2	11.0	10.1	10.0	10.7	10.6	10.4
	7	Males	17.7	18.2	18.1	16.1	18.0	17.8			
		Females	17.1	18.0	17.1	16.9	17.0	16.5			
	14	Males	32.1	30.4	32.9	31.2	33.8	31.8			
		Females	31.0	31.4	31.0	30.5	31.2	31.2			
	21	Males	54.9	57.9	52.9	53.6	53.0	57.1			
		Females	52.5	50.8	53.0	51.1	51.8	55.7			

Appendix 6 (continued)
F_0 Litter Weights (Grams)
Pubertal Toxicity Study of Vinclozolin, Flutamide and Phenobarbital in Male Sprague Dawley Rats and Methoxychlor, Ethinyl Estradiol and Phenobarbital in Female Sprague Dawley Rats When Administered in Corn Oil by Oral Gavage

F_0 Dam Number	Postnatal Day	Pup Sex	Mean Weight	Individual Pup Weights							
19	4	Males	11.9	12.3	11.7	12.0	11.6				
		Females	11.0	10.6	10.5	11.7	11.0	11.6	10.9	10.7
	7	Males	17.6	17.1	18.1	18.0	17.2			
		Females	16.7	17.6	17.3	15.9	16.7	16.3	16.5	
	14	Males	33.7	33.3	32.7	35.0	33.6			
		Females	32.3	32.1	33.1	32.9	32.7	31.1	31.8	
	21	Males	52.5	55.7	50.0	54.0	50.3			
		Females	49.8	52.0	50.4	52.0	47.0	49.8	47.7	
20	4	Males	10.1	10.4	10.4	9.8	10.1	9.9	10.0	
		Females	9.7	10.2	8.6	10.2	10.4	8.6	10.0	
	7	Males	14.6	14.6	15.0	13.9	15.1	14.4		
		Females	14.1	15.5	15.4	12.8	12.1	14.7		
	14	Males	27.7	26.1	29.1	27.7	27.5	27.9		
		Females	25.9	28.5	25.8	24.5	27.6	23.2		
	21	Males	46.5	49.3	46.5	47.1	46.4	43.1		
		Females	44.6	44.7	46.3	41.5	44.2	46.3		
25	4	Males	13.1	13.4	13.8	12.9	12.3	13.0	13.1	
		Females	12.1	12.4	12.0	12.8	10.8	12.7		
	7	Males	19.7	18.4	20.4	19.8	20.0	19.6		
		Females	18.4	19.3	16.2	18.9	18.0	19.4		
	14	Males	34.3	34.8	32.2	35.6	34.1	34.9		
		Females	33.1	30.9	34.0	32.6	33.9	34.3		
	21	Males	60.8	58.3	56.4	63.2	63.5	62.8		
		Females	58.4	61.8	53.1	59.0	58.9	59.1		

Appendix 6 (continued)
F_0 Litter Weights (Grams)
Pubertal Toxicity Study of Vinclozolin, Flutamide and Phenobarbital in Male Sprague Dawley Rats and Methoxychlor, Ethinyl Estradiol and Phenobarbital in Female Sprague Dawley Rats When Administered in Corn Oil by Oral Gavage

F_0 Dam Number	Postnatal Day	Pup Sex	Mean Weight	Individual Pup Weights								
26	4	Males	9.7	9.8	9.3	8.6	10.4	9.2	10.1	10.6		
		Females	10.0	10.3	10.5	10.6	8.8	9.7	10.1	9.8	10.5	
	7	Males	15.8	17.1	16.4	14.7	17.0	13.7				
		Females	16.3	15.6	16.2	15.4	17.0	17.2				
	14	Males	30.4	32.5	32.8	31.1	26.0	29.5				
		Females	31.1	30.8	30.3	31.6	32.4	30.5				
	21	Males	52.0	50.4	54.6	44.9	52.5	57.8				
		Females	53.2	54.6	51.2	52.7	55.4	51.9				
27	4	Males	9.9	10.0	10.7	9.8	10.1	9.3	9.7	10.1	9.9	
		Females	9.5	10.0	9.9	9.2	8.9	9.9	9.5			
	7	Males	16.0	15.4	15.5	16.2	16.2	16.6				
		Females	15.9	16.4	15.9	14.8	15.8	16.5				
	14	Males	33.9	34.1	34.0	33.5	33.6	34.4				
		Females	34.1	31.9	34.2	34.6	34.5	35.4				
	21	Males	57.0	58.3	57.4	55.0	58.4	55.8				
		Females	56.8	52.9	58.3	56.3	58.0	58.3				
28	4	Males	11.8	11.5	11.8	12.8	11.9	11.2	11.9	11.7		
		Females	11.6	12.0	11.3	11.5	11.3	11.4	11.9			
	7	Males	18.7	19.7	18.0	18.7	18.7	18.3				
		Females	18.3	19.1	17.9	18.1	18.4	17.8				
	14	Males	31.3	33.0	30.8	30.3	31.1	31.2				
		Females	30.2	31.1	29.5	31.0	29.5	29.7				
	21	Males	52.3	52.5	48.1	55.4	56.6	48.7				
		Females	51.8	48.9	52.3	49.8	55.8	52.0				

Appendix 6 (continued)
F_0 Litter Weights (Grams)
Pubertal Toxicity Study of Vinclozolin, Flutamide and Phenobarbital in Male Sprague Dawley Rats and Methoxychlor, Ethinyl Estradiol and Phenobarbital in Female Sprague Dawley Rats When Administered in Corn Oil by Oral Gavage

F_0 Dam Number	Postnatal Day	Pup Sex	Mean Weight	Individual Pup Weights									
31	4	Males	11.7	11.9	11.0	12.4	11.5						
		Females	11.3	11.6	11.7	11.2	11.1	11.2	11.7	11.7	9.9	11.2
	7	Males	18.0	18.1	18.2	18.7	16.9						
		Females	17.4	18.2	17.7	15.5	18.2	17.1	17.6				
	14	Males	33.5	32.8	35.3	32.5	33.5						
		Females	32.5	31.2	33.1	32.1	32.8	32.7	33.3				
	21	Males	55.6	52.9	56.9	56.1	56.5						
		Females	53.4	51.1	50.2	53.5	56.6	56.2	52.7				
32	4	Males	11.9	12.5	12.3	11.4	11.7	11.8	12.6	11.3	11.4		
		Females	11.4	11.8	11.3	11.5	11.1						
	7	Males	18.5	19.4	17.1	18.4	19.4	18.7	18.0				
		Females	18.1	17.2	18.6	18.8	18.0						
	14	Males	36.3	35.7	34.3	38.1	36.5	34.7	38.4				
		Females	35.6	36.6	35.5	36.9	33.4						
	21	Males	58.7	56.4	62.3	58.9	57.5	59.5	57.7				
		Females	55.9	53.1	58.9	58.9	52.8						
33	4	Males	12.2	13.1	11.4	12.4	12.6	11.4	11.3	12.7	12.8		
		Females	11.6	11.4	11.6	12.3	11.5	11.5					
	7	Males	19.0	19.9	19.8	19.7	17.9	17.9					
		Females	18.4	18.4	19.0	19.3	17.9	17.7					
	14	Males	35.1	32.9	36.7	33.0	38.1	34.9					
		Females	34.0	34.4	33.1	36.1	34.2	32.2					
	21	Males	61.8	64.7	63.9	61.1	59.7	59.5					
		Females	59.0	62.5	57.8	58.0	56.7	59.9					

Appendix 6 (continued)
F_0 Litter Weights (Grams)
Pubertal Toxicity Study of Vinclozolin, Flutamide and Phenobarbital in Male Sprague Dawley Rats and Methoxychlor, Ethinyl Estradiol and Phenobarbital in Female Sprague Dawley Rats When Administered in Corn Oil by Oral Gavage

F_0 Dam Number	Postnatal Day	Pup Sex	Mean Weight	Individual Pup Weights							
34	4	Males	11.2	11.2	10.8	11.6	11.5	11.1			
		Females	10.8	10.5	11.3	11.7	10.8	10.3	10.9	10.5
	7	Males	16.3	16.8	15.9	16.0	17.0	16.1			
		Females	15.9	15.2	17.5	15.5	14.7	16.6			
	14	Males	32.8	33.3	33.4	31.2	35.0	30.9			
		Females	31.7	29.3	33.6	33.0	30.9	31.5			
	21	Males	57.1	58.0	57.4	53.4	55.6	61.1			
		Females	54.0	53.2	53.1	55.0	53.9	54.9			
35	4	Males	10.8	10.6	10.3	11.0	10.8	10.7	11.4		
		Females	9.8	10.6	10.1	9.8	8.9	9.9	9.1	10.5
	7	Males	16.4	16.2	16.5	16.0	17.1	16.4			
		Females	15.5	14.8	16.1	15.1	15.5	16.1			
	14	Males	33.5	33.1	33.7	33.6	33.6	33.6			
		Females	31.7	33.1	32.1	30.9	32.8	29.8			
	21	Males	55.4	54.3	53.8	57.5	56.0	55.2			
		Females	52.2	51.9	54.9	52.0	51.5	50.7			
36	4	Males	13.0	13.6	13.1	12.1	13.0	13.5	13.1	12.8
		Females	12.7	12.4	12.6	13.2				
	7	Males	18.9	18.5	19.3	18.8	17.7	19.6	19.8	18.3
		Females	18.4	18.6	19.0	17.6				
	14	Males	34.4	36.5	35.2	33.8	34.0	34.8	33.5	33.1
		Females	33.4	34.2	33.0	33.0				
	21	Males	62.2	63.2	62.8	63.4	61.2	65.2	60.9	58.5
		Females	59.7	59.8	60.9	58.4				

Appendix 6 (continued)
F_0 Litter Weights (Grams)
Pubertal Toxicity Study of Vinclozolin, Flutamide and Phenobarbital in Male Sprague Dawley Rats and Methoxychlor, Ethinyl Estradiol and Phenobarbital in Female Sprague Dawley Rats When Administered in Corn Oil by Oral Gavage

F_0 Dam Number	Postnatal Day	Pup Sex	Mean Weight	Individual Pup Weights									
37	4	Males	11.5	11.7	12.0	10.2	12.2						
		Females	11.1	11.5	11.5	11.4	10.8	10.8	10.1	11.4			
	7	Males	17.3	15.2	18.0	18.5	17.3						
		Females	16.7	17.1	16.9	15.3	16.4	17.8	16.7				
	14	Males	33.0	35.7	29.7	33.0	33.4						
		Females	32.1	32.7	30.3	33.1	32.9	31.9	31.8				
	21	Males	53.2	49.4	54.5	55.2	53.7						
		Females	50.8	51.2	51.6	54.1	51.7	51.7	44.3				
40	4	Males	11.4	10.8	12.3	11.3	11.3	11.6	11.9	11.3	10.8	10.9	11.5
		Females	10.9	11.0	11.1	10.5							
	7	Males	17.4	17.7	17.3	18.0	15.8	18.3	17.2	17.6			
		Females	16.6	16.7	16.7	16.4							
	14	Males	32.5	33.0	30.6	33.0	31.7	33.1	33.5	32.8			
		Females	31.7	31.8	32.0	31.3							
	21	Males	56.6	57.9	54.6	53.9	59.7	58.2	54.9	57.3			
		Females	54.4	56.1	55.2	52.0							
41	4	Males	11.9	11.8	12.1	12.0	12.4	11.3					
		Females	12.2	12.9	11.6	12.5	11.9	12.0	12.2				
	7	Males	16.4	16.3	16.5	16.5	17.1	15.8					
		Females	16.3	16.7	16.6	15.9	16.4	16.0					
	14	Males	30.6	31.8	30.3	30.6	29.6	30.9					
		Females	29.9	29.4	31.7	28.8	29.5	29.9					
	21	Males	54.2	56.7	54.8	55.5	51.2	52.9					
		Females	53.2	54.6	55.0	52.0	52.6	51.9					

Appendix 6 (continued)
F_0 Litter Weights (Grams)
Pubertal Toxicity Study of Vinclozolin, Flutamide and Phenobarbital in Male Sprague Dawley Rats and Methoxychlor, Ethinyl Estradiol and Phenobarbital in Female Sprague Dawley Rats When Administered in Corn Oil by Oral Gavage

F_0 Dam Number	Postnatal Day	Pup Sex	Mean Weight	Individual Pup Weights						
42	4	Males	10.6	11.4	10.8	10.4	9.5	11.2	10.1	
		Females	10.6	10.9	9.3	10.3	10.7	11.6	10.9	
	7	Males	16.0	15.2	16.7	15.5	15.5	16.9		
		Females	15.7	16.2	15.3	17.1	13.5	16.6		
	14	Males	28.6	28.5	30.5	27.8	27.0	29.1		
		Females	27.7	29.8	29.5	26.8	27.1	25.5		
	21	Males	51.0	47.8	49.8	54.5	54.1	48.6		
		Females	50.4	52.4	47.3	55.2	49.0	48.3		
43	4	Males	11.9	11.8	11.6	12.2	12.5	11.2		
		Females	11.5	11.2	11.5	11.3	11.3	12.0	11.8	
	7	Males	17.4	18.2	16.8	17.4	17.6	16.9		
		Females	16.8	17.2	16.6	17.3	16.8	16.2		
	14	Males	32.8	31.9	33.6	33.2	32.5	32.9		
		Females	31.4	32.4	30.5	32.0	31.5	30.7		
	21	Males	60.1	60.5	59.0	60.3	60.4	60.3		
		Females	56.6	54.9	55.3	59.3	57.3	56.4		
44	4	Males	11.9	11.7	12.1	11.7	12.1	11.6	12.1	
		Females	11.7	12.8	11.6	11.2	11.1	12.1	11.9	11.2
	7	Males	18.5	19.1	18.1	18.5	18.5	18.2		
		Females	18.0	16.1	19.5	19.6	17.4	17.6		
	14	Males	32.3	33.7	30.9	33.9	32.4	30.8		
		Females	31.8	34.1	29.3	31.9	31.4	32.1		
	21	Males	59.3	57.1	61.5	57.3	58.1	62.4		
		Females	55.5	55.4	56.5	55.1	50.4	60.1		

Appendix 6 (continued)
F_0 Litter Weights (Grams)
Pubertal Toxicity Study of Vinclozolin, Flutamide and Phenobarbital in Male Sprague Dawley Rats and Methoxychlor, Ethinyl Estradiol and Phenobarbital in Female Sprague Dawley Rats When Administered in Corn Oil by Oral Gavage

F_0 Dam Number	Postnatal Day	Pup Sex	Mean Weight	Individual Pup Weights								
47	4	Males	11.8	12.9	12.6	11.4	11.4	10.5	12.0	12.0		
		Females	11.3	11.6	10.4	11.3	11.7					
	7	Males	18.3	18.1	19.9	19.4	17.7	18.5	16.3			
		Females	17.3	17.2	18.1	18.1	15.6					
	14	Males	35.3	36.6	36.0	35.3	35.7	35.5	32.6			
		Females	33.3	32.1	32.5	34.1	34.6					
	21	Males	60.5	62.3	60.1	63.0	60.5	58.7	58.2			
		Females	58.2	57.0	55.2	60.6	60.0					
48	4	Males	10.6	10.4	11.5	10.6	11.4	10.1	10.5	10.9	9.6	
		Females	10.1	11.4	11.9	8.1	11.4	9.6	9.8	8.8		
	7	Males	15.8	14.5	17.0	15.9	16.3	15.5				
		Females	15.5	17.0	14.0	16.5	18.0	12.2				
	14	Males	31.6	33.5	31.8	29.7	31.7	31.1				
		Females	30.9	32.4	28.7	27.8	30.8	34.9				
	21	Males	56.3	54.8	59.5	59.2	55.4	52.7				
		Females	53.8	60.0	47.5	56.4	55.4	49.7				
49	4	Males	11.3	11.0	11.8	11.1	10.4	11.5	11.6	10.7	12.2	
		Females	10.6	10.8	10.3	10.9						
	7	Males	16.4	16.9	16.1	16.2	15.6	17.2	17.9	15.2		
		Females	15.6	15.5	15.6	15.9						
	14	Males	32.2	32.6	32.1	32.8	31.7	31.2	34.3	30.5		
		Females	30.6	31.2	29.7	31.1						
	21	Males	58.2	58.0	58.2	57.1	57.5	61.8	59.4	55.1		
		Females	55.3	55.3	54.5	56.1						

Appendix 6 (continued)
F_0 Litter Weights (Grams)
Pubertal Toxicity Study of Vinclozolin, Flutamide and Phenobarbital in Male Sprague Dawley Rats and Methoxychlor, Ethinyl Estradiol and Phenobarbital in Female Sprague Dawley Rats When Administered in Corn Oil by Oral Gavage

F_0 Dam Number	Postnatal Day	Pup Sex	Mean Weight	Individual Pup Weights									
50	4	Males	10.5	10.9	10.5	11.2	9.6	10.9	9.7				
		Females	10.0	9.7	8.2	10.1	10.3	10.5	10.4	11.0			
	7	Males	15.5	16.6	15.7	14.9	14.1	16.2					
		Females	14.2	15.0	14.0	15.0	12.0	14.9					
	14	Males	29.5	30.4	30.6	30.9	26.7	28.9					
		Females	28.1	28.5	27.9	24.9	28.9	30.2					
	21	Males	53.1	54.4	55.7	56.3	48.8	50.3					
		Females	49.7	48.8	50.2	44.9	52.0	52.5					
55	4	Males	13.0	13.3	12.7								
		Females	12.3	12.0	13.2	12.1	12.9	12.2	12.9	12.1	11.1	12.1	
	7	Males	19.1	19.2	19.0								
		Females	17.9	18.7	16.7	18.1	18.0	19.4	17.6	17.5	17.5		
	14	Males	37.0	36.3	37.7								
		Females	33.8	33.3	35.8	32.4	32.7	35.5	32.9	32.9	35.1		
	21	Males	63.0	61.6	64.3								
		Females	58.9	58.7	60.7	56.7	56.1	58.1	58.4	61.8	60.4		

Appendix 7
F_1 Clinical Observations and Mortality
Pubertal Toxicity Study of Vinclozolin, Flutamide and Phenobarbital in Male Sprague Dawley Rats and Methoxychlor, Ethinyl Estradiol and Phenobarbital in Female Sprague Dawley Rats when Administered in Corn Oil by Oral Gavage

Appendix 7
F_1 Clinical Observations and Mortality
Pubertal Toxicity Study of Vinclozolin, Flutamide and Phenobarbital in Male Sprague Dawley Rats and Methoxychlor, Ethinyl Estradiol and Phenobarbital in Female Sprague Dawley Rats When Administered in Corn Oil by Oral Gavage

				Postnatal Day									
Group Sex	Animal Number	Clinical Sign	Site	2 3	2 4	2 5	2 6	3 0	3 7	4 4	5 1	5 3	5 4
1M	9026	No Abnormalities Detected		X	.	.	.	X	X	X	X	X	.
		Terminal Kill		.	.	.	.	.	.	.	.	X	.
	9027	No Abnormalities Detected		X	.	.	.	X	X	X	X	X	.
		Terminal Kill		.	.	.	.	.	.	.	.	X	.
	9028	No Abnormalities Detected		X	.	.	.	X	X	X	X	X	.
		Terminal Kill		.	.	.	.	.	.	.	.	X	.
	9029	No Abnormalities Detected		X	.	.	.	X	X	X	X	X	.
		Terminal Kill		.	.	.	.	.	.	.	.	X	.
	9030	No Abnormalities Detected		X	.	.	.	X	X	X	X	X	.
		Terminal Kill		.	.	.	.	.	.	.	.	X	.
	9031	No Abnormalities Detected		X	.	.	.	X	X	X	X	X	.
		Terminal Kill		.	.	.	.	.	.	.	.	X	.
	9032	No Abnormalities Detected		X	.	.	.	X	X	X	X	X	.
		Terminal Kill		.	.	.	.	.	.	.	.	X	.
	9033	No Abnormalities Detected		X	.	.	.	X	X	X	X	X	.
		Terminal Kill		.	.	.	.	.	.	.	.	X	.
	9034	No Abnormalities Detected		X	.	.	.	X	X	X	X	.	X
		Terminal Kill		.	.	.	.	.	.	.	.	.	X
	9035	No Abnormalities Detected		X	.	.	.	X	X	X	X	.	X
		Terminal Kill		.	.	.	.	.	.	.	.	.	X
	9036	No Abnormalities Detected		X	.	.	.	X	X	X	X	.	X
		Terminal Kill		.	.	.	.	.	.	.	.	.	X
	9037	No Abnormalities Detected		X	.	.	.	X	X	X	X	.	X
		Terminal Kill		.	.	.	.	.	.	.	.	.	X

X = Present S = Severe I = Minimal O = Moderate M = Male . = No Data
Dose Level: Group 1 – 0 mg/kg/day; Group 2 – 25 mg/kg/day Phenobarbital; Group 3 – 50 mg/kg/day Phenobarbital
Group 4 – 100 mg/kg/day Phenobarbital; Group 5 – 10 mg/kg/day Vinclozolin; Group 6 – 30 mg/kg/day Vinclozolin
Group 7 – 100 mg/kg/day Vinclozolin; Group 8 – 25 mg/kg/day Flutamide; Group 9 – 50 mg/kg/day Flutamide

Appendix 7 (continued)
F_1 Clinical Observations and Mortality
Pubertal Toxicity Study of Vinclozolin, Flutamide and Phenobarbital in Male Sprague Dawley Rats and Methoxychlor, Ethinyl Estradiol and Phenobarbital in Female Sprague Dawley Rats When Administered in Corn Oil by Oral Gavage

Group Sex	Animal Number	Clinical Sign	Site	Postnatal Day 23	24	25	26	30	37	44	51	53	54
1M	9038	No Abnormalities Detected		X	.	.	.	X	X	X	X	.	X
		Terminal Kill		.	.	.	.	.	.	.	.	.	X
	9039	No Abnormalities Detected		X	.	.	.	X	X	X	X	.	X
		Terminal Kill		.	.	.	.	.	.	.	.	.	X
	9040	No Abnormalities Detected		X	.	.	.	X	X	X	X	.	X
		Terminal Kill		.	.	.	.	.	.	.	.	.	X

X = Present S = Severe I = Minimal O = Moderate M = Male . = No Data
Dose Level: Group 1 – 0 mg/kg/day; Group 2 – 25 mg/kg/day Phenobarbital; Group 3 – 50 mg/kg/day Phenobarbital
Group 4 – 100 mg/kg/day Phenobarbital; Group 5 – 10 mg/kg/day Vinclozolin; Group 6 – 30 mg/kg/day Vinclozolin
Group 7 – 100 mg/kg/day Vinclozolin; Group 8 – 25 mg/kg/day Flutamide; Group 9 – 50 mg/kg/day Flutamide

Appendix 7 (continued)
F_1 Clinical Observations and Mortality
Pubertal Toxicity Study of Vinclozolin, Flutamide and Phenobarbital in Male Sprague Dawley Rats and Methoxychlor, Ethinyl Estradiol and Phenobarbital in Female Sprague Dawley Rats When Administered in Corn Oil by Oral Gavage

				Postnatal Day									
Group Sex	Animal Number	Clinical Sign	Site	2 3	2 4	2 5	2 6	3 0	3 7	4 4	5 1	5 3	5 4
2M	9041	No Abnormalities Detected		X	.	.	.	X	X	X	X	X	.
		Terminal Kill		.	.	.	.	.	.	.	.	X	.
	9042	No Abnormalities Detected		X	.	.	.	X	X	X	X	X	.
		Terminal Kill		.	.	.	.	.	.	.	.	X	.
	9043	No Abnormalities Detected		X	.	.	.	X	X	X	X	X	.
		Terminal Kill		.	.	.	.	.	.	.	.	X	.
	9044	No Abnormalities Detected		X	.	.	.	X	X	X	X	X	.
		Terminal Kill		.	.	.	.	.	.	.	.	X	.
	9045	No Abnormalities Detected		X	.	.	.	X	X	X	X	X	.
		Terminal Kill		.	.	.	.	.	.	.	.	X	.
	9046	No Abnormalities Detected		X	.	.	.	X	X	X	X	X	.
		Terminal Kill		.	.	.	.	.	.	.	.	X	.
	9047	No Abnormalities Detected		X	.	.	.	X	X	X	X	X	.
		Terminal Kill		.	.	.	.	.	.	.	.	X	.
	9048	No Abnormalities Detected		X	.	.	.	X	X	X	X	X	.
		Terminal Kill		.	.	.	.	.	.	.	.	X	.
	9049	No Abnormalities Detected		X	.	.	.	X	X	X	X	.	X
		Terminal Kill		.	.	.	.	.	.	.	.	.	X
	9050	No Abnormalities Detected		X	.	.	.	X	X	X	X	.	X
		Terminal Kill		.	.	.	.	.	.	.	.	.	X
	9051	No Abnormalities Detected		X	.	.	.	X	X	X	X	.	X
		Terminal Kill		.	.	.	.	.	.	.	.	.	X
	9052	No Abnormalities Detected		X	.	.	.	X	X	X	X	.	X
		Terminal Kill		.	.	.	.	.	.	.	.	.	X

X = Present S = Severe I = Minimal O = Moderate M = Male . = No Data
Dose Level: Group 1 – 0 mg/kg/day; Group 2 – 25 mg/kg/day Phenobarbital; Group 3 – 50 mg/kg/day Phenobarbital
Group 4 – 100 mg/kg/day Phenobarbital; Group 5 – 10 mg/kg/day Vinclozolin; Group 6 – 30 mg/kg/day Vinclozolin
Group 7 – 100 mg/kg/day Vinclozolin; Group 8 – 25 mg/kg/day Flutamide; Group 9 – 50 mg/kg/day Flutamide

Appendix 7 (continued)
F_1 Clinical Observations and Mortality
Pubertal Toxicity Study of Vinclozolin, Flutamide and Phenobarbital in Male Sprague Dawley Rats and Methoxychlor, Ethinyl Estradiol and Phenobarbital in Female Sprague Dawley Rats When Administered in Corn Oil by Oral Gavage

				Postnatal Day									
Group Sex	Animal Number	Clinical Sign	Site	2 3	2 4	2 5	2 6	3 0	3 7	4 4	5 1	5 3	5 4
2M	9053	No Abnormalities Detected		X	.	.	.	X	X	X	X	.	X
		Terminal Kill		.	.	.	.	.	.	.	.	.	X
	9054	No Abnormalities Detected		X	.	.	.	X	X	X	X	.	X
		Terminal Kill		.	.	.	.	.	.	.	.	.	X
	9055	No Abnormalities Detected		X	.	.	.	X	X	X	X	.	X
		Terminal Kill		.	.	.	.	.	.	.	.	.	X

X = Present S = Severe I = Minimal O = Moderate M = Male . = No Data
Dose Level: Group 1 – 0 mg/kg/day; Group 2 – 25 mg/kg/day Phenobarbital; Group 3 – 50 mg/kg/day Phenobarbital
Group 4 – 100 mg/kg/day Phenobarbital; Group 5 – 10 mg/kg/day Vinclozolin; Group 6 – 30 mg/kg/day Vinclozolin
Group 7 – 100 mg/kg/day Vinclozolin; Group 8 – 25 mg/kg/day Flutamide; Group 9 – 50 mg/kg/day Flutamide

Appendix 7 (continued)
F_1 Clinical Observations and Mortality
Pubertal Toxicity Study of Vinclozolin, Flutamide and Phenobarbital in Male Sprague Dawley Rats and Methoxychlor, Ethinyl Estradiol and Phenobarbital in Female Sprague Dawley Rats When Administered in Corn Oil by Oral Gavage

Group Sex	Animal Number	Clinical Sign	Site	Postnatal Day 23	24	25	26	30	37	44	51	53	54
3M	9056	No Abnormalities Detected		X	.	.	.	X	X	X	X	X	.
		Terminal Kill		.	.	.	.	.	.	.	.	X	.
	9057	No Abnormalities Detected		X	.	.	.	X	X	X	X	X	.
		Terminal Kill		.	.	.	.	.	.	.	.	X	.
	9058	No Abnormalities Detected		X	.	.	.	X	X	X	X	X	.
		Terminal Kill		.	.	.	.	.	.	.	.	X	.
	9059	No Abnormalities Detected		X	.	.	.	X	X	X	X	X	.
		Terminal Kill		.	.	.	.	.	.	.	.	X	.
	9060	No Abnormalities Detected		X	.	.	.	X	X	X	X	X	.
		Terminal Kill		.	.	.	.	.	.	.	.	X	.
	9061	No Abnormalities Detected		X	.	.	.	X	X	X	X	X	.
		Terminal Kill		.	.	.	.	.	.	.	.	X	.
	9062	No Abnormalities Detected		X	.	.	.	X	X	X	X	X	.
		Terminal Kill		.	.	.	.	.	.	.	.	X	.
	9063	No Abnormalities Detected		X	.	.	.	X	X	X	X	X	.
		Terminal Kill		.	.	.	.	.	.	.	.	X	.
	9064	No Abnormalities Detected		X	.	.	.	X	X	X	X	.	X
		Terminal Kill		.	.	.	.	.	.	.	.	.	X
	9065	No Abnormalities Detected		X	.	.	.	X	X	X	X	.	X
		Terminal Kill		.	.	.	.	.	.	.	.	.	X
	9066	No Abnormalities Detected		X	.	.	.	X	X	X	X	.	X
		Terminal Kill		.	.	.	.	.	.	.	.	.	X
	9067	No Abnormalities Detected		X	.	.	.	X	X	X	X	.	X
		Terminal Kill		.	.	.	.	.	.	.	.	.	X

X = Present S = Severe I = Minimal O = Moderate M = Male . = No Data
Dose Level: Group 1 – 0 mg/kg/day; Group 2 – 25 mg/kg/day Phenobarbital; Group 3 – 50 mg/kg/day Phenobarbital
Group 4 – 100 mg/kg/day Phenobarbital; Group 5 – 10 mg/kg/day Vinclozolin; Group 6 – 30 mg/kg/day Vinclozolin
Group 7 – 100 mg/kg/day Vinclozolin; Group 8 – 25 mg/kg/day Flutamide; Group 9 – 50 mg/kg/day Flutamide

Appendix 7 (continued)
F_1 Clinical Observations and Mortality
Pubertal Toxicity Study of Vinclozolin, Flutamide and Phenobarbital in Male Sprague Dawley Rats and Methoxychlor, Ethinyl Estradiol and Phenobarbital in Female Sprague Dawley Rats When Administered in Corn Oil by Oral Gavage

Group Sex	Animal Number	Clinical Sign	Site	Postnatal Day 23	24	25	26	30	37	44	51	53	54
3M	9068	No Abnormalities Detected		X	.	.	.	X	X	X	X	.	X
		Terminal Kill		.	.	.	.	.	.	.	.	.	X
	9069	No Abnormalities Detected		X	.	.	.	X	X	X	X	.	X
		Terminal Kill		.	.	.	.	.	.	.	.	.	X
	9070	No Abnormalities Detected		X	.	.	.	X	X	X	X	.	X
		Terminal Kill		.	.	.	.	.	.	.	.	.	X

X = Present S = Severe I = Minimal O = Moderate M = Male . = No Data
Dose Level: Group 1 – 0 mg/kg/day; Group 2 – 25 mg/kg/day Phenobarbital; Group 3 – 50 mg/kg/day Phenobarbital
Group 4 – 100 mg/kg/day Phenobarbital; Group 5 – 10 mg/kg/day Vinclozolin; Group 6 – 30 mg/kg/day Vinclozolin
Group 7 – 100 mg/kg/day Vinclozolin; Group 8 – 25 mg/kg/day Flutamide; Group 9 – 50 mg/kg/day Flutamide

Appendix 7 (continued)
F_1 Clinical Observations and Mortality
Pubertal Toxicity Study of Vinclozolin, Flutamide and Phenobarbital in Male Sprague Dawley Rats and Methoxychlor, Ethinyl Estradiol and Phenobarbital in Female Sprague Dawley Rats When Administered in Corn Oil by Oral Gavage

Group Sex	Animal Number	Clinical Sign	Site	Postnatal Day 23	24	25	26	30	37	44	51	53	54
4M	9071	No Abnormalities Detected		X	.	.	.	X	X	X	X	X	.
		Ataxia		.	I	I	I	.	.	.	.	.	.
		Terminal Kill		.	.	.	.	.	.	.	.	X	.
	9072	No Abnormalities Detected		X	.	.	.	X	X	X	X	X	.
		Ataxia		.	O	O	I	.	.	.	.	.	.
		Terminal Kill		.	.	.	.	.	.	.	.	X	.
	9073	No Abnormalities Detected		X	.	.	.	X	X	X	X	X	.
		Ataxia		I	I	O	O	.	.	.	.	.	.
		Terminal Kill		.	.	.	.	.	.	.	.	X	.
	9074	No Abnormalities Detected		X	.	.	.	X	X	X	X	X	.
		Ataxia		I	O	O	O	.	.	.	.	.	.
		Terminal Kill		.	.	.	.	.	.	.	.	X	.
	9075	No Abnormalities Detected		X	.	.	.	X	X	X	X	X	.
		Ataxia		.	O	O	O	.	.	.	.	.	.
		Terminal Kill		.	.	.	.	.	.	.	.	X	.
	9076	No Abnormalities Detected		X	.	.	.	X	X	X	X	X	.
		Ataxia		S	S	O	O	.	.	.	.	.	.
		Terminal Kill		.	.	.	.	.	.	.	.	X	.
	9077	No Abnormalities Detected		X	.	.	.	.	.	.	.	.	.
		Found Dead		.	X	.	.	.	.	.	.	.	.
		Prostration		X	.	.	.	.	.	.	.	.	.
	9078	No Abnormalities Detected		X	.	.	.	X	X	X	X	X	.
		Ataxia		O	S	I	I	.	.	.	.	.	.
		Terminal Kill		.	.	.	.	.	.	.	.	X	.
	9079	No Abnormalities Detected		X	.	.	.	X	X	X	X	.	X
		Ataxia		O	S	I	I	.	.	.	.	.	.
		Terminal Kill		.	.	.	.	.	.	.	.	.	X

X = Present S = Severe I = Minimal O = Moderate M = Male . = No Data
Dose Level: Group 1 – 0 mg/kg/day; Group 2 – 25 mg/kg/day Phenobarbital; Group 3 – 50 mg/kg/day Phenobarbital
Group 4 – 100 mg/kg/day Phenobarbital; Group 5 – 10 mg/kg/day Vinclozolin; Group 6 – 30 mg/kg/day Vinclozolin
Group 7 – 100 mg/kg/day Vinclozolin; Group 8 – 25 mg/kg/day Flutamide; Group 9 – 50 mg/kg/day Flutamide

Appendix 7 (continued)
F_1 Clinical Observations and Mortality
Pubertal Toxicity Study of Vinclozolin, Flutamide and Phenobarbital in Male Sprague Dawley Rats and Methoxychlor, Ethinyl Estradiol and Phenobarbital in Female Sprague Dawley Rats When Administered in Corn Oil by Oral Gavage

Group Sex	Animal Number	Clinical Sign	Site	Postnatal Day 23	24	25	26	30	37	44	51	53	54
4M	9080	No Abnormalities Detected		X	.	.	.	X	X	X	X	.	X
		Ataxia		.	S	I	I	.	.	.	.	.	.
		Prostration		X	.	.	.	.	.	.	.	.	.
		Terminal Kill		.	.	.	.	.	.	.	.	.	X
	9081	No Abnormalities Detected		X	.	.	.	X	X	X	X	.	X
		Ataxia		.	O	I	I	.	.	.	.	.	.
		Prostration		X	.	.	.	.	.	.	.	.	.
		Terminal Kill		.	.	.	.	.	.	.	.	.	X
	9082	No Abnormalities Detected		X	.	.	.	X	X	X	X	.	X
		Ataxia		O	S	I	I	.	.	.	.	.	.
		Terminal Kill		.	.	.	.	.	.	.	.	.	X
	9083	No Abnormalities Detected		X	.	.	.	X	X	X	X	.	X
		Ataxia		O	S	I	I	.	.	.	.	.	.
		Languid		.	X	.	.	.	.	.	.	.	.
		Terminal Kill		.	.	.	.	.	.	.	.	.	X
	9084	No Abnormalities Detected		X	.	.	.	X	X	X	X	.	X
		Ataxia		O	I	I	I	.	.	.	.	.	.
		Terminal Kill		.	.	.	.	.	.	.	.	.	X
	9085	No Abnormalities Detected		X	.	.	.	X	X	X	X	.	X
		Ataxia		I	O	I	I	.	.	.	.	.	.
		Terminal Kill		.	.	.	.	.	.	.	.	.	X

X = Present S = Severe I = Minimal O = Moderate M = Male . = No Data
Dose Level: Group 1 – 0 mg/kg/day; Group 2 – 25 mg/kg/day Phenobarbital; Group 3 – 50 mg/kg/day Phenobarbital
Group 4 – 100 mg/kg/day Phenobarbital; Group 5 – 10 mg/kg/day Vinclozolin; Group 6 – 30 mg/kg/day Vinclozolin
Group 7 – 100 mg/kg/day Vinclozolin; Group 8 – 25 mg/kg/day Flutamide; Group 9 – 50 mg/kg/day Flutamide

Appendix 7 (continued)
F_1 Clinical Observations and Mortality
Pubertal Toxicity Study of Vinclozolin, Flutamide and Phenobarbital in Male Sprague Dawley Rats and Methoxychlor, Ethinyl Estradiol and Phenobarbital in Female Sprague Dawley Rats When Administered in Corn Oil by Oral Gavage

				Postnatal Day									
Group Sex	Animal Number	Clinical Sign	Site	2 3	2 4	2 5	2 6	3 0	3 7	4 4	5 1	5 3	5 4
5M	9086	No Abnormalities Detected		X	.	.	.	X	X	X	X	X	.
		Terminal Kill		.	.	.	.	.	.	.	.	X	.
	9087	No Abnormalities Detected		X	.	.	.	X	X	X	X	X	.
		Terminal Kill		.	.	.	.	.	.	.	.	X	.
	9088	No Abnormalities Detected		X	.	.	.	X	X	X	X	X	.
		Terminal Kill		.	.	.	.	.	.	.	.	X	.
	9089	No Abnormalities Detected		X	.	.	.	X	X	X	X	X	.
		Terminal Kill		.	.	.	.	.	.	.	.	X	.
	9090	No Abnormalities Detected		X	.	.	.	X	X	X	X	X	.
		Terminal Kill		.	.	.	.	.	.	.	.	X	.
	9091	No Abnormalities Detected		X	.	.	.	X	X	X	X	X	.
		Terminal Kill		.	.	.	.	.	.	.	.	X	.
	9092	No Abnormalities Detected		X	.	.	.	X	X	X	X	X	.
		Terminal Kill		.	.	.	.	.	.	.	.	X	.
	9093	No Abnormalities Detected		X	.	.	.	X	X	X	X	X	.
		Terminal Kill		.	.	.	.	.	.	.	.	X	.
	9094	No Abnormalities Detected		X	.	.	.	X	X	X	X	.	X
		Terminal Kill		.	.	.	.	.	.	.	.	.	X
	9095	No Abnormalities Detected		X	.	.	.	X	X	X	X	.	X
		Terminal Kill		.	.	.	.	.	.	.	.	.	X
	9096	No Abnormalities Detected		X	.	.	.	X	X	X	X	.	X
		Terminal Kill		.	.	.	.	.	.	.	.	.	X
	9097	No Abnormalities Detected		X	.	.	.	X	X	X	X	.	X
		Terminal Kill		.	.	.	.	.	.	.	.	.	X

X = Present S = Severe I = Minimal O = Moderate M = Male . = No Data
Dose Level: Group 1 – 0 mg/kg/day; Group 2 – 25 mg/kg/day Phenobarbital; Group 3 – 50 mg/kg/day Phenobarbital
Group 4 – 100 mg/kg/day Phenobarbital; Group 5 – 10 mg/kg/day Vinclozolin; Group 6 – 30 mg/kg/day Vinclozolin
Group 7 – 100 mg/kg/day Vinclozolin; Group 8 – 25 mg/kg/day Flutamide; Group 9 – 50 mg/kg/day Flutamide

Appendix 7 (continued)
F_1 Clinical Observations and Mortality
Pubertal Toxicity Study of Vinclozolin, Flutamide and Phenobarbital in Male Sprague Dawley Rats and Methoxychlor, Ethinyl Estradiol and Phenobarbital in Female Sprague Dawley Rats When Administered in Corn Oil by Oral Gavage

				Postnatal Day									
Group Sex	Animal Number	Clinical Sign	Site	2 3	2 4	2 5	2 6	3 0	3 7	4 4	5 1	5 3	5 4
5M	9098	No Abnormalities Detected		X	.	.	.	X	X	X	X	.	X
		Terminal Kill		.	.	.	.	.	.	.	.	.	X
	9099	No Abnormalities Detected		X	.	.	.	X	X	X	X	.	X
		Terminal Kill		.	.	.	.	.	.	.	.	.	X
	9100	No Abnormalities Detected		X	.	.	.	X	X	X	X	.	X
		Terminal Kill		.	.	.	.	.	.	.	.	.	X

X = Present S = Severe I = Minimal O = Moderate M = Male . = No Data
Dose Level: Group 1 – 0 mg/kg/day; Group 2 – 25 mg/kg/day Phenobarbital; Group 3 – 50 mg/kg/day Phenobarbital
Group 4 – 100 mg/kg/day Phenobarbital; Group 5 – 10 mg/kg/day Vinclozolin; Group 6 – 30 mg/kg/day Vinclozolin
Group 7 – 100 mg/kg/day Vinclozolin; Group 8 – 25 mg/kg/day Flutamide; Group 9 – 50 mg/kg/day Flutamide

Appendix 7 (continued)
F_1 Clinical Observations and Mortality
Pubertal Toxicity Study of Vinclozolin, Flutamide and Phenobarbital in Male Sprague Dawley Rats and Methoxychlor, Ethinyl Estradiol and Phenobarbital in Female Sprague Dawley Rats When Administered in Corn Oil by Oral Gavage

				Postnatal Day									
Group Sex	Animal Number	Clinical Sign	Site	23	24	25	26	30	37	44	51	53	54
6M	9101	No Abnormalities Detected		X	.	.	.	X	X	X	X	X	.
		Terminal Kill		.	.	.	.	.	.	.	.	X	.
	9102	No Abnormalities Detected		X	.	.	.	X	X	X	X	X	.
		Terminal Kill		.	.	.	.	.	.	.	.	X	.
	9103	No Abnormalities Detected		X	.	.	.	X	X	X	X	X	.
		Terminal Kill		.	.	.	.	.	.	.	.	X	.
	9104	No Abnormalities Detected		X	.	.	.	X	X	X	X	X	.
		Terminal Kill		.	.	.	.	.	.	.	.	X	.
	9105	No Abnormalities Detected		X	.	.	.	X	X	X	X	X	.
		Terminal Kill		.	.	.	.	.	.	.	.	X	.
	9106	No Abnormalities Detected		X	.	.	.	X	X	X	X	X	.
		Terminal Kill		.	.	.	.	.	.	.	.	X	.
	9107	No Abnormalities Detected		X	.	.	.	X	X	X	X	X	.
		Terminal Kill		.	.	.	.	.	.	.	.	X	.
	9108	No Abnormalities Detected		X	.	.	.	X	X	X	X	X	.
		Terminal Kill		.	.	.	.	.	.	.	.	X	.
	9109	No Abnormalities Detected		X	.	.	.	X	X	X	X	.	X
		Terminal Kill		.	.	.	.	.	.	.	.	.	X
	9110	No Abnormalities Detected		X	.	.	.	X	X	X	X	.	X
		Terminal Kill		.	.	.	.	.	.	.	.	.	X
	9111	No Abnormalities Detected		X	.	.	.	X	X	X	X	.	X
		Terminal Kill		.	.	.	.	.	.	.	.	.	X
	9112	No Abnormalities Detected		X	.	.	.	X	X	X	X	.	X
		Terminal Kill		.	.	.	.	.	.	.	.	.	X

X = Present S = Severe I = Minimal O = Moderate M = Male . = No Data
Dose Level: Group 1 – 0 mg/kg/day; Group 2 – 25 mg/kg/day Phenobarbital; Group 3 – 50 mg/kg/day Phenobarbital
Group 4 – 100 mg/kg/day Phenobarbital; Group 5 – 10 mg/kg/day Vinclozolin; Group 6 – 30 mg/kg/day Vinclozolin
Group 7 – 100 mg/kg/day Vinclozolin; Group 8 – 25 mg/kg/day Flutamide; Group 9 – 50 mg/kg/day Flutamide

Appendix 7 (continued)
F_1 Clinical Observations and Mortality
Pubertal Toxicity Study of Vinclozolin, Flutamide and Phenobarbital in Male Sprague Dawley Rats and Methoxychlor, Ethinyl Estradiol and Phenobarbital in Female Sprague Dawley Rats When Administered in Corn Oil by Oral Gavage

Group Sex	Animal Number	Clinical Sign	Site	Postnatal Day 23	24	25	26	30	37	44	51	53	54
6M	9113	No Abnormalities Detected		X	.	.	.	X	X	X	X	.	X
		Terminal Kill		.	.	.	.	.	.	.	.	.	X
	9114	No Abnormalities Detected		X	.	.	.	X	X	X	X	.	X
		Terminal Kill		.	.	.	.	.	.	.	.	.	X
	9115	No Abnormalities Detected		X	.	.	.	X	X	X	X	.	X
		Terminal Kill		.	.	.	.	.	.	.	.	.	X

X = Present S = Severe I = Minimal O = Moderate M = Male . = No Data
Dose Level: Group 1 – 0 mg/kg/day; Group 2 – 25 mg/kg/day Phenobarbital; Group 3 – 50 mg/kg/day Phenobarbital
Group 4 – 100 mg/kg/day Phenobarbital; Group 5 – 10 mg/kg/day Vinclozolin; Group 6 – 30 mg/kg/day Vinclozolin
Group 7 – 100 mg/kg/day Vinclozolin; Group 8 – 25 mg/kg/day Flutamide; Group 9 – 50 mg/kg/day Flutamide

Appendix 7 (continued)
F_1 Clinical Observations and Mortality
Pubertal Toxicity Study of Vinclozolin, Flutamide and Phenobarbital in Male Sprague Dawley Rats and Methoxychlor, Ethinyl Estradiol and Phenobarbital in Female Sprague Dawley Rats When Administered in Corn Oil by Oral Gavage

				Postnatal Day									
Group Sex	Animal Number	Clinical Sign	Site	2 3	2 4	2 5	2 6	3 0	3 7	4 4	5 1	5 3	5 4
7M	9116	No Abnormalities Detected		X	.	.	.	X	X	X	X	X	.
		Terminal Kill		.	.	.	.	.	.	.	.	X	.
	9117	No Abnormalities Detected		X	.	.	.	X	X	X	X	X	.
		Terminal Kill		.	.	.	.	.	.	.	.	X	.
	9118	No Abnormalities Detected		X	.	.	.	X	X	X	X	X	.
		Terminal Kill		.	.	.	.	.	.	.	.	X	.
	9119	No Abnormalities Detected		X	.	.	.	X	X	X	X	X	.
		Terminal Kill		.	.	.	.	.	.	.	.	X	.
	9120	No Abnormalities Detected		X	.	.	.	X	X	X	X	X	.
		Terminal Kill		.	.	.	.	.	.	.	.	X	.
	9121	No Abnormalities Detected		X	.	.	.	X	X	X	X	X	.
		Terminal Kill		.	.	.	.	.	.	.	.	X	.
	9122	No Abnormalities Detected		X	.	.	.	X	X	X	X	X	.
		Terminal Kill		.	.	.	.	.	.	.	.	X	.
	9123	No Abnormalities Detected		X	.	.	.	X	X	X	X	X	.
		Terminal Kill		.	.	.	.	.	.	.	.	X	.
	9124	No Abnormalities Detected		X	.	.	.	X	X	X	X	.	X
		Terminal Kill		.	.	.	.	.	.	.	.	.	X
	9125	No Abnormalities Detected		X	.	.	.	X	X	X	X	.	X
		Terminal Kill		.	.	.	.	.	.	.	.	.	X
	9126	No Abnormalities Detected		X	.	.	.	X	X	X	X	.	X
		Terminal Kill		.	.	.	.	.	.	.	.	.	X
	9127	No Abnormalities Detected		X	.	.	.	X	X	X	X	.	X
		Terminal Kill		.	.	.	.	.	.	.	.	.	X

X = Present S = Severe I = Minimal O = Moderate M = Male . = No Data
Dose Level: Group 1 – 0 mg/kg/day; Group 2 – 25 mg/kg/day Phenobarbital; Group 3 – 50 mg/kg/day Phenobarbital
Group 4 – 100 mg/kg/day Phenobarbital; Group 5 – 10 mg/kg/day Vinclozolin; Group 6 – 30 mg/kg/day Vinclozolin
Group 7 – 100 mg/kg/day Vinclozolin; Group 8 – 25 mg/kg/day Flutamide; Group 9 – 50 mg/kg/day Flutamide

Appendix 7 (continued)
F_1 Clinical Observations and Mortality
Pubertal Toxicity Study of Vinclozolin, Flutamide and Phenobarbital in Male Sprague Dawley Rats and Methoxychlor, Ethinyl Estradiol and Phenobarbital in Female Sprague Dawley Rats When Administered in Corn Oil by Oral Gavage

				Postnatal Day									
Group Sex	Animal Number	Clinical Sign	Site	2 3	2 4	2 5	2 6	3 0	3 7	4 4	5 1	5 3	5 4
7M	9128	No Abnormalities Detected		X	.	.	.	X	X	X	X	.	X
		Terminal Kill		.	.	.	.	.	.	.	.	.	X
	9129	No Abnormalities Detected		X	.	.	.	X	X	X	X	.	X
		Terminal Kill		.	.	.	.	.	.	.	.	.	X
	9130	No Abnormalities Detected		X	.	.	.	X	X	X	X	.	X
		Terminal Kill		.	.	.	.	.	.	.	.	.	X

X = Present S = Severe I = Minimal O = Moderate M = Male . = No Data
Dose Level: Group 1 – 0 mg/kg/day; Group 2 – 25 mg/kg/day Phenobarbital; Group 3 – 50 mg/kg/day Phenobarbital
Group 4 – 100 mg/kg/day Phenobarbital; Group 5 – 10 mg/kg/day Vinclozolin; Group 6 – 30 mg/kg/day Vinclozolin
Group 7 – 100 mg/kg/day Vinclozolin; Group 8 – 25 mg/kg/day Flutamide; Group 9 – 50 mg/kg/day Flutamide

Appendix 7 (continued)
F_1 Clinical Observations and Mortality
Pubertal Toxicity Study of Vinclozolin, Flutamide and Phenobarbital in Male Sprague Dawley Rats and Methoxychlor, Ethinyl Estradiol and Phenobarbital in Female Sprague Dawley Rats When Administered in Corn Oil by Oral Gavage

				Postnatal Day									
Group Sex	Animal Number	Clinical Sign	Site	2 3	2 4	2 5	2 6	3 0	3 7	4 4	5 1	5 3	5 4
8M	9131	No Abnormalities Detected		X	.	.	.	X	X	X	X	X	.
		Terminal Kill		.	.	.	.	.	.	.	.	X	.
	9132	No Abnormalities Detected		X	.	.	.	X	X	X	X	X	.
		Terminal Kill		.	.	.	.	.	.	.	.	X	.
	9133	No Abnormalities Detected		X	.	.	.	X	X	X	X	X	.
		Terminal Kill		.	.	.	.	.	.	.	.	X	.
	9134	No Abnormalities Detected		X	.	.	.	X	X	X	X	X	.
		Terminal Kill		.	.	.	.	.	.	.	.	X	.
	9135	No Abnormalities Detected		X	.	.	.	X	X	X	X	X	.
		Terminal Kill		.	.	.	.	.	.	.	.	X	.
	9136	No Abnormalities Detected		X	.	.	.	X	X	X	X	X	.
		Terminal Kill		.	.	.	.	.	.	.	.	X	.
	9137	No Abnormalities Detected		X	.	.	.	X	X	X	X	X	.
		Terminal Kill		.	.	.	.	.	.	.	.	X	.
	9138	No Abnormalities Detected		X	.	.	.	X	X	X	X	X	.
		Terminal Kill		.	.	.	.	.	.	.	.	X	.
	9139	No Abnormalities Detected		X	.	.	.	X	X	X	X	.	X
		Terminal Kill		.	.	.	.	.	.	.	.	.	X
	9140	No Abnormalities Detected		X	.	.	.	X	X	X	X	.	X
		Terminal Kill		.	.	.	.	.	.	.	.	.	X
	9141	No Abnormalities Detected		X	.	.	.	X	X	X	X	.	X
		Terminal Kill		.	.	.	.	.	.	.	.	.	X
	9142	No Abnormalities Detected		X	.	.	.	X	X	X	X	.	X
		Terminal Kill		.	.	.	.	.	.	.	.	.	X

X = Present S = Severe I = Minimal O = Moderate M = Male . = No Data
Dose Level: Group 1 – 0 mg/kg/day; Group 2 – 25 mg/kg/day Phenobarbital; Group 3 – 50 mg/kg/day Phenobarbital
Group 4 – 100 mg/kg/day Phenobarbital; Group 5 – 10 mg/kg/day Vinclozolin; Group 6 – 30 mg/kg/day Vinclozolin
Group 7 – 100 mg/kg/day Vinclozolin; Group 8 – 25 mg/kg/day Flutamide; Group 9 – 50 mg/kg/day Flutamide

Appendix 7 (continued)
F_1 Clinical Observations and Mortality
Pubertal Toxicity Study of Vinclozolin, Flutamide and Phenobarbital in Male Sprague Dawley Rats and Methoxychlor, Ethinyl Estradiol and Phenobarbital in Female Sprague Dawley Rats When Administered in Corn Oil by Oral Gavage

Group Sex	Animal Number	Clinical Sign	Site	Postnatal Day 23	24	25	26	30	37	44	51	53	54
8M	9143	No Abnormalities Detected		X	.	.	.	X	X	X	X	.	X
		Terminal Kill		.	.	.	.	.	.	.	.	.	X
	9144	No Abnormalities Detected		X	.	.	.	X	X	X	X	.	X
		Terminal Kill		.	.	.	.	.	.	.	.	.	X
	9145	No Abnormalities Detected		X	.	.	.	X	X	X	X	.	X
		Terminal Kill		.	.	.	.	.	.	.	.	.	X

X = Present S = Severe I = Minimal O = Moderate M = Male . = No Data
Dose Level: Group 1 – 0 mg/kg/day; Group 2 – 25 mg/kg/day Phenobarbital; Group 3 – 50 mg/kg/day Phenobarbital
Group 4 – 100 mg/kg/day Phenobarbital; Group 5 – 10 mg/kg/day Vinclozolin; Group 6 – 30 mg/kg/day Vinclozolin
Group 7 – 100 mg/kg/day Vinclozolin; Group 8 – 25 mg/kg/day Flutamide; Group 9 – 50 mg/kg/day Flutamide

Appendix 7 (continued)
F_1 Clinical Observations and Mortality
Pubertal Toxicity Study of Vinclozolin, Flutamide and Phenobarbital in Male Sprague Dawley Rats and Methoxychlor, Ethinyl Estradiol and Phenobarbital in Female Sprague Dawley Rats When Administered in Corn Oil by Oral Gavage

				Postnatal Day									
Group Sex	Animal Number	Clinical Sign	Site	23	24	25	26	30	37	44	51	53	54
9M	9146	No Abnormalities Detected		X	.	.	.	X	X	X	X	X	.
		Terminal Kill		.	.	.	.	.	.	.	.	X	.
	9147	No Abnormalities Detected		X	.	.	.	X	X	X	X	X	.
		Terminal Kill		.	.	.	.	.	.	.	.	X	.
	9148	No Abnormalities Detected		X	.	.	.	X	X	X	X	X	.
		Terminal Kill		.	.	.	.	.	.	.	.	X	.
	9149	No Abnormalities Detected		X	.	.	.	X	X	X	X	X	.
		Terminal Kill		.	.	.	.	.	.	.	.	X	.
	9150	No Abnormalities Detected		X	.	.	.	X	X	X	X	X	.
		Terminal Kill		.	.	.	.	.	.	.	.	X	.
	9151	No Abnormalities Detected		X	.	.	.	X	X	X	X	X	.
		Terminal Kill		.	.	.	.	.	.	.	.	X	.
	9152	No Abnormalities Detected		X	.	.	.	X	X	X	X	X	.
		Terminal Kill		.	.	.	.	.	.	.	.	X	.
	9153	No Abnormalities Detected		X	.	.	.	X	X	X	X	X	.
		Terminal Kill		.	.	.	.	.	.	.	.	X	.
	9154	No Abnormalities Detected		X	.	.	.	X	X	X	X	.	X
		Terminal Kill		.	.	.	.	.	.	.	.	.	X
	9155	No Abnormalities Detected		X	.	.	.	X	X	X	X	.	X
		Terminal Kill		.	.	.	.	.	.	.	.	.	X
	9156	No Abnormalities Detected		X	.	.	.	X	X	X	X	.	X
		Terminal Kill		.	.	.	.	.	.	.	.	.	X
	9157	No Abnormalities Detected		X	.	.	.	X	X	X	X	.	X
		Terminal Kill		.	.	.	.	.	.	.	.	.	X

X = Present S = Severe I = Minimal O = Moderate M = Male . = No Data
Dose Level: Group 1 – 0 mg/kg/day; Group 2 – 25 mg/kg/day Phenobarbital; Group 3 – 50 mg/kg/day Phenobarbital
Group 4 – 100 mg/kg/day Phenobarbital; Group 5 – 10 mg/kg/day Vinclozolin; Group 6 – 30 mg/kg/day Vinclozolin
Group 7 – 100 mg/kg/day Vinclozolin; Group 8 – 25 mg/kg/day Flutamide; Group 9 – 50 mg/kg/day Flutamide

Appendix 7 (continued)
F_1 Clinical Observations and Mortality
Pubertal Toxicity Study of Vinclozolin, Flutamide and Phenobarbital in Male Sprague Dawley Rats and Methoxychlor, Ethinyl Estradiol and Phenobarbital in Female Sprague Dawley Rats When Administered in Corn Oil by Oral Gavage

Group Sex	Animal Number	Clinical Sign	Site	Postnatal Day 23	24	25	26	30	37	44	51	53	54
9M	9158	No Abnormalities Detected		X	.	.	.	X	X	X	X	.	X
		Terminal Kill		.	.	.	.	.	.	.	.	.	X
	9159	No Abnormalities Detected		X	.	.	.	X	X	X	X	.	X
		Terminal Kill		.	.	.	.	.	.	.	.	.	X
	9160	No Abnormalities Detected		X	.	.	.	X	X	X	X	.	X
		Terminal Kill		.	.	.	.	.	.	.	.	.	X

X = Present S = Severe I = Minimal O = Moderate M = Male . = No Data
Dose Level: Group 1 – 0 mg/kg/day; Group 2 – 25 mg/kg/day Phenobarbital; Group 3 – 50 mg/kg/day Phenobarbital
Group 4 – 100 mg/kg/day Phenobarbital; Group 5 – 10 mg/kg/day Vinclozolin; Group 6 – 30 mg/kg/day Vinclozolin
Group 7 – 100 mg/kg/day Vinclozolin; Group 8 – 25 mg/kg/day Flutamide; Group 9 – 50 mg/kg/day Flutamide

Appendix 7 (continued)
F_1 Clinical Observations and Mortality
Pubertal Toxicity Study of Vinclozolin, Flutamide and Phenobarbital in Male Sprague Dawley Rats and Methoxychlor, Ethinyl Estradiol and Phenobarbital in Female Sprague Dawley Rats When Administered in Corn Oil by Oral Gavage

Group Sex	Animal Number	Clinical Sign	Site	Postnatal Day 22	23	24	25	26	29	36	42	43
1F	9161	No Abnormalities Detected		X	.	.	.	.	X	X	X	.
		Terminal Kill		.	.	.	.	.	.	.	X	.
	9162	No Abnormalities Detected		X	.	.	.	.	X	X	X	.
		Terminal Kill		.	.	.	.	.	.	.	X	.
	9163	No Abnormalities Detected		X	.	.	.	.	X	X	X	.
		Terminal Kill		.	.	.	.	.	.	.	X	.
	9164	No Abnormalities Detected		X	.	.	.	.	X	X	X	.
		Terminal Kill		.	.	.	.	.	.	.	X	.
	9165	No Abnormalities Detected		X	.	.	.	.	X	X	X	.
		Terminal Kill		.	.	.	.	.	.	.	X	.
	9166	No Abnormalities Detected		X	.	.	.	.	X	X	X	.
		Terminal Kill		.	.	.	.	.	.	.	X	.
	9167	No Abnormalities Detected		X	.	.	.	.	X	X	X	.
		Terminal Kill		.	.	.	.	.	.	.	X	.
	9168	No Abnormalities Detected		X	.	.	.	.	X	X	X	.
		Terminal Kill		.	.	.	.	.	.	.	X	.
	9169	No Abnormalities Detected		X	.	.	.	.	X	X	.	X
		Terminal Kill		.	.	.	.	.	.	.	.	X
	9170	No Abnormalities Detected		X	.	.	.	.	X	X	.	X
		Terminal Kill		.	.	.	.	.	.	.	.	X
	9171	No Abnormalities Detected		X	.	.	.	.	X	X	.	X
		Terminal Kill		.	.	.	.	.	.	.	.	X
	9172	No Abnormalities Detected		X	.	.	.	.	X	X	.	X
		Terminal Kill		.	.	.	.	.	.	.	.	X

X = Present S = Severe I = Minimal O = Moderate F = Female . = No data
Dose Level: Group 1 - 0 mg/kg/day; Group 2 - 0.0025 mg/kg/day Ethinyl Estradiol; Group 3 - 0.005 mg/kg/day Ethinyl Estradiol
Group 4 - 12.5 mg/kg/day Methoxychlor; Group 5 - 25 mg/kg/day Methoxychlor; Group 6 - 50 mg/kg/day Methoxychlor
Group 7 - 25 mg/kg/day Phenobarbital; Group 8 - 50 mg/kg/day Phenobarbital; Group 9 - 100 mg/kg/day Phenobarbital

Appendix 7 (continued)
F_1 Clinical Observations and Mortality
Pubertal Toxicity Study of Vinclozolin, Flutamide and Phenobarbital in Male Sprague Dawley Rats and Methoxychlor, Ethinyl Estradiol and Phenobarbital in Female Sprague Dawley Rats When Administered in Corn Oil by Oral Gavage

Group Sex	Animal Number	Clinical Sign	Site	Postnatal Day 2 2	2 3	2 4	2 5	2 6	2 9	3 6	4 2	4 3
1F	9173	No Abnormalities Detected		X	.	.	.	.	X	X	.	X
		Terminal Kill		.	.	.	.	.	.	.	.	X
	9174	No Abnormalities Detected		X	.	.	.	.	X	X	.	X
		Terminal Kill		.	.	.	.	.	.	.	.	X
	9175	No Abnormalities Detected		X	.	.	.	.	X	X	.	X
		Terminal Kill		.	.	.	.	.	.	.	.	X

X = Present S = Severe I = Minimal O = Moderate F = Female . = No data
Dose Level: Group 1 – 0 mg/kg/day; Group 2 – 0.0025 mg/kg/day Ethinyl Estradiol; Group 3 – 0.005 mg/kg/day Ethinyl Estradiol
Group 4 – 12.5 mg/kg/day Methoxychlor; Group 5 – 25 mg/kg/day Methoxychlor; Group 6 – 50 mg/kg/day Methoxychlor
Group 7 – 25 mg/kg/day Phenobarbital; Group 8 – 50 mg/kg/day Phenobarbital; Group 9 – 100 mg/kg/day Phenobarbital

Appendix 7 (continued)
F_1 Clinical Observations and Mortality
Pubertal Toxicity Study of Vinclozolin, Flutamide and Phenobarbital in Male Sprague Dawley Rats and Methoxychlor, Ethinyl Estradiol and Phenobarbital in Female Sprague Dawley Rats When Administered in Corn Oil by Oral Gavage

				Postnatal Day								
Group Sex	Animal Number	Clinical Sign	Site	2 2	2 3	2 4	2 5	2 6	2 9	3 6	4 2	4 3
2F	9176	No Abnormalities Detected		X	.	.	.	.	X	X	X	.
		Terminal Kill		.	.	.	.	.	.	.	X	.
	9177	No Abnormalities Detected		X	.	.	.	.	X	X	X	.
		Terminal Kill		.	.	.	.	.	.	.	X	.
	9178	No Abnormalities Detected		X	.	.	.	.	X	X	X	.
		Terminal Kill		.	.	.	.	.	.	.	X	.
	9179	No Abnormalities Detected		X	.	.	.	.	X	X	X	.
		Terminal Kill		.	.	.	.	.	.	.	X	.
	9180	No Abnormalities Detected		X	.	.	.	.	X	X	X	.
		Terminal Kill		.	.	.	.	.	.	.	X	.
	9181	No Abnormalities Detected		X	.	.	.	.	X	X	X	.
		Terminal Kill		.	.	.	.	.	.	.	X	.
	9182	No Abnormalities Detected		X	.	.	.	.	X	X	X	.
		Terminal Kill		.	.	.	.	.	.	.	X	.
	9183	No Abnormalities Detected		X	.	.	.	.	X	X	X	.
		Terminal Kill		.	.	.	.	.	.	.	X	.
	9184	No Abnormalities Detected		X	.	.	.	.	X	X	.	X
		Terminal Kill		.	.	.	.	.	.	.	.	X
	9185	No Abnormalities Detected		X	.	.	.	.	X	X	.	X
		Terminal Kill		.	.	.	.	.	.	.	.	X
	9186	No Abnormalities Detected		X	.	.	.	.	X	X	.	X
		Terminal Kill		.	.	.	.	.	.	.	.	X
	9187	No Abnormalities Detected		X	.	.	.	.	X	X	.	X
		Terminal Kill		.	.	.	.	.	.	.	.	X

X = Present S = Severe I = Minimal O = Moderate F = Female . = No data
Dose Level: Group 1 – 0 mg/kg/day; Group 2 – 0.0025 mg/kg/day Ethinyl Estradiol; Group 3 – 0.005 mg/kg/day Ethinyl Estradiol
Group 4 – 12.5 mg/kg/day Methoxychlor; Group 5 – 25 mg/kg/day Methoxychlor; Group 6 – 50 mg/kg/day Methoxychlor
Group 7 – 25 mg/kg/day Phenobarbital; Group 8 – 50 mg/kg/day Phenobarbital; Group 9 – 100 mg/kg/day Phenobarbital

Appendix 7 (continued)
F_1 Clinical Observations and Mortality
Pubertal Toxicity Study of Vinclozolin, Flutamide and Phenobarbital in Male Sprague Dawley Rats and Methoxychlor, Ethinyl Estradiol and Phenobarbital in Female Sprague Dawley Rats When Administered in Corn Oil by Oral Gavage

Group Sex	Animal Number	Clinical Sign	Site	Postnatal Day 2 2	2 3	2 4	2 5	2 6	2 9	3 6	4 2	4 3
2F	9188	No Abnormalities Detected		X	.	.	.	.	X	X	.	X
		Terminal Kill		.	.	.	.	.	.	.	.	X
	9189	No Abnormalities Detected		X	.	.	.	.	X	X	.	X
		Terminal Kill		.	.	.	.	.	.	.	.	X
	9190	No Abnormalities Detected		X	.	.	.	.	X	X	.	X
		Terminal Kill		.	.	.	.	.	.	.	.	X

X = Present S = Severe I = Minimal O = Moderate F = Female . = No data
Dose Level: Group 1 – 0 mg/kg/day; Group 2 – 0.0025 mg/kg/day Ethinyl Estradiol; Group 3 – 0.005 mg/kg/day Ethinyl Estradiol
Group 4 – 12.5 mg/kg/day Methoxychlor; Group 5 – 25 mg/kg/day Methoxychlor; Group 6 – 50 mg/kg/day Methoxychlor
Group 7 – 25 mg/kg/day Phenobarbital; Group 8 – 50 mg/kg/day Phenobarbital; Group 9 – 100 mg/kg/day Phenobarbital

Appendix 7 (continued)
F_1 Clinical Observations and Mortality
Pubertal Toxicity Study of Vinclozolin, Flutamide and Phenobarbital in Male Sprague Dawley Rats and Methoxychlor, Ethinyl Estradiol and Phenobarbital in Female Sprague Dawley Rats When Administered in Corn Oil by Oral Gavage

Group Sex	Animal Number	Clinical Sign	Site	Postnatal Day 22	23	24	25	26	29	36	42	43
3F	9191	No Abnormalities Detected		X	.	.	.	.	X	X	X	.
		Terminal Kill		.	.	.	.	.	.	.	X	.
	9192	No Abnormalities Detected		X	.	.	.	.	X	X	X	.
		Terminal Kill		.	.	.	.	.	.	.	X	.
	9193	No Abnormalities Detected		X	.	.	.	.	X	X	X	.
		Terminal Kill		.	.	.	.	.	.	.	X	.
	9194	No Abnormalities Detected		X	.	.	.	.	X	X	X	.
		Terminal Kill		.	.	.	.	.	.	.	X	.
	9195	No Abnormalities Detected		X	.	.	.	.	X	X	X	.
		Terminal Kill		.	.	.	.	.	.	.	X	.
	9196	No Abnormalities Detected		X	.	.	.	.	X	X	X	.
		Terminal Kill		.	.	.	.	.	.	.	X	.
	9197	No Abnormalities Detected		X	.	.	.	.	X	X	X	.
		Terminal Kill		.	.	.	.	.	.	.	X	.
	9198	No Abnormalities Detected		X	.	.	.	.	X	X	X	.
		Terminal Kill		.	.	.	.	.	.	.	X	.
	9199	No Abnormalities Detected		X	.	.	.	.	X	X	.	X
		Terminal Kill		.	.	.	.	.	.	.	.	X
	9200	No Abnormalities Detected		X	.	.	.	.	X	X	.	X
		Terminal Kill		.	.	.	.	.	.	.	.	X
	9201	No Abnormalities Detected		X	.	.	.	.	X	X	.	X
		Terminal Kill		.	.	.	.	.	.	.	.	X
	9202	No Abnormalities Detected		X	.	.	.	.	X	X	.	X
		Terminal Kill		.	.	.	.	.	.	.	.	X

X = Present S = Severe I = Minimal O = Moderate F = Female . = No data
Dose Level: Group 1 - 0 mg/kg/day; Group 2 - 0.0025 mg/kg/day Ethinyl Estradiol; Group 3 - 0.005 mg/kg/day Ethinyl Estradiol
Group 4 - 12.5 mg/kg/day Methoxychlor; Group 5 - 25 mg/kg/day Methoxychlor; Group 6 - 50 mg/kg/day Methoxychlor
Group 7 - 25 mg/kg/day Phenobarbital; Group 8 - 50 mg/kg/day Phenobarbital; Group 9 - 100 mg/kg/day Phenobarbital

Appendix 7 (continued)
F_1 Clinical Observations and Mortality
Pubertal Toxicity Study of Vinclozolin, Flutamide and Phenobarbital in Male Sprague Dawley Rats and Methoxychlor, Ethinyl Estradiol and Phenobarbital in Female Sprague Dawley Rats When Administered in Corn Oil by Oral Gavage

Group Sex	Animal Number	Clinical Sign	Site	Postnatal Day 2 2	2 3	2 4	2 5	2 6	2 9	3 6	4 2	4 3
3F	9203	No Abnormalities Detected		X	.	.	.	.	X	X	.	X
		Terminal Kill		.	.	.	.	.	.	.	.	X
	9204	No Abnormalities Detected		X	.	.	.	.	X	X	.	X
		Terminal Kill		.	.	.	.	.	.	.	.	X
	9205	No Abnormalities Detected		X	.	.	.	.	X	X	.	X
		Terminal Kill		.	.	.	.	.	.	.	.	X

X = Present S = Severe I = Minimal O = Moderate F = Female . = No data
Dose Level: Group 1 – 0 mg/kg/day; Group 2 – 0.0025 mg/kg/day Ethinyl Estradiol; Group 3 – 0.005 mg/kg/day Ethinyl Estradiol
Group 4 – 12.5 mg/kg/day Methoxychlor; Group 5 – 25 mg/kg/day Methoxychlor; Group 6 – 50 mg/kg/day Methoxychlor
Group 7 – 25 mg/kg/day Phenobarbital; Group 8 – 50 mg/kg/day Phenobarbital; Group 9 – 100 mg/kg/day Phenobarbital

Appendix 7 (continued)
F_1 Clinical Observations and Mortality
Pubertal Toxicity Study of Vinclozolin, Flutamide and Phenobarbital in Male Sprague Dawley Rats and Methoxychlor, Ethinyl Estradiol and Phenobarbital in Female Sprague Dawley Rats When Administered in Corn Oil by Oral Gavage

Group Sex	Animal Number	Clinical Sign	Site	Postnatal Day 22	23	24	25	26	29	36	42	43
4F	9206	No Abnormalities Detected		X	.	.	.	.	X	X	X	.
		Terminal Kill		.	.	.	.	.	.	.	X	.
	9207	No Abnormalities Detected		X	.	.	.	.	X	X	X	.
		Terminal Kill		.	.	.	.	.	.	.	X	.
	9208	No Abnormalities Detected		X	.	.	.	.	X	X	X	.
		Terminal Kill		.	.	.	.	.	.	.	X	.
	9209	No Abnormalities Detected		X	.	.	.	.	X	X	X	.
		Terminal Kill		.	.	.	.	.	.	.	X	.
	9210	No Abnormalities Detected		X	.	.	.	.	X	X	X	.
		Terminal Kill		.	.	.	.	.	.	.	X	.
	9211	No Abnormalities Detected		X	.	.	.	.	X	X	X	.
		Terminal Kill		.	.	.	.	.	.	.	X	.
	9212	No Abnormalities Detected		X	.	.	.	.	X	X	X	.
		Terminal Kill		.	.	.	.	.	.	.	X	.
	9213	No Abnormalities Detected		X	.	.	.	.	X	X	X	.
		Terminal Kill		.	.	.	.	.	.	.	X	.
	9214	No Abnormalities Detected		X	.	.	.	.	X	X	.	X
		Terminal Kill		.	.	.	.	.	.	.	.	X
	9215	No Abnormalities Detected		X	.	.	.	.	X	X	.	X
		Terminal Kill		.	.	.	.	.	.	.	.	X
	9216	No Abnormalities Detected		X	.	.	.	.	X	X	.	X
		Terminal Kill		.	.	.	.	.	.	.	.	X
	9217	No Abnormalities Detected		X	.	.	.	.	X	X	.	X
		Terminal Kill		.	.	.	.	.	.	.	.	X

X = Present S = Severe I = Minimal O = Moderate F = Female . = No data
Dose Level: Group 1 – 0 mg/kg/day; Group 2 – 0.0025 mg/kg/day Ethinyl Estradiol; Group 3 – 0.005 mg/kg/day Ethinyl Estradiol
Group 4 – 12.5 mg/kg/day Methoxychlor; Group 5 – 25 mg/kg/day Methoxychlor; Group 6 – 50 mg/kg/day Methoxychlor
Group 7 – 25 mg/kg/day Phenobarbital; Group 8 – 50 mg/kg/day Phenobarbital; Group 9 – 100 mg/kg/day Phenobarbital

Appendix 7 (continued)
F_1 Clinical Observations and Mortality
Pubertal Toxicity Study of Vinclozolin, Flutamide and Phenobarbital in Male Sprague Dawley Rats and Methoxychlor, Ethinyl Estradiol and Phenobarbital in Female Sprague Dawley Rats When Administered in Corn Oil by Oral Gavage

				Postnatal Day								
Group Sex	Animal Number	Clinical Sign	Site	2 2	2 3	2 4	2 5	2 6	2 9	3 6	4 2	4 3
4F	9218	No Abnormalities Detected		X	.	.	.	.	X	X	.	X
		Terminal Kill		.	.	.	.	.	.	.	.	X
	9219	No Abnormalities Detected		X	.	.	.	.	X	X	.	X
		Terminal Kill		.	.	.	.	.	.	.	.	X
	9220	No Abnormalities Detected		X	.	.	.	.	X	X	.	X
		Terminal Kill		.	.	.	.	.	.	.	.	X

X = Present S = Severe I = Minimal O = Moderate F = Female . = No data
Dose Level: Group 1 – 0 mg/kg/day; Group 2 – 0.0025 mg/kg/day Ethinyl Estradiol; Group 3 – 0.005 mg/kg/day Ethinyl Estradiol
Group 4 – 12.5 mg/kg/day Methoxychlor; Group 5 – 25 mg/kg/day Methoxychlor; Group 6 – 50 mg/kg/day Methoxychlor
Group 7 – 25 mg/kg/day Phenobarbital; Group 8 – 50 mg/kg/day Phenobarbital; Group 9 – 100 mg/kg/day Phenobarbital

Appendix 7 (continued)
F_1 Clinical Observations and Mortality
Pubertal Toxicity Study of Vinclozolin, Flutamide and Phenobarbital in Male Sprague Dawley Rats and Methoxychlor, Ethinyl Estradiol and Phenobarbital in Female Sprague Dawley Rats When Administered in Corn Oil by Oral Gavage

Group Sex	Animal Number	Clinical Sign	Site	Postnatal Day 22	23	24	25	26	29	36	42	43
5F	9221	No Abnormalities Detected		X	.	.	.	.	X	X	X	.
		Terminal Kill		.	.	.	.	.	.	.	X	.
	9222	No Abnormalities Detected		X	.	.	.	.	X	X	X	.
		Terminal Kill		.	.	.	.	.	.	.	X	.
	9223	No Abnormalities Detected		X	.	.	.	.	X	X	X	.
		Terminal Kill		.	.	.	.	.	.	.	X	.
	9224	No Abnormalities Detected		X	.	.	.	.	X	X	X	.
		Terminal Kill		.	.	.	.	.	.	.	X	.
	9225	No Abnormalities Detected		X	.	.	.	.	X	X	X	.
		Terminal Kill		.	.	.	.	.	.	.	X	.
	9226	No Abnormalities Detected		X	.	.	.	.	X	X	X	.
		Terminal Kill		.	.	.	.	.	.	.	X	.
	9227	No Abnormalities Detected		X	.	.	.	.	X	X	X	.
		Terminal Kill		.	.	.	.	.	.	.	X	.
	9228	No Abnormalities Detected		X	.	.	.	.	X	X	X	.
		Terminal Kill		.	.	.	.	.	.	.	X	.
	9229	No Abnormalities Detected		X	.	.	.	.	X	X	.	X
		Terminal Kill		.	.	.	.	.	.	.	.	X
	9230	No Abnormalities Detected		X	.	.	.	.	X	X	.	X
		Terminal Kill		.	.	.	.	.	.	.	.	X
	9231	No Abnormalities Detected		X	.	.	.	.	X	X	.	X
		Terminal Kill		.	.	.	.	.	.	.	.	X
	9232	No Abnormalities Detected		X	.	.	.	.	X	X	.	X
		Terminal Kill		.	.	.	.	.	.	.	.	X

X = Present S = Severe I = Minimal O = Moderate F = Female . = No data
Dose Level: Group 1 – 0 mg/kg/day; Group 2 – 0.0025 mg/kg/day Ethinyl Estradiol; Group 3 – 0.005 mg/kg/day Ethinyl Estradiol
Group 4 – 12.5 mg/kg/day Methoxychlor; Group 5 – 25 mg/kg/day Methoxychlor; Group 6 – 50 mg/kg/day Methoxychlor
Group 7 – 25 mg/kg/day Phenobarbital; Group 8 – 50 mg/kg/day Phenobarbital; Group 9 – 100 mg/kg/day Phenobarbital

Appendix 7 (continued)
F_1 Clinical Observations and Mortality
Pubertal Toxicity Study of Vinclozolin, Flutamide and Phenobarbital in Male Sprague Dawley Rats and Methoxychlor, Ethinyl Estradiol and Phenobarbital in Female Sprague Dawley Rats When Administered in Corn Oil by Oral Gavage

Group Sex	Animal Number	Clinical Sign	Site	Postnatal Day 2 2	2 3	2 4	2 5	2 6	2 9	3 6	4 2	4 3
5F	9233	No Abnormalities Detected		X	.	.	.	.	X	X	.	X
		Terminal Kill		.	.	.	.	.	.	.	.	X
	9234	No Abnormalities Detected		X	.	.	.	.	X	X	.	X
		Terminal Kill		.	.	.	.	.	.	.	.	X
	9235	No Abnormalities Detected		X	.	.	.	.	X	X	.	X
		Terminal Kill		.	.	.	.	.	.	.	.	X

X = Present S = Severe I = Minimal O = Moderate F = Female . = No data
Dose Level: Group 1 – 0 mg/kg/day; Group 2 – 0.0025 mg/kg/day Ethinyl Estradiol; Group 3 – 0.005 mg/kg/day Ethinyl Estradiol
Group 4 – 12.5 mg/kg/day Methoxychlor; Group 5 – 25 mg/kg/day Methoxychlor; Group 6 – 50 mg/kg/day Methoxychlor
Group 7 – 25 mg/kg/day Phenobarbital; Group 8 – 50 mg/kg/day Phenobarbital; Group 9 – 100 mg/kg/day Phenobarbital

Appendix 7 (continued)
F_1 Clinical Observations and Mortality
Pubertal Toxicity Study of Vinclozolin, Flutamide and Phenobarbital in Male Sprague Dawley Rats and Methoxychlor, Ethinyl Estradiol and Phenobarbital in Female Sprague Dawley Rats When Administered in Corn Oil by Oral Gavage

Group Sex	Animal Number	Clinical Sign	Site	Postnatal Day 22	23	24	25	26	29	36	42	43
6F	9236	No Abnormalities Detected		X	.	.	.	.	X	X	X	.
		Terminal Kill		.	.	.	.	.	.	.	X	.
	9237	No Abnormalities Detected		X	.	.	.	.	X	X	X	.
		Terminal Kill		.	.	.	.	.	.	.	X	.
	9238	No Abnormalities Detected		X	.	.	.	.	X	X	X	.
		Terminal Kill		.	.	.	.	.	.	.	X	.
	9239	No Abnormalities Detected		X	.	.	.	.	X	X	X	.
		Terminal Kill		.	.	.	.	.	.	.	X	.
	9240	No Abnormalities Detected		X	.	.	.	.	X	X	X	.
		Terminal Kill		.	.	.	.	.	.	.	X	.
	9241	No Abnormalities Detected		X	.	.	.	.	X	X	X	.
		Terminal Kill		.	.	.	.	.	.	.	X	.
	9242	No Abnormalities Detected		X	.	.	.	.	X	X	X	.
		Terminal Kill		.	.	.	.	.	.	.	X	.
	9243	No Abnormalities Detected		X	.	.	.	.	X	X	X	.
		Terminal Kill		.	.	.	.	.	.	.	X	.
	9244	No Abnormalities Detected		X	.	.	.	.	X	X	.	X
		Terminal Kill		.	.	.	.	.	.	.	.	X
	9245	No Abnormalities Detected		X	.	.	.	.	X	X	.	X
		Terminal Kill		.	.	.	.	.	.	.	.	X
	9246	No Abnormalities Detected		X	.	.	.	.	X	X	.	X
		Terminal Kill		.	.	.	.	.	.	.	.	X
	9247	No Abnormalities Detected		X	.	.	.	.	X	X	.	X
		Terminal Kill		.	.	.	.	.	.	.	.	X

X = Present S = Severe I = Minimal O = Moderate F = Female . = No data
Dose Level: Group 1 – 0 mg/kg/day; Group 2 – 0.0025 mg/kg/day Ethinyl Estradiol; Group 3 – 0.005 mg/kg/day Ethinyl Estradiol
Group 4 – 12.5 mg/kg/day Methoxychlor; Group 5 – 25 mg/kg/day Methoxychlor; Group 6 – 50 mg/kg/day Methoxychlor
Group 7 – 25 mg/kg/day Phenobarbital; Group 8 – 50 mg/kg/day Phenobarbital; Group 9 – 100 mg/kg/day Phenobarbital

Appendix 7 (continued)
F_1 Clinical Observations and Mortality
Pubertal Toxicity Study of Vinclozolin, Flutamide and Phenobarbital in Male Sprague Dawley Rats and Methoxychlor, Ethinyl Estradiol and Phenobarbital in Female Sprague Dawley Rats When Administered in Corn Oil by Oral Gavage

				Postnatal Day								
Group Sex	Animal Number	Clinical Sign	Site	2 2	2 3	2 4	2 5	2 6	2 9	3 6	4 2	4 3
6F	9248	No Abnormalities Detected		X	.	.	.	.	X	X	.	X
		Terminal Kill		.	.	.	.	.	.	.	.	X
	9249	No Abnormalities Detected		X	.	.	.	.	X	X	.	X
		Terminal Kill		.	.	.	.	.	.	.	.	X
	9250	No Abnormalities Detected		X	.	.	.	.	X	X	.	X
		Terminal Kill		.	.	.	.	.	.	.	.	X

X = Present S = Severe I = Minimal O = Moderate F = Female . = No data
Dose Level: Group 1 – 0 mg/kg/day; Group 2 – 0.0025 mg/kg/day Ethinyl Estradiol; Group 3 – 0.005 mg/kg/day Ethinyl Estradiol
Group 4 – 12.5 mg/kg/day Methoxychlor; Group 5 – 25 mg/kg/day Methoxychlor; Group 6 – 50 mg/kg/day Methoxychlor
Group 7 – 25 mg/kg/day Phenobarbital; Group 8 – 50 mg/kg/day Phenobarbital; Group 9 – 100 mg/kg/day Phenobarbital

Appendix 7 (continued)
F_1 Clinical Observations and Mortality
Pubertal Toxicity Study of Vinclozolin, Flutamide and Phenobarbital in Male Sprague Dawley Rats and Methoxychlor, Ethinyl Estradiol and Phenobarbital in Female Sprague Dawley Rats When Administered in Corn Oil by Oral Gavage

Group Sex	Animal Number	Clinical Sign	Site	Postnatal Day 22	23	24	25	26	29	36	42	43
7F	9251	No Abnormalities Detected		X	.	.	.	.	X	X	X	.
		Terminal Kill		.	.	.	.	.	.	.	X	.
	9252	No Abnormalities Detected		X	.	.	.	.	X	X	X	.
		Terminal Kill		.	.	.	.	.	.	.	X	.
	9253	No Abnormalities Detected		X	.	.	.	.	X	.	.	.
		Missing anatomy	Tip of Tail	.	.	.	.	.	.	X	X	.
		Terminal Kill		.	.	.	.	.	.	.	X	.
	9254	No Abnormalities Detected		X	.	.	.	.	X	X	X	.
		Terminal Kill		.	.	.	.	.	.	.	X	.
	9255	No Abnormalities Detected		X	.	.	.	.	X	X	X	.
		Terminal Kill		.	.	.	.	.	.	.	X	.
	9256	No Abnormalities Detected		X	.	.	.	.	X	X	X	.
		Terminal Kill		.	.	.	.	.	.	.	X	.
	9257	No Abnormalities Detected		X	.	.	.	.	X	X	X	.
		Terminal Kill		.	.	.	.	.	.	.	X	.
	9258	No Abnormalities Detected		X	.	.	.	.	X	X	X	.
		Terminal Kill		.	.	.	.	.	.	.	X	.
	9259	No Abnormalities Detected		X	.	.	.	.	X	X	.	X
		Terminal Kill		.	.	.	.	.	.	.	.	X
	9260	No Abnormalities Detected		X	.	.	.	.	X	X	.	X
		Terminal Kill		.	.	.	.	.	.	.	.	X
	9261	No Abnormalities Detected		X	.	.	.	.	X	X	.	X
		Terminal Kill		.	.	.	.	.	.	.	.	X
	9262	No Abnormalities Detected		X	.	.	.	.	X	X	.	X
		Terminal Kill		.	.	.	.	.	.	.	.	X
	9263	No Abnormalities Detected		X	.	.	.	.	X	X	.	X
		Terminal Kill		.	.	.	.	.	.	.	.	X

X = Present S = Severe I = Minimal O = Moderate F = Female . = No data
Dose Level: Group 1 – 0 mg/kg/day; Group 2 – 0.0025 mg/kg/day Ethinyl Estradiol; Group 3 – 0.005 mg/kg/day Ethinyl Estradiol
Group 4 – 12.5 mg/kg/day Methoxychlor; Group 5 – 25 mg/kg/day Methoxychlor; Group 6 – 50 mg/kg/day Methoxychlor
Group 7 – 25 mg/kg/day Phenobarbital; Group 8 – 50 mg/kg/day Phenobarbital; Group 9 – 100 mg/kg/day Phenobarbital

Appendix 7 (continued)
F_1 Clinical Observations and Mortality
Pubertal Toxicity Study of Vinclozolin, Flutamide and Phenobarbital in Male Sprague Dawley Rats and Methoxychlor, Ethinyl Estradiol and Phenobarbital in Female Sprague Dawley Rats When Administered in Corn Oil by Oral Gavage

Group Sex	Animal Number	Clinical Sign	Site	Postnatal Day 2 2	2 3	2 4	2 5	2 6	2 9	3 6	4 2	4 3
7F	9264	No Abnormalities Detected		X	.	.	.	.	X	X	.	X
		Terminal Kill		.	.	.	.	.	.	.	.	X
	9265	No Abnormalities Detected		X	.	.	.	.	X	X	.	X
		Terminal Kill		.	.	.	.	.	.	.	.	X

X = Present S = Severe I = Minimal O = Moderate F = Female . = No data
Dose Level: Group 1 – 0 mg/kg/day; Group 2 – 0.0025 mg/kg/day Ethinyl Estradiol; Group 3 – 0.005 mg/kg/day Ethinyl Estradiol
Group 4 – 12.5 mg/kg/day Methoxychlor; Group 5 – 25 mg/kg/day Methoxychlor; Group 6 – 50 mg/kg/day Methoxychlor
Group 7 – 25 mg/kg/day Phenobarbital; Group 8 – 50 mg/kg/day Phenobarbital; Group 9 – 100 mg/kg/day Phenobarbital

Appendix 7 (continued)
F_1 Clinical Observations and Mortality
Pubertal Toxicity Study of Vinclozolin, Flutamide and Phenobarbital in Male Sprague Dawley Rats and Methoxychlor, Ethinyl Estradiol and Phenobarbital in Female Sprague Dawley Rats When Administered in Corn Oil by Oral Gavage

Group Sex	Animal Number	Clinical Sign	Site	Postnatal Day 22	23	24	25	26	29	36	42	43
8F	9266	No Abnormalities Detected		X	.	.	.	.	X	X	X	.
		Terminal Kill		.	.	.	.	.	.	.	X	.
	9267	No Abnormalities Detected		X	.	.	.	.	X	X	X	.
		Terminal Kill		.	.	.	.	.	.	.	X	.
	9268	No Abnormalities Detected		X	.	.	.	.	X	X	X	.
		Terminal Kill		.	.	.	.	.	.	.	X	.
	9269	No Abnormalities Detected		X	.	.	.	.	X	X	X	.
		Terminal Kill		.	.	.	.	.	.	.	X	.
	9270	No Abnormalities Detected		X	.	.	.	.	X	X	X	.
		Terminal Kill		.	.	.	.	.	.	.	X	.
	9271	No Abnormalities Detected		X	.	.	.	.	X	X	X	.
		Terminal Kill		.	.	.	.	.	.	.	X	.
	9272	No Abnormalities Detected		X	.	.	.	.	X	X	X	.
		Terminal Kill		.	.	.	.	.	.	.	X	.
	9273	No Abnormalities Detected		X	.	.	.	.	X	X	X	.
		Terminal Kill		.	.	.	.	.	.	.	X	.
	9274	No Abnormalities Detected		X	.	.	.	.	X	X	.	X
		Terminal Kill		.	.	.	.	.	.	.	.	X
	9275	No Abnormalities Detected		X	.	.	.	.	X	X	.	X
		Terminal Kill		.	.	.	.	.	.	.	.	X
	9276	No Abnormalities Detected		X	.	.	.	.	X	X	.	X
		Terminal Kill		.	.	.	.	.	.	.	.	X
	9277	No Abnormalities Detected		X	.	.	.	.	X	X	.	X
		Terminal Kill		.	.	.	.	.	.	.	.	X

X = Present S = Severe I = Minimal O = Moderate F = Female . = No data
Dose Level: Group 1 – 0 mg/kg/day; Group 2 – 0.0025 mg/kg/day Ethinyl Estradiol; Group 3 – 0.005 mg/kg/day Ethinyl Estradiol
Group 4 – 12.5 mg/kg/day Methoxychlor; Group 5 – 25 mg/kg/day Methoxychlor; Group 6 – 50 mg/kg/day Methoxychlor
Group 7 – 25 mg/kg/day Phenobarbital; Group 8 – 50 mg/kg/day Phenobarbital; Group 9 – 100 mg/kg/day Phenobarbital

Appendix 7 (continued)
F_1 Clinical Observations and Mortality
Pubertal Toxicity Study of Vinclozolin, Flutamide and Phenobarbital in Male Sprague Dawley Rats and Methoxychlor, Ethinyl Estradiol and Phenobarbital in Female Sprague Dawley Rats When Administered in Corn Oil by Oral Gavage

				Postnatal Day								
Group Sex	Animal Number	Clinical Sign	Site	2 2	2 3	2 4	2 5	2 6	2 9	3 6	4 2	4 3
8F	9278	No Abnormalities Detected		X	.	.	.	.	X	X	.	X
		Terminal Kill		.	.	.	.	.	.	.	.	X
	9279	No Abnormalities Detected		X	.	.	.	.	X	X	.	X
		Terminal Kill		.	.	.	.	.	.	.	.	X
	9280	No Abnormalities Detected		X	.	.	.	.	X	X	.	X
		Terminal Kill		.	.	.	.	.	.	.	.	X

X = Present S = Severe I = Minimal O = Moderate F = Female . = No data
Dose Level: Group 1 – 0 mg/kg/day; Group 2 – 0.0025 mg/kg/day Ethinyl Estradiol; Group 3 – 0.005 mg/kg/day Ethinyl Estradiol
Group 4 – 12.5 mg/kg/day Methoxychlor; Group 5 – 25 mg/kg/day Methoxychlor; Group 6 – 50 mg/kg/day Methoxychlor
Group 7 – 25 mg/kg/day Phenobarbital; Group 8 – 50 mg/kg/day Phenobarbital; Group 9 – 100 mg/kg/day Phenobarbital

Appendix 7 (continued)
F_1 Clinical Observations and Mortality
Pubertal Toxicity Study of Vinclozolin, Flutamide and Phenobarbital in Male Sprague Dawley Rats and Methoxychlor, Ethinyl Estradiol and Phenobarbital in Female Sprague Dawley Rats When Administered in Corn Oil by Oral Gavage

Group Sex	Animal Number	Clinical Sign	Site	Postnatal Day 22	23	24	25	26	29	36	42	43
9F	9281	No Abnormalities Detected		X	.	.	.	.	X	X	X	.
		Ataxia		I	O	O	I	.	.	.	.	.
		Terminal Kill		.	.	.	.	.	.	.	X	.
	9282	No Abnormalities Detected		X	.	.	.	.	X	X	X	.
		Ataxia		I	.	.	I	.	.	.	.	.
		Prostration		.	X	.	.	.	.	.	.	.
		Terminal Kill		.	.	.	.	.	.	.	X	.
	9283	No Abnormalities Detected		X	.	.	.	.	X	X	X	.
		Ataxia		O	O	.	I	.	.	.	.	.
		Terminal Kill		.	.	.	.	.	.	.	X	.
	9284	No Abnormalities Detected		X	.	.	.	.	X	X	X	.
		Ataxia		I	O	I	I	.	.	.	.	.
		Terminal Kill		.	.	.	.	.	.	.	X	.
	9285	No Abnormalities Detected		X	.	.	.	.	X	X	X	.
		Ataxia		I	O	.	O	.	.	.	.	.
		Prostration		.	.	X	.	.	.	.	.	.
		Terminal Kill		.	.	.	.	.	.	.	X	.
	9286	No Abnormalities Detected		X	.	.	.	.	X	X	X	.
		Ataxia		I	.	O	I	.	.	.	.	.
		Prostration		.	X	.	.	.	.	.	.	.
		Terminal Kill		.	.	.	.	.	.	.	X	.
	9287	No Abnormalities Detected		X	.	.	.	.	X	X	X	.
		Ataxia		I	S	O	I	I	.	.	.	.
		Terminal Kill		.	.	.	.	.	.	.	X	.

X = Present S = Severe I = Minimal O = Moderate F = Female . = No data
Dose Level: Group 1 – 0 mg/kg/day; Group 2 – 0.0025 mg/kg/day Ethinyl Estradiol; Group 3 – 0.005 mg/kg/day Ethinyl Estradiol
Group 4 – 12.5 mg/kg/day Methoxychlor; Group 5 – 25 mg/kg/day Methoxychlor; Group 6 – 50 mg/kg/day Methoxychlor
Group 7 – 25 mg/kg/day Phenobarbital; Group 8 – 50 mg/kg/day Phenobarbital; Group 9 – 100 mg/kg/day Phenobarbital

Appendix 7 (continued)
F_1 Clinical Observations and Mortality
Pubertal Toxicity Study of Vinclozolin, Flutamide and Phenobarbital in Male Sprague Dawley Rats and Methoxychlor, Ethinyl Estradiol and Phenobarbital in Female Sprague Dawley Rats When Administered in Corn Oil by Oral Gavage

Group Sex	Animal Number	Clinical Sign	Site	Postnatal Day 22	23	24	25	26	29	36	42	43
9F	9288	No Abnormalities Detected		X	.	.	.	.	X	X	X	.
		Ataxia		O	I	O	I	I	.	.	.	.
		Prostration		.	X	.	.	.	.	.	.	.
		Terminal Kill		.	.	.	.	.	.	.	X	.
	9289	No Abnormalities Detected		X	.	.	.	.	X	X	.	X
		Ataxia		I	S	O	I	.	.	.	.	.
		Terminal Kill		.	.	.	.	.	.	.	.	X
	9290	No Abnormalities Detected		X	.	.	.	.	X	X	.	X
		Ataxia		O	O	S	I	.	.	.	.	.
		Terminal Kill		.	.	.	.	.	.	.	.	X
	9291	No Abnormalities Detected		X	.	.	.	.	X	X	.	X
		Ataxia		O	.	O	I	.	.	.	.	.
		Prostration		.	X	.	.	.	.	.	.	.
		Terminal Kill		.	.	.	.	.	.	.	.	X
	9292	No Abnormalities Detected		X	.	.	.	.	X	X	.	X
		Ataxia		I	.	I	I	.	.	.	.	.
		Prostration		.	X	.	.	.	.	.	.	.
		Terminal Kill		.	.	.	.	.	.	.	.	X
	9293	No Abnormalities Detected		X	.	.	.	.	X	X	.	X
		Ataxia		I	O	S	I	.	.	.	.	.
		Terminal Kill		.	.	.	.	.	.	.	.	X
	9294	No Abnormalities Detected		X	.	.	.	.	X	X	.	X
		Ataxia		O	O	.	I	.	.	.	.	.
		Prostration		.	.	X	.	.	.	.	.	.
		Terminal Kill		.	.	.	.	.	.	.	.	X

X = Present S = Severe I = Minimal O = Moderate F = Female . = No data
Dose Level: Group 1 – 0 mg/kg/day; Group 2 – 0.0025 mg/kg/day Ethinyl Estradiol; Group 3 – 0.005 mg/kg/day Ethinyl Estradiol
Group 4 – 12.5 mg/kg/day Methoxychlor; Group 5 – 25 mg/kg/day Methoxychlor; Group 6 – 50 mg/kg/day Methoxychlor
Group 7 – 25 mg/kg/day Phenobarbital; Group 8 – 50 mg/kg/day Phenobarbital; Group 9 – 100 mg/kg/day Phenobarbital

Appendix 7 (continued)
F_1 Clinical Observations and Mortality
Pubertal Toxicity Study of Vinclozolin, Flutamide and Phenobarbital in Male Sprague Dawley Rats and Methoxychlor, Ethinyl Estradiol and Phenobarbital in Female Sprague Dawley Rats When Administered in Corn Oil by Oral Gavage

Group Sex	Animal Number	Clinical Sign	Site	Postnatal Day 2 2	2 3	2 4	2 5	2 6	2 9	3 6	4 2	4 3
9F	9295	No Abnormalities Detected		X	.	.	.	.	X	X	.	X
		Ataxia		O	I	O	O	I	.	.	.	.
		Prostration		.	X	.	.	.	.	.	.	.
		Terminal Kill		.	.	.	.	.	.	.	.	X

X = Present S = Severe I = Minimal O = Moderate F = Female . = No data
Dose Level: Group 1 – 0 mg/kg/day; Group 2 – 0.0025 mg/kg/day Ethinyl Estradiol; Group 3 – 0.005 mg/kg/day Ethinyl Estradiol
Group 4 – 12.5 mg/kg/day Methoxychlor; Group 5 – 25 mg/kg/day Methoxychlor; Group 6 – 50 mg/kg/day Methoxychlor
Group 7 – 25 mg/kg/day Phenobarbital; Group 8 – 50 mg/kg/day Phenobarbital; Group 9 – 100 mg/kg/day Phenobarbital

Appendix 8
F_1 Individual Body Weights
Pubertal Toxicity Study of Vinclozolin, Flutamide and Phenobarbital in Male Sprague Dawley Rats and Methoxychlor, Ethinyl Estradiol and Phenobarbital in Female Sprague Dawley Rats when Administered in Corn Oil by Oral Gavage

Appendix 8
F_1 Individual Body Weights (Grams)
Pubertal Toxicity Study of Vinclozolin, Flutamide and Phenobarbital in Male Sprague Dawley Rats and Methoxychlor, Ethinyl Estradiol and Phenobarbital in Female Sprague Dawley Rats When Administered in Corn Oil by Oral Gavage

		Postnatal Day															
Group Sex	Animal Number	23	24	25	26	27	28	29	30	31	32	33	34	35	36	37	38
1M	9026	75.4	83.7	92.4	98.2	104.5	114.4	121.5	127.8	137.7	148.5	155.6	165.2	174.7	185.4	194.0	201.9
	9027	72.6	80.1	87.8	95.2	100.1	108.5	115.7	121.9	132.1	137.8	145.0	154.6	166.5	170.5	179.9	188.5
	9028	72.5	81.0	89.4	96.4	101.7	111.7	121.3	128.0	139.5	148.6	157.1	167.2	176.6	186.0	198.0	207.4
	9029	68.1	75.7	81.6	88.2	93.1	99.2	107.6	113.6	121.0	129.1	138.5	147.1	153.6	158.4	170.0	177.9
	9030	66.7	73.8	79.8	85.3	89.4	97.2	103.6	109.6	117.0	125.0	133.1	141.9	149.9	158.7	166.5	178.4
	9031	70.1	77.7	84.4	91.0	96.5	104.2	109.9	119.2	126.7	135.6	145.9	153.1	162.1	172.7	178.9	191.5
	9032	67.9	75.9	83.5	90.4	97.0	103.8	110.6	119.0	126.7	135.0	142.7	153.8	160.7	170.4	178.7	188.3
	9033	68.4	73.9	80.4	86.7	91.4	99.1	106.1	111.3	122.4	131.4	139.3	146.6	155.5	161.9	170.4	181.5
	9034	66.4	72.1	77.8	84.2	89.7	94.7	105.2	111.7	119.3	128.3	137.2	146.0	155.0	163.9	174.1	186.4
	9035	64.1	69.7	75.8	83.4	88.3	95.2	102.3	109.7	118.0	126.1	135.1	143.6	152.2	161.0	169.3	182.2
	9036	63.6	71.4	77.4	84.6	90.3	98.4	105.9	113.6	121.8	130.3	138.3	147.7	155.6	163.9	172.3	180.3
	9037	65.4	72.1	78.0	83.9	90.1	98.0	105.1	111.4	119.0	128.0	133.2	143.3	150.1	154.6	165.6	174.9
	9038	63.6	70.2	76.0	82.7	90.0	96.0	105.4	111.0	122.7	130.4	138.4	146.0	158.0	166.9	175.6	185.6
	9039	62.8	69.3	77.2	83.4	89.6	99.2	105.5	111.0	122.1	130.0	141.8	151.7	158.3	170.4	183.2	190.6
	9040	63.5	70.9	79.6	86.8	92.5	100.9	109.5	115.1	123.8	131.0	144.5	149.8	158.0	168.9	177.2	188.2

Dose Level: Group 1 - 0 mg/kg/day; Group 2 - 25 mg/kg/day Phenobarbital; Group 3 - 50 mg/kg/day Phenobarbital
Group 4 - 100 mg/kg/day Phenobarbital; Group 5 - 10 mg/kg/day Vinclozolin; Group 6 - 30 mg/kg/day Vinclozolin
Group 7 - 100 mg/kg/day Vinclozolin; Group 8 - 25 mg/kg/day Flutamide; Group 9 - 50 mg/kg/day Flutamide

M = Males
. = No Data

Appendix 8 (continued)
F_1 Individual Body Weightss (Grams)
Pubertal Toxicity Study of Vinclozolin, Flutamide and Phenobarbital in Male Sprague Dawley Rats and Methoxychlor, Ethinyl Estradiol and Phenobarbital in Female Sprague Dawley Rats When Administered in Corn Oil by Oral Gavage

		Postnatal Day															
Group Sex	Animal Number	39	40	41	42	43	44	45	46	47	48	49	50	51	52	53	54
1M	9026	214.1	225.0	237.7	244.9	251.5	257.0	268.6	279.2	292.0	299.1	313.0	324.5	332.7	343.7	357.9	.
	9027	196.5	205.9	211.8	222.6	230.7	235.2	245.0	252.8	264.0	266.3	274.2	280.8	292.6	298.1	305.5	.
	9028	216.1	225.0	239.6	245.7	254.1	261.0	273.5	285.0	290.7	302.1	308.9	325.7	329.6	339.9	351.7	.
	9029	185.2	197.1	204.4	208.2	218.8	222.4	229.9	236.5	247.3	254.2	261.3	269.7	276.7	287.3	294.1	.
	9030	184.3	194.2	204.2	211.1	221.1	227.4	239.6	245.8	254.7	263.4	274.2	282.7	289.5	297.0	308.4	.
	9031	197.2	208.4	219.5	226.6	233.9	243.9	253.3	263.6	270.9	283.9	292.0	302.8	308.6	319.0	331.9	.
	9032	195.3	206.0	216.6	221.1	229.0	242.0	250.0	262.8	267.4	274.6	287.4	295.8	299.2	313.6	324.4	.
	9033	188.1	196.6	206.2	211.2	219.7	226.7	237.0	242.0	251.4	258.6	266.0	273.9	277.7	286.1	293.6	.
	9034	193.1	203.6	212.3	217.6	227.7	236.1	245.7	254.3	263.9	272.1	282.7	290.0	300.4	311.7	324.4	325.0
	9035	188.9	196.8	206.3	211.9	221.5	230.1	241.1	253.4	264.0	268.0	280.1	290.7	297.6	308.1	322.2	328.2
	9036	190.5	201.1	212.4	218.8	227.2	238.1	246.6	255.6	264.6	273.8	287.0	295.4	299.2	311.5	320.6	330.3
	9037	179.8	191.1	196.4	204.7	213.7	220.6	229.2	234.2	242.8	253.1	262.7	268.9	278.6	287.5	297.2	305.9
	9038	194.0	203.5	213.9	219.6	231.5	240.2	251.7	257.1	267.8	277.1	284.8	282.9	297.0	307.0	315.5	325.0
	9039	199.1	210.6	221.2	227.5	237.7	247.2	255.9	263.3	271.9	287.6	297.4	305.7	316.4	323.7	337.0	340.8
	9040	196.2	209.7	217.7	224.1	234.0	242.7	253.0	262.9	270.2	286.1	292.7	306.0	310.1	320.2	330.4	340.0

Dose Level: Group 1 – 0 mg/kg/day; Group 2 – 25 mg/kg/day Phenobarbital; Group 3 – 50 mg/kg/day Phenobarbital
Group 4 – 100 mg/kg/day Phenobarbital; Group 5 – 10 mg/kg/day Vinclozolin; Group 6 – 30 mg/kg/day Vinclozolin
Group 7 – 100 mg/kg/day Vinclozolin; Group 8 – 25 mg/kg/day Flutamide; Group 9 – 50 mg/kg/day Flutamide

M = Males
. = No Data

Appendix 8 (continued)
F_1 Individual Body Weights (Grams)
Pubertal Toxicity Study of Vinclozolin, Flutamide and Phenobarbital in Male Sprague Dawley Rats and Methoxychlor, Ethinyl Estradiol and Phenobarbital in Female Sprague Dawley Rats When Administered in Corn Oil by Oral Gavage

		Postnatal Day															
Group Sex	Animal Number	23	24	25	26	27	28	29	30	31	32	33	34	35	36	37	38
2M	9041	75.5	85.2	91.9	98.4	102.2	110.5	117.4	124.8	132.9	144.0	152.4	163.0	171.0	181.3	194.3	199.6
	9042	76.7	84.5	92.5	96.4	103.0	111.9	118.5	126.7	134.9	141.7	151.6	161.5	169.6	179.5	188.5	198.8
	9043	69.7	76.5	82.9	86.0	91.8	99.9	106.9	112.6	122.1	129.6	137.4	146.5	152.7	159.8	168.2	175.5
	9044	69.9	78.8	85.5	91.5	95.0	103.5	110.9	119.7	126.5	137.0	145.7	156.5	164.7	176.3	182.8	190.4
	9045	70.4	79.9	86.0	91.3	95.9	101.6	108.8	115.0	124.0	131.5	137.8	148.3	158.1	169.2	176.7	184.6
	9046	66.6	74.4	80.6	87.2	89.8	98.4	101.9	111.3	116.4	127.0	134.8	144.7	153.2	164.8	171.2	178.7
	9047	66.2	74.9	80.7	87.4	92.6	100.4	108.3	115.6	123.9	132.3	140.5	150.9	157.5	165.8	174.2	184.3
	9048	64.2	71.7	77.6	84.7	89.3	96.6	106.0	112.4	119.1	128.6	136.9	144.5	151.7	160.7	169.6	177.1
	9049	68.8	74.8	80.6	85.1	90.8	95.9	105.6	110.8	118.9	125.9	135.1	141.3	148.3	159.2	166.0	173.4
	9050	65.3	73.6	78.9	83.7	89.1	95.8	103.1	108.0	117.7	124.5	133.6	141.0	149.7	158.0	174.2	175.2
	9051	64.4	71.7	78.5	84.8	88.8	96.7	104.4	111.7	120.3	129.3	138.9	147.9	156.0	166.2	166.1	184.1
	9052	63.5	71.4	76.5	82.2	86.2	94.1	101.5	107.3	115.3	124.5	131.9	141.2	150.7	159.2	167.6	176.9
	9053	63.5	72.8	79.7	84.6	91.2	98.8	108.1	116.9	125.9	136.8	145.1	152.9	162.9	174.2	184.6	192.5
	9054	63.0	72.7	79.2	84.1	91.2	98.5	105.1	113.4	120.8	129.4	138.1	146.7	157.7	167.6	178.2	180.1
	9055	62.1	71.3	77.4	82.1	86.6	93.9	102.3	108.7	115.8	124.7	132.6	141.1	150.3	159.8	167.7	184.5

Dose Level: Group 1 – 0 mg/kg/day; Group 2 – 25 mg/kg/day Phenobarbital; Group 3 – 50 mg/kg/day Phenobarbital
Group 4 – 100 mg/kg/day Phenobarbital; Group 5 – 10 mg/kg/day Vinclozolin; Group 6 – 30 mg/kg/day Vinclozolin
Group 7 – 100 mg/kg/day Vinclozolin; Group 8 – 25 mg/kg/day Flutamide; Group 9 – 50 mg/kg/day Flutamide

M = Males
. = No Data

Appendix 8 (continued)
F_1 Individual Body Weights (Grams)
Pubertal Toxicity Study of Vinclozolin, Flutamide and Phenobarbital in Male Sprague Dawley Rats and Methoxychlor, Ethinyl Estradiol and Phenobarbital in Female Sprague Dawley Rats When Administered in Corn Oil by Oral Gavage

		Postnatal Day															
Group Sex	Animal Number	39	40	41	42	43	44	45	46	47	48	49	50	51	52	53	54
2M	9041	210.8	221.1	229.4	236.8	246.6	250.9	263.1	272.4	282.2	293.0	301.9	308.9	319.2	330.6	340.2	.
	9042	205.9	220.4	227.1	235.8	245.1	255.8	265.1	273.4	284.9	293.7	304.7	314.9	324.3	333.2	344.4	.
	9043	184.2	193.0	200.4	206.1	212.2	216.7	226.5	232.3	239.9	245.3	256.6	264.0	268.8	279.5	287.7	.
	9044	198.3	207.5	214.2	223.8	228.9	237.0	247.5	254.5	262.5	270.3	282.6	288.1	289.5	300.6	309.3	.
	9045	195.9	204.8	212.1	218.8	227.7	236.8	247.0	257.2	265.7	272.9	284.8	293.8	303.6	312.1	320.8	.
	9046	187.9	196.6	205.3	213.0	220.8	227.9	242.4	249.2	257.0	267.6	277.4	286.6	293.4	305.4	309.3	.
	9047	190.6	202.4	205.6	214.3	224.6	229.7	242.3	250.8	257.9	265.9	274.2	285.9	290.8	303.8	314.8	.
	9048	185.0	193.3	199.0	206.9	214.3	220.0	228.5	236.1	243.7	251.1	259.3	270.5	273.0	282.9	292.3	.
	9049	180.2	190.7	193.4	201.4	207.8	216.1	223.2	232.7	240.3	247.6	255.5	263.4	270.7	280.8	290.1	299.0
	9050	179.7	189.6	201.9	206.4	217.5	224.6	230.9	238.8	247.2	256.2	265.5	268.8	278.6	286.6	296.1	304.5
	9051	193.8	201.3	213.1	220.9	228.4	234.0	243.6	253.3	258.7	269.1	276.8	284.6	294.1	300.0	312.9	317.4
	9052	182.9	192.8	204.2	207.8	219.1	225.0	233.9	238.5	248.6	257.3	268.0	274.3	276.8	288.2	298.5	305.8
	9053	203.4	212.9	222.8	229.5	240.1	248.0	261.1	270.7	279.3	292.0	300.5	309.4	318.2	330.0	340.8	351.4
	9054	194.1	202.2	210.3	220.1	228.9	235.1	246.4	253.7	263.9	267.9	277.9	286.5	290.7	300.1	308.5	312.6
	9055	187.1	197.3	205.2	212.6	223.6	230.2	242.1	249.8	260.2	270.0	285.3	288.1	300.5	308.6	317.1	327.7

Dose Level: Group 1 – 0 mg/kg/day; Group 2 – 25 mg/kg/day Phenobarbital; Group 3 – 50 mg/kg/day Phenobarbital
Group 4 – 100 mg/kg/day Phenobarbital; Group 5 – 10 mg/kg/day Vinclozolin; Group 6 – 30 mg/kg/day Vinclozolin
Group 7 – 100 mg/kg/day Vinclozolin; Group 8 – 25 mg/kg/day Flutamide; Group 9 – 50 mg/kg/day Flutamide

M = Males
. = No Data

Appendix 8 (continued)
F_1 Individual Body Weights (Grams)
Pubertal Toxicity Study of Vinclozolin, Flutamide and Phenobarbital in Male Sprague Dawley Rats and Methoxychlor, Ethinyl Estradiol and Phenobarbital in Female Sprague Dawley Rats When Administered in Corn Oil by Oral Gavage

		Postnatal Day															
Group Sex	Animal Number	23	24	25	26	27	28	29	30	31	32	33	34	35	36	37	38
3M	9056	75.2	83.1	91.2	98.2	102.7	111.4	119.6	128.3	138.6	146.1	156.4	167.3	179.0	188.7	199.0	209.3
	9057	73.6	83.8	90.4	99.8	106.2	115.5	124.3	133.4	144.3	155.6	165.7	177.6	191.6	198.9	212.3	225.5
	9058	70.8	80.2	86.6	91.2	96.5	103.6	111.4	118.6	126.6	135.7	146.6	154.5	161.8	174.4	182.6	189.0
	9059	70.1	76.7	84.2	91.1	102.6	105.3	110.5	117.4	123.2	133.4	141.0	149.6	157.2	165.3	173.8	178.7
	9060	69.5	79.6	87.6	93.5	99.1	107.7	115.1	123.3	131.3	143.0	148.7	159.8	168.8	177.5	187.3	195.6
	9061	69.4	77.9	85.3	91.2	95.9	105.2	112.1	119.1	129.0	136.9	148.3	158.7	167.4	176.7	183.1	193.1
	9062	67.1	75.6	81.4	86.3	77.6	94.5	102.1	107.0	115.1	123.6	130.8	138.6	144.8	153.6	161.8	169.1
	9063	69.5	77.9	84.0	89.9	79.7	96.5	107.5	112.9	120.6	130.1	136.8	146.6	159.1	166.1	174.5	183.8
	9064	68.0	75.7	80.3	85.9	79.6	97.1	106.3	112.4	120.6	129.5	134.1	148.1	155.4	164.5	172.4	181.2
	9065	66.6	76.5	87.6	90.2	98.0	105.9	112.0	120.2	127.1	137.7	146.8	156.3	166.0	175.3	188.2	197.9
	9066	65.2	75.0	80.4	84.9	89.3	95.7	103.8	112.5	120.5	129.6	140.1	146.7	156.1	166.1	175.3	185.0
	9067	64.0	73.7	81.3	87.3	92.6	99.1	107.1	112.4	120.5	129.7	138.7	147.0	158.0	170.5	180.6	187.0
	9068	63.6	70.8	77.6	82.8	87.6	93.2	99.9	108.1	115.1	123.3	127.9	138.8	147.9	155.1	163.0	171.5
	9069	63.2	71.1	80.0	82.9	89.8	96.8	104.8	111.6	120.6	127.6	136.2	145.2	154.1	166.0	172.3	181.1
	9070	61.8	69.3	77.1	81.6	86.0	90.9	97.0	105.7	113.1	122.3	127.7	135.9	145.6	154.6	162.0	167.6

Dose Level: Group 1 – 0 mg/kg/day; Group 2 – 25 mg/kg/day Phenobarbital; Group 3 – 50 mg/kg/day Phenobarbital
Group 4 – 100 mg/kg/day Phenobarbital; Group 5 – 10 mg/kg/day Vinclozolin; Group 6 – 30 mg/kg/day Vinclozolin
Group 7 – 100 mg/kg/day Vinclozolin; Group 8 – 25 mg/kg/day Flutamide; Group 9 – 50 mg/kg/day Flutamide

M = Males
. = No Data

Appendix 8 (continued)
F_1 Individual Body Weights (Grams)
Pubertal Toxicity Study of Vinclozolin, Flutamide and Phenobarbital in Male Sprague Dawley Rats and Methoxychlor, Ethinyl Estradiol and Phenobarbital in Female Sprague Dawley Rats When Administered in Corn Oil by Oral Gavage

		Postnatal Day															
Group Sex	Animal Number	39	40	41	42	43	44	45	46	47	48	49	50	51	52	53	54
3M	9056	218.0	227.0	235.7	243.1	255.5	263.0	270.3	281.0	289.9	299.9	302.9	313.4	320.8	325.6	337.0	.
	9057	237.0	245.1	257.9	267.5	277.2	283.3	295.4	306.0	314.9	325.0	331.7	338.4	341.3	351.5	363.6	.
	9058	200.3	206.3	214.7	222.9	234.7	241.2	252.6	256.0	267.1	276.2	287.2	294.2	302.4	311.6	315.8	.
	9059	188.3	197.9	201.8	211.8	218.0	226.3	240.1	246.0	255.2	263.7	275.4	287.0	287.5	304.8	309.5	.
	9060	204.5	211.7	218.9	229.0	237.9	246.2	259.9	267.1	276.4	285.1	296.1	308.4	313.2	324.1	334.2	.
	9061	203.6	212.3	221.4	230.1	241.0	249.1	258.4	268.0	275.7	285.1	291.7	305.4	303.1	318.0	326.5	.
	9062	176.3	187.7	194.1	199.2	208.9	214.8	225.0	233.1	241.0	250.0	259.1	263.8	265.1	275.6	283.5	.
	9063	192.7	201.7	212.2	217.2	228.5	235.8	244.3	253.4	260.4	270.9	281.5	287.3	292.3	301.2	310.8	.
	9064	190.6	198.1	205.1	215.0	219.9	229.5	236.2	245.5	254.4	260.2	270.6	277.7	286.2	296.2	302.7	310.8
	9065	205.6	217.0	223.7	234.5	244.0	248.8	260.4	271.4	283.5	289.9	299.1	308.3	313.7	325.7	335.8	349.4
	9066	196.2	206.7	213.7	224.3	228.8	238.8	245.7	254.7	263.1	270.5	278.7	288.4	290.2	302.4	309.5	322.2
	9067	199.2	210.1	222.0	233.4	239.8	247.2	259.8	268.6	281.4	289.4	303.9	317.1	321.0	335.6	342.9	359.9
	9068	178.6	189.0	195.2	200.4	210.8	218.4	228.2	233.3	241.0	246.3	260.0	266.9	270.3	281.9	289.0	303.5
	9069	190.6	201.9	204.1	216.1	221.4	226.0	237.5	246.3	251.8	263.1	270.9	276.5	279.5	292.5	311.4	309.5
	9070	178.4	187.7	193.6	202.7	209.0	216.3	226.0	233.8	243.5	250.0	260.1	267.5	273.6	283.5	295.7	304.5

Dose Level: Group 1 – 0 mg/kg/day; Group 2 – 25 mg/kg/day Phenobarbital; Group 3 – 50 mg/kg/day Phenobarbital
Group 4 – 100 mg/kg/day Phenobarbital; Group 5 – 10 mg/kg/day Vinclozolin; Group 6 – 30 mg/kg/day Vinclozolin
Group 7 – 100 mg/kg/day Vinclozolin; Group 8 – 25 mg/kg/day Flutamide; Group 9 – 50 mg/kg/day Flutamide

M = Males
. = No Data

Appendix 8 (continued)
F_1 Individual Body Weights (Grams)
Pubertal Toxicity Study of Vinclozolin, Flutamide and Phenobarbital in Male Sprague Dawley Rats and Methoxychlor, Ethinyl Estradiol and Phenobarbital in Female Sprague Dawley Rats When Administered in Corn Oil by Oral Gavage

		Postnatal Day															
Group Sex	Animal Number	23	24	25	26	27	28	29	30	31	32	33	34	35	36	37	38
4M	9071	72.8	82.6	89.9	94.1	98.3	104.8	113.9	117.3	127.0	136.5	144.5	152.2	160.7	167.5	176.9	185.0
	9072	74.8	85.0	94.8	100.9	108.2	117.2	126.2	133.4	143.9	150.9	164.7	170.9	179.5	189.2	200.0	212.1
	9073	72.3	79.3	84.9	90.4	95.4	101.5	105.7	114.5	122.8	129.0	136.0	143.4	151.9	160.0	165.3	171.7
	9074	72.1	79.1	85.7	91.9	97.5	104.5	111.9	115.5	123.8	132.3	142.9	151.0	158.7	168.5	180.0	188.8
	9075	69.7	79.4	86.0	92.1	97.9	105.7	110.5	118.9	126.1	134.1	140.9	149.8	159.7	167.1	175.2	186.0
	9076	68.5	73.3	78.5	84.4	89.1	95.3	104.1	107.9	115.0	124.4	131.2	138.6	148.8	157.3	163.5	173.9
	9077	69.7	68.4*	.	.	.	.	.	.	.	.	.	.	.	.	.	.
	9078	67.8	65.1	75.9	82.0	87.2	95.8	102.5	106.6	116.0	120.8	128.6	136.5	147.1	156.5	166.0	174.2
	9079	65.9	73.4	79.6	83.8	88.2	94.6	101.2	107.9	115.7	123.3	131.2	139.8	147.0	157.8	162.4	171.2
	9080	64.5	59.2	70.2	77.9	83.5	90.2	97.8	101.2	106.9	117.4	123.8	128.4	140.3	149.1	157.6	165.9
	9081	65.4	69.2	78.6	83.4	87.5	94.0	99.9	106.2	110.4	121.8	128.5	132.0	143.5	152.7	158.6	166.9
	9082	63.3	63.2	71.0	78.6	83.5	90.9	95.6	101.4	108.0	117.2	125.1	129.5	140.2	148.6	155.8	166.3
	9083	60.7	68.0	72.1	77.3	81.3	87.8	93.4	101.3	106.8	113.0	122.2	127.0	140.0	146.7	153.0	162.1
	9084	63.2	68.1	74.5	79.5	84.4	90.3	96.9	105.3	111.5	118.6	127.0	133.3	145.8	149.9	158.4	167.8
	9085	62.3	71.8	76.6	81.3	85.3	91.6	98.2	104.7	111.5	118.1	126.0	131.9	140.1	147.8	155.0	164.2

Dose Level: Group 1 – 0 mg/kg/day; Group 2 – 25 mg/kg/day Phenobarbital; Group 3 – 50 mg/kg/day Phenobarbital
Group 4 – 100 mg/kg/day Phenobarbital; Group 5 – 10 mg/kg/day Vinclozolin; Group 6 – 30 mg/kg/day Vinclozolin
Group 7 – 100 mg/kg/day Vinclozolin; Group 8 – 25 mg/kg/day Flutamide; Group 9 – 50 mg/kg/day Flutamide

M = Males
. = No Data
* = Animal found dead, value excluded from calculations.

Appendix 8 (continued)
F_1 Individual Body Weights (Grams)
Pubertal Toxicity Study of Vinclozolin, Flutamide and Phenobarbital in Male Sprague Dawley Rats and Methoxychlor, Ethinyl Estradiol and Phenobarbital in Female Sprague Dawley Rats When Administered in Corn Oil by Oral Gavage

		Postnatal Day															
Group Sex	Animal Number	39	40	41	42	43	44	45	46	47	48	49	50	51	52	53	54
4M	9071	195.5	203.2	208.4	217.1	227.7	233.4	247.0	252.0	263.4	267.8	277.1	285.3	294.1	298.9	309.3	.
	9072	222.6	228.7	238.7	244.1	257.4	264.0	273.4	281.3	288.6	302.1	316.3	333.3	333.4	339.5	338.2	.
	9073	180.0	185.2	193.1	198.2	205.7	212.4	219.4	226.8	232.2	240.4	247.0	252.7	258.1	263.1	269.4	.
	9074	197.1	209.2	216.2	225.4	233.6	242.6	253.0	259.5	268.5	278.1	290.4	297.1	305.9	312.7	321.5	.
	9075	195.3	203.2	212.3	222.0	231.5	238.3	246.4	252.9	265.2	277.7	287.1	291.3	299.1	310.6	316.9	.
	9076	181.3	190.1	195.4	204.0	212.8	218.2	229.9	233.7	241.6	247.6	256.0	261.9	267.0	274.6	281.3	.
	9077	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.
	9078	184.4	192.8	201.8	209.3	218.3	227.8	235.1	240.3	250.7	260.3	268.4	280.3	281.9	292.8	303.0	.
	9079	178.6	187.2	195.3	203.7	211.8	220.0	231.9	235.4	246.9	253.6	262.4	271.3	276.7	284.8	296.1	301.9
	9080	173.2	184.0	193.8	197.6	208.5	215.1	223.3	228.9	237.4	247.1	254.2	260.2	264.9	278.3	281.6	292.9
	9081	175.8	183.1	192.6	197.0	205.4	209.6	222.6	222.7	233.1	240.9	248.7	257.7	261.6	274.7	276.6	287.3
	9082	170.9	178.0	189.1	195.7	204.8	210.9	221.5	227.1	238.1	246.7	257.4	265.9	272.3	287.4	293.7	303.7
	9083	169.8	177.3	186.5	196.8	202.7	208.7	219.8	222.5	234.0	238.7	249.6	256.3	263.7	275.1	282.8	294.6
	9084	175.4	182.7	191.4	199.2	207.4	211.6	219.4	225.4	231.3	239.4	246.6	258.2	260.4	268.3	273.0	282.9
	9085	171.1	176.9	183.7	189.8	195.1	200.8	209.7	216.7	222.6	231.6	240.5	245.0	252.9	255.4	266.4	272.9

Dose Level: Group 1 – 0 mg/kg/day; Group 2 – 25 mg/kg/day Phenobarbital; Group 3 – 50 mg/kg/day Phenobarbital
Group 4 – 100 mg/kg/day Phenobarbital; Group 5 – 10 mg/kg/day Vinclozolin; Group 6 – 30 mg/kg/day Vinclozolin
Group 7 – 100 mg/kg/day Vinclozolin; Group 8 – 25 mg/kg/day Flutamide; Group 9 – 50 mg/kg/day Flutamide

M = Males
. = No Data

Appendix 8 (continued)
F_1 Individual Body Weights (Grams)
Pubertal Toxicity Study of Vinclozolin, Flutamide and Phenobarbital in Male Sprague Dawley Rats and Methoxychlor, Ethinyl Estradiol and Phenobarbital in Female Sprague Dawley Rats When Administered in Corn Oil by Oral Gavage

		Postnatal Day															
Group Sex	Animal Number	23	24	25	26	27	28	29	30	31	32	33	34	35	36	37	38
5M	9086	73.6	79.5	88.0	95.9	104.6	109.9	119.1	126.5	139.9	148.0	158.4	168.0	176.9	185.6	194.4	205.3
	9087	69.8	76.3	83.0	88.8	92.1	98.1	103.9	110.8	119.2	127.2	133.9	143.1	152.2	158.9	168.9	176.8
	9088	72.0	80.1	87.0	95.3	101.6	110.7	121.0	124.7	137.4	144.4	155.3	167.3	174.4	184.5	192.6	204.4
	9089	70.5	78.2	84.7	91.7	98.8	106.5	114.0	120.6	130.2	137.2	146.2	156.7	163.6	171.2	180.4	192.0
	9090	68.9	76.0	82.8	91.2	99.4	105.2	115.5	121.4	133.6	142.0	149.3	160.3	169.7	176.8	188.4	199.4
	9091	69.1	74.4	81.9	87.6	95.2	100.9	109.8	115.5	124.1	131.4	139.5	147.6	155.3	163.2	171.5	179.0
	9092	66.6	74.1	81.9	91.5	98.7	105.7	114.5	122.5	132.3	140.6	150.3	159.4	169.4	176.4	183.8	195.3
	9093	67.7	73.6	80.6	86.3	93.5	98.8	108.7	113.2	122.5	126.9	137.1	144.2	151.4	162.2	169.9	181.8
	9094	67.1	74.0	80.8	86.6	93.4	101.1	110.4	116.8	123.8	131.1	140.8	147.7	155.6	164.0	168.9	178.8
	9095	67.1	75.5	83.7	89.9	97.9	105.6	113.9	120.7	132.2	141.9	151.3	161.1	170.9	179.1	190.4	202.6
	9096	65.0	71.7	78.7	85.8	92.7	100.5	108.2	116.4	125.1	133.9	141.2	153.0	160.9	171.3	178.6	193.2
	9097	67.5	76.5	83.3	90.0	98.3	105.6	114.1	123.0	134.9	143.0	152.1	164.2	171.0	180.7	191.2	202.1
	9098	62.2	69.8	77.5	83.7	92.4	98.6	108.2	117.0	124.8	134.5	145.3	153.0	162.4	169.9	180.0	191.4
	9099	63.4	70.2	77.9	82.6	91.2	94.6	104.5	111.5	121.1	128.3	136.8	143.5	151.2	159.2	168.6	176.1
	9100	64.3	71.4	79.5	85.9	90.9	96.0	106.0	112.0	119.7	127.1	135.7	142.6	153.0	158.2	167.7	179.0

Dose Level: Group 1 – 0 mg/kg/day; Group 2 – 25 mg/kg/day Phenobarbital; Group 3 – 50 mg/kg/day Phenobarbital
Group 4 – 100 mg/kg/day Phenobarbital; Group 5 – 10 mg/kg/day Vinclozolin; Group 6 – 30 mg/kg/day Vinclozolin
Group 7 – 100 mg/kg/day Vinclozolin; Group 8 – 25 mg/kg/day Flutamide; Group 9 – 50 mg/kg/day Flutamide

M = Males
. = No Data

Appendix 8 (continued)
F_1 Individual Body Weights (Grams)
Pubertal Toxicity Study of Vinclozolin, Flutamide and Phenobarbital in Male Sprague Dawley Rats and Methoxychlor, Ethinyl Estradiol and Phenobarbital in Female Sprague Dawley Rats When Administered in Corn Oil by Oral Gavage

		Postnatal Day															
Group Sex	Animal Number	39	40	41	42	43	44	45	46	47	48	49	50	51	52	53	54
5M	9086	216.7	227.3	233.3	242.4	252.9	262.8	270.4	279.5	290.0	300.3	311.4	318.3	326.7	333.4	353.1	.
	9087	185.2	195.0	204.6	212.0	223.2	229.4	236.7	246.9	255.5	264.9	275.2	282.5	290.3	300.3	311.1	.
	9088	213.7	223.0	229.1	241.7	248.5	256.0	261.8	272.9	279.4	289.9	300.3	304.5	311.7	324.6	332.5	.
	9089	197.2	209.4	213.2	225.0	232.8	239.1	249.1	258.0	266.0	273.2	279.4	291.9	297.0	306.7	318.0	.
	9090	208.1	220.3	228.1	238.8	246.7	255.6	263.8	275.7	285.3	295.4	307.0	321.8	328.3	340.5	352.9	.
	9091	189.1	197.9	205.1	213.1	223.1	226.8	236.7	244.7	251.5	259.0	267.0	273.2	282.9	292.1	301.8	.
	9092	204.4	212.4	224.0	230.1	236.9	243.5	259.1	268.3	277.1	288.9	298.1	308.1	315.9	328.3	340.2	.
	9093	188.1	198.5	206.4	214.2	222.6	229.9	240.9	249.3	257.4	264.5	274.9	283.7	287.6	302.7	308.5	.
	9094	188.4	197.2	202.1	209.7	217.3	225.8	236.8	243.9	254.1	261.5	270.4	283.3	287.9	299.1	312.6	319.4
	9095	213.0	225.1	238.0	246.4	256.8	267.9	279.4	293.2	304.8	314.0	327.1	338.0	341.9	359.8	372.8	382.1
	9096	201.0	210.6	217.6	227.2	233.4	248.6	252.2	262.6	270.7	282.6	291.1	299.9	302.9	315.8	323.3	331.7
	9097	213.5	221.8	233.7	241.4	250.2	259.4	268.1	277.9	288.3	298.3	310.8	320.7	329.4	341.8	352.3	366.1
	9098	199.3	210.5	220.4	228.3	236.1	243.3	255.3	268.1	274.9	286.7	292.3	306.5	313.4	320.9	331.5	340.6
	9099	182.7	191.6	199.6	205.4	216.5	221.8	229.7	239.5	246.1	257.9	266.1	272.1	280.6	288.6	298.1	302.3
	9100	184.8	195.0	205.5	210.5	220.6	228.8	235.7	246.8	254.7	265.9	277.2	288.2	296.0	304.8	316.8	328.1

Dose Level: Group 1 – 0 mg/kg/day; Group 2 – 25 mg/kg/day Phenobarbital; Group 3 – 50 mg/kg/day Phenobarbital
Group 4 – 100 mg/kg/day Phenobarbital; Group 5 – 10 mg/kg/day Vinclozolin; Group 6 – 30 mg/kg/day Vinclozolin
Group 7 – 100 mg/kg/day Vinclozolin; Group 8 – 25 mg/kg/day Flutamide; Group 9 – 50 mg/kg/day Flutamide

M = Males
. = No Data

Appendix 8 (continued)
F_1 Individual Body Weights (Grams)
Pubertal Toxicity Study of Vinclozolin, Flutamide and Phenobarbital in Male Sprague Dawley Rats and Methoxychlor, Ethinyl Estradiol and Phenobarbital in Female Sprague Dawley Rats When Administered in Corn Oil by Oral Gavage

		Postnatal Day															
Group Sex	Animal Number	23	24	25	26	27	28	29	30	31	32	33	34	35	36	37	38
6M	9101	74.3	81.4	88.4	96.2	103.2	111.0	119.6	126.6	138.3	144.7	154.2	167.0	173.8	182.7	193.7	203.6
	9102	73.2	79.2	87.6	97.2	105.0	113.8	120.3	127.4	139.9	147.8	159.6	170.7	180.4	191.2	200.5	210.9
	9103	73.0	80.3	88.1	95.7	101.8	110.5	115.7	121.9	134.9	140.6	149.7	155.6	166.7	173.4	182.9	191.2
	9104	73.3	78.4	86.2	96.0	100.4	106.9	116.5	123.4	133.0	143.3	152.0	163.2	173.5	176.8	189.9	199.7
	9105	71.9	79.2	86.6	92.6	98.0	107.5	114.1	120.9	129.8	136.3	143.7	153.2	159.6	170.2	177.8	184.4
	9106	70.6	76.8	84.2	91.6	96.3	105.4	113.9	121.6	130.9	141.3	150.2	159.4	168.9	177.7	189.5	200.2
	9107	64.2	72.6	78.0	85.8	93.2	101.0	109.7	117.6	126.9	135.4	143.1	152.1	159.8	165.8	174.3	183.3
	9108	66.5	74.3	79.7	88.1	92.6	99.5	106.0	114.7	122.5	129.1	138.0	145.9	152.2	161.1	170.1	178.0
	9109	63.4	71.0	76.4	85.4	94.8	101.2	110.3	118.5	127.3	138.6	148.5	158.5	167.5	175.7	190.2	201.9
	9110	68.6	77.4	87.3	93.4	101.5	109.7	119.7	125.6	138.6	147.2	154.2	167.9	175.5	184.5	196.6	208.3
	9111	67.2	74.1	82.0	87.4	96.7	104.8	110.9	117.7	129.2	136.0	144.7	155.2	163.9	173.9	181.0	192.8
	9112	61.2	67.3	72.7	76.8	86.0	89.6	97.8	104.4	113.0	120.0	128.8	136.1	146.1	156.8	163.1	174.2
	9113	66.7	73.7	80.1	87.9	95.1	101.9	109.5	118.6	128.8	140.9	148.9	163.0	171.9	183.6	191.7	202.9
	9114	64.2	72.5	76.6	86.4	92.3	99.4	106.3	114.4	124.9	135.2	145.0	155.6	165.6	174.7	184.0	197.5
	9115	62.9	70.5	80.2	82.6	89.3	98.6	104.2	110.5	119.2	130.5	137.1	147.0	154.7	163.8	173.8	185.4

Dose Level: Group 1 – 0 mg/kg/day; Group 2 – 25 mg/kg/day Phenobarbital; Group 3 – 50 mg/kg/day Phenobarbital
Group 4 – 100 mg/kg/day Phenobarbital; Group 5 – 10 mg/kg/day Vinclozolin; Group 6 – 30 mg/kg/day Vinclozolin
Group 7 – 100 mg/kg/day Vinclozolin; Group 8 – 25 mg/kg/day Flutamide; Group 9 – 50 mg/kg/day Flutamide
M = Males
. = No Data

Appendix 8 (continued)
F_1 Individual Body Weights (Grams)
Pubertal Toxicity Study of Vinclozolin, Flutamide and Phenobarbital in Male Sprague Dawley Rats and Methoxychlor, Ethinyl Estradiol and Phenobarbital in Female Sprague Dawley Rats When Administered in Corn Oil by Oral Gavage

		Postnatal Day															
Group Sex	Animal Number	39	40	41	42	43	44	45	46	47	48	49	50	51	52	53	54
6M	9101	214.6	223.4	233.3	240.1	252.5	258.0	271.2	280.0	289.5	299.8	308.4	320.7	328.4	337.7	345.9	.
	9102	224.4	234.5	244.9	254.8	263.6	272.0	281.9	296.6	305.1	316.7	327.5	340.4	343.7	357.7	369.9	.
	9103	201.5	210.0	222.4	231.3	241.2	247.3	256.4	267.1	277.7	286.7	297.8	310.4	316.8	330.0	343.9	.
	9104	207.3	218.0	225.8	236.8	245.6	248.4	260.5	268.2	278.1	286.0	295.5	305.7	314.4	325.4	339.3	.
	9105	195.4	202.4	211.1	217.0	226.5	231.7	242.9	249.4	260.2	267.9	277.3	286.9	296.3	305.1	315.1	.
	9106	209.4	216.0	228.8	236.1	244.4	251.4	261.7	273.0	278.1	290.2	304.1	309.2	317.4	332.6	340.9	.
	9107	192.6	200.3	209.1	217.4	225.5	232.5	241.5	252.2	258.1	267.4	281.0	289.3	295.2	310.4	319.1	.
	9108	186.8	199.6	206.8	213.6	220.2	230.7	237.3	247.8	257.9	265.9	277.1	286.3	288.3	305.1	314.5	.
	9109	208.7	220.7	228.2	241.0	251.1	258.9	269.5	280.1	289.3	298.6	313.0	322.3	332.4	347.7	362.1	373.6
	9110	218.5	226.2	238.3	246.1	257.4	265.6	278.3	290.4	297.4	312.3	320.4	334.3	343.4	353.2	367.1	377.8
	9111	197.3	207.8	221.1	227.3	237.0	243.6	252.2	263.1	271.7	281.4	293.5	293.9	304.7	311.6	328.0	333.8
	9112	182.1	193.4	203.5	211.0	221.5	228.6	237.1	245.4	258.4	263.9	274.0	286.2	291.4	302.4	312.4	320.2
	9113	213.2	226.3	233.2	244.3	254.2	261.2	273.1	283.0	291.3	300.4	311.9	322.6	333.9	346.8	356.5	367.5
	9114	208.4	219.8	230.8	243.5	254.1	259.4	268.8	280.6	291.6	301.9	313.7	322.7	332.3	343.7	356.0	366.7
	9115	190.4	204.5	212.4	218.9	228.8	233.1	243.5	253.7	261.8	270.1	282.2	291.6	300.8	306.8	318.1	331.6

Dose Level: Group 1 – 0 mg/kg/day; Group 2 – 25 mg/kg/day Phenobarbital; Group 3 – 50 mg/kg/day Phenobarbital
Group 4 – 100 mg/kg/day Phenobarbital; Group 5 – 10 mg/kg/day Vinclozolin; Group 6 – 30 mg/kg/day Vinclozolin
Group 7 – 100 mg/kg/day Vinclozolin; Group 8 – 25 mg/kg/day Flutamide; Group 9 – 50 mg/kg/day Flutamide

M = Males
. = No Data

Appendix 8 (continued)
F_1 Individual Body Weights (Grams)
Pubertal Toxicity Study of Vinclozolin, Flutamide and Phenobarbital in Male Sprague Dawley Rats and Methoxychlor, Ethinyl Estradiol and Phenobarbital in Female Sprague Dawley Rats When Administered in Corn Oil by Oral Gavage

		Postnatal Day															
Group Sex	Animal Number	23	24	25	26	27	28	29	30	31	32	33	34	35	36	37	38
7M	9116	73.8	81.2	89.1	95.8	103.2	110.0	116.3	115.3	129.7	140.2	148.0	158.6	167.2	176.1	182.2	193.6
	9117	72.3	79.8	86.7	92.2	99.5	107.9	112.5	119.7	129.8	139.9	143.8	156.0	165.2	175.6	184.1	196.3
	9118	73.1	81.2	88.3	97.0	102.4	111.9	120.2	116.7	136.2	144.9	154.4	165.2	173.9	183.5	193.5	205.3
	9119	70.1	77.0	81.9	88.9	94.8	102.3	110.7	118.1	128.0	136.3	144.2	154.7	164.1	174.2	184.2	194.4
	9120	71.0	76.8	83.8	91.9	97.3	106.8	115.2	120.7	129.2	137.0	146.3	156.4	165.0	172.7	180.1	191.2
	9121	68.0	74.4	80.8	85.4	93.8	101.7	110.1	115.9	124.7	134.2	141.5	153.1	161.8	168.2	176.5	186.0
	9122	66.4	71.4	78.4	85.2	90.9	96.8	103.8	110.8	117.9	125.0	134.3	142.9	151.1	159.8	166.7	178.3
	9123	67.6	71.5	78.7	84.3	90.9	96.4	105.8	110.1	117.7	124.4	131.4	140.2	147.9	159.8	162.8	174.0
	9124	67.5	73.9	83.6	88.5	97.8	105.3	115.4	122.9	133.3	140.4	151.5	164.4	168.7	179.3	192.4	204.9
	9125	70.3	76.3	85.2	91.9	98.7	106.1	113.7	120.2	130.2	136.3	145.7	154.1	162.9	172.5	182.7	192.0
	9126	67.9	73.2	81.0	85.9	94.0	102.2	107.6	113.0	124.1	129.8	139.8	148.9	157.5	162.9	172.3	180.6
	9127	64.3	70.0	76.0	79.9	85.3	90.7	97.4	103.8	112.2	120.4	128.7	136.3	141.7	150.3	156.9	167.9
	9128	62.3	67.9	75.9	80.4	87.0	93.2	99.7	106.9	115.9	123.2	132.5	139.9	148.8	158.2	168.4	174.7
	9129	62.5	68.1	74.5	80.9	86.3	92.5	100.7	107.4	115.0	120.3	126.3	136.9	146.4	151.9	157.9	164.7
	9130	61.5	68.0	74.1	79.3	85.5	92.8	101.3	107.9	117.7	123.4	132.5	141.4	150.2	157.6	164.1	175.6

Dose Level: Group 1 – 0 mg/kg/day; Group 2 – 25 mg/kg/day Phenobarbital; Group 3 – 50 mg/kg/day Phenobarbital
Group 4 – 100 mg/kg/day Phenobarbital; Group 5 – 10 mg/kg/day Vinclozolin; Group 6 – 30 mg/kg/day Vinclozolin
Group 7 – 100 mg/kg/day Vinclozolin; Group 8 – 25 mg/kg/day Flutamide; Group 9 – 50 mg/kg/day Flutamide

M = Males
. = No Data

Appendix 8 (continued)
F_1 Individual Body Weights (Grams)
Pubertal Toxicity Study of Vinclozolin, Flutamide and Phenobarbital in Male Sprague Dawley Rats and Methoxychlor, Ethinyl Estradiol and Phenobarbital in Female Sprague Dawley Rats When Administered in Corn Oil by Oral Gavage

		Postnatal Day															
Group Sex	Animal Number	39	40	41	42	43	44	45	46	47	48	49	50	51	52	53	54
7M	9116	200.8	212.4	222.3	229.2	239.9	247.9	257.4	267.4	272.2	284.5	293.2	298.4	309.8	314.7	325.3	.
	9117	202.8	213.4	224.6	231.2	239.5	249.3	256.9	266.3	273.6	286.5	299.1	305.2	311.3	327.0	333.6	.
	9118	212.4	225.9	234.2	242.1	252.0	260.4	266.3	278.8	288.2	297.4	307.6	314.1	321.9	333.1	342.5	.
	9119	202.1	214.3	222.5	228.7	242.6	248.7	260.0	266.6	275.1	282.9	292.4	300.0	306.8	318.3	320.7	.
	9120	199.2	205.6	214.5	226.6	233.1	240.8	247.5	259.1	267.7	277.1	284.2	295.6	303.5	315.2	320.4	.
	9121	195.6	203.7	211.7	220.3	229.2	236.2	243.6	253.0	265.3	272.4	280.1	292.0	298.7	309.8	319.8	.
	9122	185.8	193.3	202.2	211.2	220.8	225.1	239.1	244.7	248.9	256.6	268.4	274.4	282.3	288.4	300.7	.
	9123	182.4	192.0	200.6	208.0	220.3	226.5	235.8	243.2	250.1	261.1	270.6	277.8	284.7	294.4	308.2	.
	9124	212.2	223.8	231.0	240.5	251.8	257.4	265.5	278.2	284.5	292.5	304.5	313.5	319.2	327.5	337.8	344.5
	9125	202.1	212.2	219.5	231.2	235.6	242.7	252.9	266.3	276.5	286.2	296.5	308.9	312.0	326.4	337.3	345.8
	9126	190.4	197.4	204.9	212.9	220.2	231.0	235.7	246.5	256.2	262.8	272.9	281.6	289.0	300.6	308.8	314.5
	9127	175.2	183.9	188.7	195.8	204.1	209.1	219.3	224.0	232.2	241.1	250.6	257.4	262.7	272.0	281.1	288.2
	9128	184.2	192.4	199.8	208.1	220.1	224.9	236.6	243.1	252.6	260.8	274.3	280.2	279.2	289.8	296.5	309.9
	9129	171.6	178.5	184.3	189.5	197.1	200.7	208.5	214.4	220.7	226.0	236.1	239.2	245.3	251.4	259.8	268.2
	9130	184.0	192.4	202.4	209.5	218.3	225.3	236.7	245.6	255.9	262.6	273.7	279.6	288.8	295.1	305.0	313.9

Dose Level: Group 1 – 0 mg/kg/day; Group 2 – 25 mg/kg/day Phenobarbital; Group 3 – 50 mg/kg/day Phenobarbital
Group 4 – 100 mg/kg/day Phenobarbital; Group 5 – 10 mg/kg/day Vinclozolin; Group 6 – 30 mg/kg/day Vinclozolin
Group 7 – 100 mg/kg/day Vinclozolin; Group 8 – 25 mg/kg/day Flutamide; Group 9 – 50 mg/kg/day Flutamide
M = Males
. = No Data

Appendix 8 (continued)
F_1 Individual Body Weights (Grams)
Pubertal Toxicity Study of Vinclozolin, Flutamide and Phenobarbital in Male Sprague Dawley Rats and Methoxychlor, Ethinyl Estradiol and Phenobarbital in Female Sprague Dawley Rats When Administered in Corn Oil by Oral Gavage

		Postnatal Day															
Group Sex	Animal Number	23	24	25	26	27	28	29	30	31	32	33	34	35	36	37	38
8M	9131	72.7	79.2	86.1	94.1	100.2	108.1	117.2	123.0	132.5	143.3	153.9	162.6	174.2	182.4	192.2	200.0
	9132	71.3	78.5	84.3	91.6	97.1	103.2	113.0	117.7	126.2	134.8	140.8	151.3	160.3	164.4	174.5	178.6
	9133	69.6	76.0	82.5	89.7	95.1	100.9	109.7	115.8	126.4	132.0	141.8	151.6	159.8	167.0	177.7	184.5
	9134	69.4	77.0	84.0	91.4	97.8	103.1	112.8	118.2	127.8	138.8	145.6	157.0	164.2	174.1	181.8	193.1
	9135	69.6	77.3	84.4	91.4	98.2	104.1	112.8	119.3	129.6	137.6	144.5	156.3	167.3	174.0	183.3	194.4
	9136	65.6	73.7	78.7	84.5	89.4	95.2	100.6	106.3	115.8	119.7	127.9	133.8	141.6	147.6	155.0	164.1
	9137	65.9	71.2	77.5	82.8	88.3	96.2	101.3	108.3	117.8	127.4	134.5	142.5	151.0	159.4	167.8	177.1
	9138	68.9	75.3	82.2	86.8	92.1	99.6	108.1	113.2	121.6	129.6	137.1	147.0	153.3	164.6	172.8	179.1
	9139	68.6	76.8	83.7	89.3	94.4	102.5	108.8	113.1	120.8	129.9	134.4	142.7	150.5	156.0	163.3	174.1
	9140	65.1	72.8	79.7	83.9	92.5	97.2	103.9	110.4	116.7	125.4	130.3	140.5	149.5	158.5	166.9	176.4
	9141	62.6	69.8	77.4	83.3	90.0	96.4	105.2	111.6	121.9	129.8	140.7	148.9	157.7	166.4	177.6	189.0
	9142	63.2	70.4	77.4	82.0	89.9	95.8	104.4	112.0	119.9	129.0	138.0	144.4	152.4	160.2	170.7	181.7
	9143	65.6	71.9	79.3	84.9	89.1	96.3	102.1	108.7	118.5	125.1	131.4	139.0	147.7	153.5	162.6	169.6
	9144	63.1	69.2	75.1	80.3	86.0	91.4	98.3	105.6	114.3	121.8	128.0	139.6	145.6	154.6	164.9	172.7
	9145	62.8	68.4	75.6	81.8	86.2	94.1	101.1	107.9	116.4	125.3	134.6	138.6	147.9	155.1	164.6	174.5

Dose Level: Group 1 – 0 mg/kg/day; Group 2 – 25 mg/kg/day Phenobarbital; Group 3 – 50 mg/kg/day Phenobarbital
Group 4 – 100 mg/kg/day Phenobarbital; Group 5 – 10 mg/kg/day Vinclozolin; Group 6 – 30 mg/kg/day Vinclozolin
Group 7 – 100 mg/kg/day Vinclozolin; Group 8 – 25 mg/kg/day Flutamide; Group 9 – 50 mg/kg/day Flutamide

M = Males
. = No Data

Appendix 8 (continued)
F_1 Individual Body Weights (Grams)
Pubertal Toxicity Study of Vinclozolin, Flutamide and Phenobarbital in Male Sprague Dawley Rats and Methoxychlor, Ethinyl Estradiol and Phenobarbital in Female Sprague Dawley Rats When Administered in Corn Oil by Oral Gavage

		Postnatal Day															
Group Sex	Animal Number	39	40	41	42	43	44	45	46	47	48	49	50	51	52	53	54
8M	9131	209.1	219.1	226.8	236.9	245.8	254.3	262.0	272.1	283.6	293.3	301.7	317.1	321.9	325.7	334.7	.
	9132	189.1	197.1	202.9	209.4	219.8	224.0	234.0	242.6	250.6	254.7	264.2	271.4	276.8	287.1	292.9	.
	9133	194.6	204.0	213.8	222.8	231.7	239.8	250.1	256.8	266.9	276.4	287.0	294.5	298.7	308.1	321.6	.
	9134	201.9	211.1	221.5	229.8	240.0	245.2	254.7	263.5	270.2	275.7	291.4	295.6	304.0	317.2	326.6	.
	9135	205.5	213.6	224.4	230.4	239.2	251.1	257.7	264.1	270.6	280.5	290.3	300.5	306.5	316.1	329.0	.
	9136	170.8	178.2	185.8	195.3	202.9	212.7	219.2	226.8	235.2	243.8	256.8	262.7	269.9	275.6	288.3	.
	9137	188.8	195.9	201.6	211.0	222.7	231.7	237.2	249.7	254.8	265.6	272.5	283.9	287.1	297.5	309.7	.
	9138	189.9	198.2	202.1	210.9	223.1	228.0	240.8	245.9	253.6	261.4	268.5	278.8	280.5	291.7	302.7	.
	9139	178.6	186.0	190.9	198.4	208.0	214.9	224.3	232.0	238.4	247.3	256.8	264.3	273.0	288.1	290.9	300.2
	9140	189.2	194.0	204.4	212.5	219.8	226.2	234.5	243.2	253.5	257.1	266.6	274.3	279.1	289.0	298.4	304.5
	9141	198.0	210.2	215.8	226.2	235.0	247.1	256.4	266.8	277.2	283.7	295.3	305.8	314.9	329.3	337.2	351.6
	9142	187.1	196.5	201.9	211.6	221.3	228.8	241.3	249.6	257.8	265.2	274.0	284.1	290.4	301.5	312.3	317.2
	9143	178.1	184.3	190.1	200.4	207.0	214.3	222.4	231.7	238.8	247.4	255.4	263.1	268.6	279.5	289.0	293.7
	9144	180.7	192.1	199.2	210.5	215.9	226.3	238.1	246.2	252.0	261.5	271.5	281.1	289.5	301.4	313.8	320.3
	9145	183.9	190.1	197.6	205.3	215.8	221.4	230.0	239.0	245.0	255.5	267.2	273.5	278.1	292.1	303.4	309.4

Dose Level: Group 1 – 0 mg/kg/day; Group 2 – 25 mg/kg/day Phenobarbital; Group 3 – 50 mg/kg/day Phenobarbital
Group 4 – 100 mg/kg/day Phenobarbital; Group 5 – 10 mg/kg/day Vinclozolin; Group 6 – 30 mg/kg/day Vinclozolin
Group 7 – 100 mg/kg/day Vinclozolin; Group 8 – 25 mg/kg/day Flutamide; Group 9 – 50 mg/kg/day Flutamide

M = Males
. = No Data

Appendix 8 (continued)
F_1 Individual Body Weights (Grams)
Pubertal Toxicity Study of Vinclozolin, Flutamide and Phenobarbital in Male Sprague Dawley Rats and Methoxychlor, Ethinyl Estradiol and Phenobarbital in Female Sprague Dawley Rats When Administered in Corn Oil by Oral Gavage

		Postnatal Day															
Group Sex	Animal Number	23	24	25	26	27	28	29	30	31	32	33	34	35	36	37	38
9M	9146	71.7	78.7	85.8	93.0	99.0	107.3	115.0	122.7	132.5	142.7	149.7	159.0	168.5	178.3	184.0	194.7
	9147	74.3	80.4	89.1	95.1	101.8	108.1	117.8	124.3	135.6	144.8	155.1	162.7	174.0	182.0	188.3	203.6
	9148	71.7	76.3	85.4	92.0	100.4	107.2	116.5	121.5	131.2	142.1	150.6	162.3	172.7	182.7	190.2	202.1
	9149	72.0	76.9	82.4	87.9	87.8	98.9	107.2	113.4	121.7	130.0	139.2	148.5	156.9	165.1	172.4	183.1
	9150	68.1	76.1	82.3	87.8	88.9	98.7	104.4	110.9	123.0	128.5	136.5	145.4	155.4	162.2	173.9	183.4
	9151	67.9	75.4	81.8	88.2	92.1	99.0	108.0	114.9	124.8	134.8	144.8	157.3	165.1	175.6	185.4	190.6
	9152	64.8	69.6	74.5	79.3	84.9	89.9	96.8	103.0	111.1	117.0	122.2	133.3	137.6	144.6	149.1	155.7
	9153	65.1	71.5	79.1	84.3	89.5	94.3	103.0	108.9	115.6	123.5	131.7	139.6	148.0	157.0	163.1	173.0
	9154	68.2	72.4	80.2	85.2	94.0	99.6	107.0	114.6	123.9	128.6	136.7	144.4	153.8	162.3	169.9	177.6
	9155	66.2	74.3	79.7	84.3	93.0	98.0	105.9	110.5	119.6	128.2	137.0	144.1	154.4	161.1	179.0	180.6
	9156	65.9	72.6	80.6	86.7	92.7	100.2	108.7	115.0	126.7	132.4	140.0	148.4	157.6	165.1	169.8	183.8
	9157	65.2	73.7	81.7	87.9	93.4	102.3	110.1	116.3	125.0	132.6	142.2	153.1	158.8	168.8	172.9	188.9
	9158	60.3	68.0	73.6	77.9	84.5	90.2	96.6	101.2	110.7	116.1	123.1	131.6	138.2	146.6	153.8	161.9
	9159	63.6	69.8	77.4	84.0	89.0	97.0	105.6	112.2	122.4	130.1	138.8	146.6	154.3	164.2	172.2	182.2
	9160	60.9	66.7	71.8	77.5	84.2	91.5	98.7	104.1	112.6	118.9	126.3	135.5	142.9	151.9	158.1	166.1

Dose Level: Group 1 – 0 mg/kg/day; Group 2 – 25 mg/kg/day Phenobarbital; Group 3 – 50 mg/kg/day Phenobarbital
Group 4 – 100 mg/kg/day Phenobarbital; Group 5 – 10 mg/kg/day Vinclozolin; Group 6 – 30 mg/kg/day Vinclozolin
Group 7 – 100 mg/kg/day Vinclozolin; Group 8 – 25 mg/kg/day Flutamide; Group 9 – 50 mg/kg/day Flutamide

M = Males
. = No Data

Appendix 8 (continued)
F_1 Individual Body Weights (Grams)
Pubertal Toxicity Study of Vinclozolin, Flutamide and Phenobarbital in Male Sprague Dawley Rats and Methoxychlor, Ethinyl Estradiol and Phenobarbital in Female Sprague Dawley Rats When Administered in Corn Oil by Oral Gavage

		Postnatal Day															
Group Sex	Animal Number	39	40	41	42	43	44	45	46	47	48	49	50	51	52	53	54
9M	9146	203.2	211.9	221.8	229.8	238.6	245.1	256.7	266.6	278.2	283.1	291.2	306.8	310.5	322.4	331.3	.
	9147	210.3	221.0	229.0	238.2	246.5	253.5	264.6	275.7	281.8	287.4	302.8	306.0	314.8	325.9	335.8	.
	9148	212.7	222.9	232.3	242.3	251.6	260.8	271.4	281.6	290.1	299.6	309.8	320.0	323.3	332.4	347.8	.
	9149	189.8	199.3	203.8	211.6	219.4	230.4	235.9	241.7	252.4	255.6	263.2	272.6	270.7	281.8	288.3	.
	9150	191.0	200.8	209.3	213.2	224.3	231.5	241.0	249.2	258.9	265.7	275.5	285.1	290.6	301.6	313.6	.
	9151	201.0	212.7	219.6	229.5	237.3	247.8	255.7	263.1	273.8	280.2	296.1	302.8	308.2	319.5	329.1	.
	9152	163.6	165.8	172.4	178.4	186.2	190.1	196.7	201.8	209.2	215.4	220.8	229.6	232.8	244.0	252.7	.
	9153	181.3	189.5	197.8	204.9	214.4	221.4	230.4	239.0	246.1	250.6	260.4	270.0	272.1	280.9	289.7	.
	9154	186.2	190.4	199.0	203.9	213.8	220.5	226.3	234.0	242.8	249.6	258.0	266.7	271.0	281.8	290.7	292.3
	9155	189.9	196.1	204.3	212.4	219.1	228.1	235.8	243.5	250.6	260.5	268.9	277.5	284.6	297.7	308.5	316.7
	9156	190.1	200.4	206.8	215.7	222.9	230.5	240.9	247.2	258.4	264.8	274.4	284.4	290.4	302.8	312.6	321.8
	9157	199.2	207.6	216.6	225.1	235.2	244.5	250.4	260.9	269.9	278.7	288.8	299.5	300.7	311.3	323.5	331.6
	9158	167.4	180.9	184.8	197.6	203.9	208.6	217.4	224.6	229.8	236.6	245.1	254.1	257.3	266.2	276.3	282.2
	9159	189.6	198.7	205.6	215.4	223.4	227.6	237.4	245.9	254.9	264.2	271.3	278.1	283.2	293.0	303.7	311.0
	9160	171.9	180.9	184.9	193.4	200.3	205.0	212.4	221.1	226.6	237.3	246.8	253.0	256.9	267.6	274.6	286.5

Dose Level: Group 1 – 0 mg/kg/day; Group 2 – 25 mg/kg/day Phenobarbital; Group 3 – 50 mg/kg/day Phenobarbital
Group 4 – 100 mg/kg/day Phenobarbital; Group 5 – 10 mg/kg/day Vinclozolin; Group 6 – 30 mg/kg/day Vinclozolin
Group 7 – 100 mg/kg/day Vinclozolin; Group 8 – 25 mg/kg/day Flutamide; Group 9 – 50 mg/kg/day Flutamide
M = Males
. = No Data

Appendix 8 (continued)
F_1 Individual Body Weights (Grams)
Pubertal Toxicity Study of Vinclozolin, Flutamide and Phenobarbital in Male Sprague Dawley Rats and Methoxychlor, Ethinyl Estradiol and Phenobarbital in Female Sprague Dawley Rats When Administered in Corn Oil by Oral Gavage

		Postnatal Day										
Group Sex	Animal Number	22	23	24	25	26	27	28	29	30	31	32
1F	9161	63.7	70.8	76.9	83.1	89.0	94.3	102.2	108.3	113.6	121.3	128.4
	9162	60.4	64.6	69.9	75.0	79.6	84.0	91.0	96.7	101.6	108.3	115.4
	9163	62.8	66.5	73.3	78.3	85.1	91.0	97.6	103.2	109.8	115.4	122.8
	9164	60.5	67.2	73.7	80.7	84.9	90.2	92.2	103.0	107.2	116.1	122.5
	9165	59.7	65.6	71.4	76.3	84.6	90.8	98.0	106.2	110.7	118.1	127.4
	9166	60.8	65.0	70.4	75.1	80.8	87.4	92.8	99.7	105.2	110.6	118.1
	9167	60.5	66.5	73.3	78.9	84.4	87.7	93.7	100.9	105.1	114.0	121.3
	9168	60.1	64.5	70.4	75.8	81.7	86.9	92.9	100.0	105.8	112.5	119.0
	9169	58.3	63.1	69.1	75.0	79.5	85.2	91.2	99.1	103.9	111.7	117.8
	9170	59.2	64.5	69.6	75.0	78.2	85.1	92.3	95.4	101.5	108.6	114.0
	9171	57.2	63.1	70.0	75.2	81.3	86.4	95.1	102.2	109.5	117.2	126.0
	9172	55.7	61.3	66.5	71.8	76.5	82.6	88.5	94.4	99.5	104.7	110.2
	9173	54.1	60.0	65.3	71.1	75.5	81.9	87.1	93.3	99.3	108.6	113.8
	9174	55.8	61.5	67.5	72.1	76.3	82.8	89.2	95.3	101.5	106.4	113.4
	9175	54.2	59.7	66.7	70.1	76.7	83.1	89.5	94.8	100.2	108.0	115.8

Dose Level: Group 1 – 0 mg/kg/day; Group 2 – 0.0025 mg/kg/day Ethinyl Estradiol; Group 3 – 0.005 mg/kg/day Ethinyl Estradiol
Group 4 – 12.5 mg/kg/day Methoxychlor; Group 5 – 25 mg/kg/day Methoxychlor; Group 6 – 50 mg/kg/day Methoxychlor
Group 7 – 25 mg/kg/day Phenobarbital; Group 8 – 50 mg/kg/day Phenobarbital; Group 9 – 100 mg/kg/day Phenobarbital
F = Females
. = No Data

Appendix 8 (continued)
F_1 Individual Body Weights (Grams)
Pubertal Toxicity Study of Vinclozolin, Flutamide and Phenobarbital in Male Sprague Dawley Rats and Methoxychlor, Ethinyl Estradiol and Phenobarbital in Female Sprague Dawley Rats When Administered in Corn Oil by Oral Gavage

		Postnatal Day										
Group Sex	Animal Number	33	34	35	36	37	38	39	40	41	42	43
1F	9161	133.3	143.8	150.0	152.2	161.3	166.3	173.0	173.9	180.2	179.6	.
	9162	120.5	127.7	132.1	130.7	143.2	150.8	152.4	159.4	156.8	169.3	.
	9163	129.4	136.1	141.3	145.7	151.6	155.8	160.7	164.9	167.3	168.1	.
	9164	129.3	136.3	141.1	148.1	155.6	164.5	168.4	175.3	180.9	184.4	.
	9165	132.6	141.2	149.3	150.5	158.5	167.1	171.0	172.0	178.5	180.6	.
	9166	122.6	133.8	138.6	141.8	146.9	154.8	158.5	162.9	163.1	168.1	.
	9167	128.7	133.5	140.4	143.1	149.8	156.4	158.9	160.1	167.0	169.5	.
	9168	126.3	133.4	138.9	144.9	152.9	162.5	162.6	170.2	169.3	163.5	.
	9169	124.0	128.3	135.6	139.9	148.4	155.1	152.8	159.2	164.4	167.5	166.1
	9170	118.5	126.5	128.4	138.8	141.2	151.6	148.7	157.0	161.7	167.7	167.9
	9171	130.3	134.7	140.2	150.2	157.4	163.5	165.8	169.1	171.5	175.9	178.9
	9172	115.5	121.8	123.5	132.7	136.5	141.4	144.3	150.2	151.7	156.0	155.4
	9173	119.3	125.6	131.8	138.6	146.0	150.0	156.4	162.4	163.1	169.4	173.1
	9174	119.1	126.5	134.0	137.9	143.5	150.9	153.9	160.1	163.2	169.5	175.1
	9175	121.6	129.5	133.1	138.9	146.5	149.5	155.9	160.7	157.2	158.3	166.9

Dose Level: Group 1 – 0 mg/kg/day; Group 2 – 0.0025 mg/kg/day Ethinyl Estradiol; Group 3 – 0.005 mg/kg/day Ethinyl Estradiol
Group 4 – 12.5 mg/kg/day Methoxychlor; Group 5 – 25 mg/kg/day Methoxychlor; Group 6 – 50 mg/kg/day Methoxychlor
Group 7 – 25 mg/kg/day Phenobarbital; Group 8 – 50 mg/kg/day Phenobarbital; Group 9 – 100 mg/kg/day Phenobarbital
F = Females
. = No Data

Appendix 8 (continued)
F_1 Individual Body Weights (Grams)
Pubertal Toxicity Study of Vinclozolin, Flutamide and Phenobarbital in Male Sprague Dawley Rats and Methoxychlor, Ethinyl Estradiol and Phenobarbital in Female Sprague Dawley Rats When Administered in Corn Oil by Oral Gavage

		Postnatal Day										
Group Sex	Animal Number	22	23	24	25	26	27	28	29	30	31	32
2F	9176	64.5	72.0	79.6	85.6	93.7	99.8	109.6	116.0	122.2	131.7	137.7
	9177	62.8	68.4	75.3	79.2	84.7	90.0	96.5	106.0	108.5	116.1	122.5
	9178	61.8	68.0	73.1	78.0	85.2	89.8	95.2	101.8	106.4	115.2	120.7
	9179	60.5	66.9	73.8	80.6	86.8	92.0	100.3	106.3	111.8	119.9	126.6
	9180	59.1	65.6	70.1	76.1	82.8	88.4	95.8	99.6	104.6	112.1	116.1
	9181	58.9	65.3	70.0	76.1	81.9	86.5	92.7	96.0	104.9	108.7	114.7
	9182	58.9	64.1	70.3	74.8	80.6	87.8	93.4	99.4	105.3	112.2	118.9
	9183	57.5	63.0	69.6	74.4	79.3	88.2	95.3	100.3	106.0	110.6	119.8
	9184	58.0	63.8	68.7	72.8	78.5	83.9	90.5	96.6	104.3	111.9	118.7
	9185	59.2	64.6	72.0	76.3	81.3	86.9	92.4	97.4	100.9	105.3	109.1
	9186	55.5	62.7	68.9	71.8	77.6	82.0	88.1	93.7	100.2	104.6	109.3
	9187	54.5	62.7	71.0	75.4	80.9	87.6	93.3	101.5	105.4	107.0	114.7
	9188	53.6	60.9	66.9	71.9	76.2	81.1	87.5	93.2	97.2	102.9	107.6
	9189	55.0	60.7	66.8	71.2	77.7	82.7	88.3	93.9	101.6	105.5	116.3
	9190	52.8	60.0	65.1	70.1	75.0	79.2	88.3	92.1	97.2	105.0	111.5

Dose Level: Group 1 – 0 mg/kg/day; Group 2 – 0.0025 mg/kg/day Ethinyl Estradiol; Group 3 – 0.005 mg/kg/day Ethinyl Estradiol
Group 4 – 12.5 mg/kg/day Methoxychlor; Group 5 – 25 mg/kg/day Methoxychlor; Group 6 – 50 mg/kg/day Methoxychlor
Group 7 – 25 mg/kg/day Phenobarbital; Group 8 – 50 mg/kg/day Phenobarbital; Group 9 – 100 mg/kg/day Phenobarbital
F = Females
. = No Data

Appendix 8 (continued)
F_1 Individual Body Weights (Grams)
Pubertal Toxicity Study of Vinclozolin, Flutamide and Phenobarbital in Male Sprague Dawley Rats and Methoxychlor, Ethinyl Estradiol and Phenobarbital in Female Sprague Dawley Rats When Administered in Corn Oil by Oral Gavage

		Postnatal Day										
Group Sex	Animal Number	33	34	35	36	37	38	39	40	41	42	43
2F	9176	144.5	151.9	159.8	164.2	167.2	179.8	181.2	185.6	183.3	189.1	.
	9177	126.2	135.9	139.2	148.3	151.6	150.4	158.7	161.3	167.3	172.6	.
	9178	128.3	135.3	143.4	146.8	156.1	165.1	165.5	172.5	177.5	182.9	.
	9179	132.6	139.0	146.0	150.9	161.1	168.5	171.2	167.5	178.3	181.9	.
	9180	120.3	127.0	131.9	137.7	143.7	149.6	154.1	158.0	162.9	167.8	.
	9181	118.1	125.7	131.9	137.8	142.6	147.5	154.8	158.6	162.2	163.5	.
	9182	125.4	130.3	137.4	139.8	147.4	149.8	159.8	161.5	164.6	167.2	.
	9183	124.5	130.7	137.6	135.4	144.5	153.7	154.1	150.6	159.2	162.8	.
	9184	125.3	133.6	139.2	147.7	154.7	159.5	166.0	173.5	175.5	176.6	177.0
	9185	114.8	121.3	127.2	130.4	137.9	140.9	145.7	151.0	149.8	152.3	154.2
	9186	117.7	123.7	130.3	139.8	145.6	154.5	160.8	167.8	174.4	178.3	176.8
	9187	117.8	123.8	130.1	138.0	146.1	148.4	155.4	160.0	161.1	165.8	167.7
	9188	114.3	120.4	123.7	130.3	134.7	140.8	144.5	150.6	152.5	157.5	159.4
	9189	119.9	125.8	129.9	135.1	138.1	146.7	150.1	153.9	157.1	155.6	164.6
	9190	115.9	124.7	123.4	131.2	138.1	145.0	146.4	153.3	156.6	161.2	161.2

Dose Level: Group 1 – 0 mg/kg/day; Group 2 – 0.0025 mg/kg/day Ethinyl Estradiol; Group 3 – 0.005 mg/kg/day Ethinyl Estradiol
Group 4 – 12.5 mg/kg/day Methoxychlor; Group 5 – 25 mg/kg/day Methoxychlor; Group 6 – 50 mg/kg/day Methoxychlor
Group 7 – 25 mg/kg/day Phenobarbital; Group 8 – 50 mg/kg/day Phenobarbital; Group 9 – 100 mg/kg/day Phenobarbital
F = Females
. = No Data

Appendix 8 (continued)
F_1 Individual Body Weights (Grams)
Pubertal Toxicity Study of Vinclozolin, Flutamide and Phenobarbital in Male Sprague Dawley Rats and Methoxychlor, Ethinyl Estradiol and Phenobarbital in Female Sprague Dawley Rats When Administered in Corn Oil by Oral Gavage

		Postnatal Day										
Group Sex	Animal Number	22	23	24	25	26	27	28	29	30	31	32
3F	9191	63.5	69.9	76.5	83.0	89.1	94.9	99.6	107.4	115.8	122.1	130.7
	9192	64.3	71.0	77.6	83.8	90.8	95.1	103.4	109.9	117.4	128.4	136.3
	9193	60.9	67.5	73.5	78.4	83.8	88.7	95.3	100.8	106.5	109.8	118.2
	9194	62.2	68.5	73.7	80.0	86.6	92.9	101.9	106.5	113.8	118.7	125.7
	9195	61.0	67.2	71.2	77.2	82.1	86.9	93.0	97.8	104.6	111.5	117.7
	9196	60.2	65.8	72.6	77.7	84.0	91.3	99.2	102.4	108.5	115.0	121.7
	9197	58.9	65.3	71.8	78.6	84.0	90.6	96.0	101.4	107.2	115.6	122.1
	9198	58.1	63.9	69.2	75.8	81.9	87.8	93.7	99.5	105.0	112.2	118.4
	9199	56.7	63.7	69.3	74.7	79.8	84.5	91.2	97.2	101.0	108.8	112.5
	9200	55.8	60.7	66.9	71.3	77.7	82.4	88.9	92.7	100.0	105.2	111.9
	9201	54.8	61.1	67.0	71.1	75.4	79.4	86.5	90.3	95.6	100.6	107.6
	9202	54.3	61.1	65.9	70.8	76.5	79.6	87.4	90.9	95.8	102.1	106.1
	9203	53.6	58.0	63.8	69.6	73.9	78.9	84.7	89.1	93.9	100.7	107.2
	9204	53.3	58.4	65.1	69.9	74.2	80.3	86.1	89.5	96.8	103.8	111.0
	9205	52.8	61.3	68.5	71.9	77.7	82.3	89.7	94.0	100.9	107.1	113.8

Dose Level: Group 1 – 0 mg/kg/day; Group 2 – 0.0025 mg/kg/day Ethinyl Estradiol; Group 3 – 0.005 mg/kg/day Ethinyl Estradiol
Group 4 – 12.5 mg/kg/day Methoxychlor; Group 5 – 25 mg/kg/day Methoxychlor; Group 6 – 50 mg/kg/day Methoxychlor
Group 7 – 25 mg/kg/day Phenobarbital; Group 8 – 50 mg/kg/day Phenobarbital; Group 9 – 100 mg/kg/day Phenobarbital
F = Females
. = No Data

Appendix 8 (continued)
F_1 Individual Body Weights (Grams)
Pubertal Toxicity Study of Vinclozolin, Flutamide and Phenobarbital in Male Sprague Dawley Rats and Methoxychlor, Ethinyl Estradiol and Phenobarbital in Female Sprague Dawley Rats When Administered in Corn Oil by Oral Gavage

		Postnatal Day										
Group Sex	Animal Number	33	34	35	36	37	38	39	40	41	42	43
3F	9191	136.0	145.7	151.8	158.6	162.8	169.1	175.1	183.2	183.8	182.6	.
	9192	143.0	153.5	160.2	170.2	180.2	188.5	188.3	194.7	202.8	209.6	.
	9193	125.4	133.5	140.8	147.0	151.9	152.7	160.8	167.3	172.1	173.1	.
	9194	134.3	142.3	148.9	153.3	158.8	161.9	169.4	171.7	177.3	176.8	.
	9195	123.5	131.5	137.1	144.2	147.7	156.1	161.4	161.6	161.7	170.4	.
	9196	128.0	134.7	140.1	144.1	150.9	158.1	157.9	158.8	167.3	171.6	.
	9197	128.4	137.5	145.3	148.9	155.3	161.7	167.7	172.8	175.3	179.9	.
	9198	123.4	131.2	138.7	137.1	146.8	152.7	158.9	155.7	155.1	168.2	.
	9199	116.0	126.3	130.6	138.6	141.0	149.3	154.8	157.8	159.8	168.3	170.2
	9200	119.6	125.7	134.2	141.2	149.3	156.8	162.7	165.0	169.4	178.7	183.3
	9201	113.6	121.3	128.3	129.9	134.7	143.8	148.7	152.6	152.7	150.7	157.0
	9202	112.9	118.2	124.0	126.6	135.7	139.9	144.1	145.2	151.8	158.5	161.1
	9203	109.8	119.4	123.7	129.7	133.1	138.8	143.9	148.1	145.6	152.7	157.3
	9204	102.7	122.2	128.1	133.7	141.0	140.6	148.2	154.2	156.6	157.3	163.8
	9205	118.2	127.2	134.6	140.0	145.9	151.7	158.6	166.7	169.8	174.8	173.7

Dose Level: Group 1 – 0 mg/kg/day; Group 2 – 0.0025 mg/kg/day Ethinyl Estradiol; Group 3 – 0.005 mg/kg/day Ethinyl Estradiol
Group 4 – 12.5 mg/kg/day Methoxychlor; Group 5 – 25 mg/kg/day Methoxychlor; Group 6 – 50 mg/kg/day Methoxychlor
Group 7 – 25 mg/kg/day Phenobarbital; Group 8 – 50 mg/kg/day Phenobarbital; Group 9 – 100 mg/kg/day Phenobarbital
F = Females
. = No Data

Appendix 8 (continued)
F_1 Individual Body Weights (Grams)
Pubertal Toxicity Study of Vinclozolin, Flutamide and Phenobarbital in Male Sprague Dawley Rats and Methoxychlor, Ethinyl Estradiol and Phenobarbital in Female Sprague Dawley Rats When Administered in Corn Oil by Oral Gavage

		Postnatal Day										
Group Sex	Animal Number	22	23	24	25	26	27	28	29	30	31	32
4F	9206	66.2	71.8	78.2	85.2	91.4	95.6	100.3	107.0	111.6	118.1	121.8
	9207	64.7	71.7	78.0	85.9	91.8	98.2	106.9	115.8	123.7	131.8	140.5
	9208	63.9	68.6	73.0	78.7	82.7	88.9	96.6	102.4	107.8	113.3	121.8
	9209	62.0	67.9	76.5	80.0	88.2	93.0	100.5	105.6	112.1	121.8	124.9
	9210	60.5	64.8	69.9	74.9	81.0	86.1	92.1	97.9	104.2	109.7	117.7
	9211	59.6	65.6	70.9	75.9	83.6	88.2	93.0	99.6	105.7	109.0	114.8
	9212	58.5	66.1	71.0	76.7	81.2	86.4	93.4	98.2	103.8	110.5	115.2
	9213	57.4	64.6	69.5	76.4	79.6	83.7	90.9	96.3	100.4	107.4	112.4
	9214	58.6	65.0	71.4	79.2	82.8	88.0	93.2	99.2	105.8	111.9	115.8
	9215	57.0	63.3	69.8	75.1	80.8	86.7	92.6	97.7	102.7	107.5	112.6
	9216	54.3	61.1	66.2	69.0	74.3	80.2	84.1	88.3	94.0	97.0	101.9
	9217	56.5	62.9	67.7	74.3	79.5	84.6	92.6	96.3	100.8	103.6	112.9
	9218	55.9	63.5	68.1	74.2	79.1	85.4	91.9	99.6	104.0	111.2	119.6
	9219	57.0	64.4	68.9	74.4	79.4	83.9	91.6	97.4	105.5	110.2	119.1
	9220	52.5	59.1	64.4	69.1	72.3	77.2	83.0	88.4	92.7	99.2	105.8

Dose Level: Group 1 – 0 mg/kg/day; Group 2 – 0.0025 mg/kg/day Ethinyl Estradiol; Group 3 – 0.005 mg/kg/day Ethinyl Estradiol
Group 4 – 12.5 mg/kg/day Methoxychlor; Group 5 – 25 mg/kg/day Methoxychlor; Group 6 – 50 mg/kg/day Methoxychlor
Group 7 – 25 mg/kg/day Phenobarbital; Group 8 – 50 mg/kg/day Phenobarbital; Group 9 – 100 mg/kg/day Phenobarbital
F = Females
. = No Data

Appendix 8 (continued)
F_1 Individual Body Weights (Grams)
Pubertal Toxicity Study of Vinclozolin, Flutamide and Phenobarbital in Male Sprague Dawley Rats and Methoxychlor, Ethinyl Estradiol and Phenobarbital in Female Sprague Dawley Rats When Administered in Corn Oil by Oral Gavage

		Postnatal Day										
Group Sex	Animal Number	33	34	35	36	37	38	39	40	41	42	43
4F	9206	127.8	117.8	138.7	144.0	145.8	154.6	157.7	161.4	162.5	164.3	.
	9207	144.7	129.0	153.7	157.0	162.8	173.6	175.2	180.9	181.9	184.5	.
	9208	129.4	117.9	138.1	139.8	152.0	157.1	163.7	166.6	164.0	172.6	.
	9209	129.8	138.6	146.7	152.9	155.3	163.8	167.3	175.7	176.3	177.1	.
	9210	123.0	130.8	138.0	139.7	147.2	152.1	158.4	163.0	160.8	170.3	.
	9211	122.4	130.8	136.6	138.0	146.1	150.5	157.1	163.1	161.3	170.5	.
	9212	120.2	130.2	135.9	140.9	146.3	153.5	160.2	163.1	166.8	167.2	.
	9213	117.8	125.8	131.2	136.5	140.8	148.7	149.4	153.3	161.0	164.4	.
	9214	123.3	133.3	141.0	144.1	146.8	157.7	163.3	167.4	171.3	168.3	173.7
	9215	119.4	125.6	130.3	136.1	141.8	145.3	149.6	152.0	153.4	158.3	165.7
	9216	108.6	113.3	117.2	123.6	128.8	132.4	133.4	139.8	143.0	148.2	152.9
	9217	118.8	127.4	131.0	132.4	141.1	146.4	150.6	150.5	146.8	159.1	163.1
	9218	125.7	132.9	131.4	142.0	150.0	155.5	151.4	160.5	162.7	166.9	169.5
	9219	124.3	131.6	139.3	141.4	142.1	154.8	152.9	157.9	161.5	160.1	169.5
	9220	110.4	115.8	119.6	128.3	132.2	135.7	140.4	145.0	151.4	155.0	152.5

Dose Level: Group 1 – 0 mg/kg/day; Group 2 – 0.0025 mg/kg/day Ethinyl Estradiol; Group 3 – 0.005 mg/kg/day Ethinyl Estradiol
Group 4 – 12.5 mg/kg/day Methoxychlor; Group 5 – 25 mg/kg/day Methoxychlor; Group 6 – 50 mg/kg/day Methoxychlor
Group 7 – 25 mg/kg/day Phenobarbital; Group 8 – 50 mg/kg/day Phenobarbital; Group 9 – 100 mg/kg/day Phenobarbital
F = Females
. = No Data

Appendix 8 (continued)
F_1 Individual Body Weights (Grams)
Pubertal Toxicity Study of Vinclozolin, Flutamide and Phenobarbital in Male Sprague Dawley Rats and Methoxychlor, Ethinyl Estradiol and Phenobarbital in Female Sprague Dawley Rats When Administered in Corn Oil by Oral Gavage

		Postnatal Day										
Group Sex	Animal Number	22	23	24	25	26	27	28	29	30	31	32
5F	9221	64.1	69.2	75.0	81.1	86.0	92.8	97.9	103.2	111.1	117.8	124.9
	9222	65.7	70.5	79.0	84.0	91.3	96.5	102.4	110.7	118.0	123.0	130.6
	9223	60.5	67.6	73.6	78.8	86.4	90.5	95.7	100.9	109.1	115.8	122.4
	9224	61.3	66.9	72.1	77.9	82.3	87.5	92.4	98.5	105.9	110.8	115.6
	9225	61.1	68.5	73.7	81.6	87.2	93.2	98.7	103.9	109.9	117.7	123.7
	9226	60.3	67.2	72.2	79.8	85.6	91.0	97.2	101.4	109.5	115.9	120.4
	9227	59.6	67.6	75.0	81.1	85.1	91.1	98.1	101.9	106.0	116.7	123.4
	9228	59.2	64.3	69.6	75.2	80.4	85.6	92.8	98.9	106.7	112.6	121.4
	9229	57.5	62.4	68.6	74.0	80.3	91.0	92.5	97.4	102.9	108.1	112.6
	9230	47.3	53.2	59.1	63.7	68.1	71.7	77.3	81.4	87.7	93.5	100.9
	9231	56.9	64.7	71.5	76.8	83.6	88.4	92.9	99.1	107.4	113.7	119.8
	9232	56.6	64.0	69.4	74.9	79.1	83.8	91.0	94.3	102.4	107.1	113.8
	9233	55.5	60.4	66.3	71.1	76.8	80.7	85.8	91.3	97.5	101.0	107.8
	9234	54.7	60.5	66.5	72.7	77.7	84.0	90.4	95.7	104.3	107.5	114.6
	9235	54.5	59.8	65.3	71.2	76.2	81.8	87.5	90.1	97.7	100.3	104.6

Dose Level: Group 1 - 0 mg/kg/day; Group 2 - 0.0025 mg/kg/day Ethinyl Estradiol; Group 3 - 0.005 mg/kg/day Ethinyl Estradiol
Group 4 - 12.5 mg/kg/day Methoxychlor; Group 5 - 25 mg/kg/day Methoxychlor; Group 6 - 50 mg/kg/day Methoxychlor
Group 7 - 25 mg/kg/day Phenobarbital; Group 8 - 50 mg/kg/day Phenobarbital; Group 9 - 100 mg/kg/day Phenobarbital
F = Females
. = No Data

Appendix 8 (continued)
F_1 Individual Body Weights (Grams)
Pubertal Toxicity Study of Vinclozolin, Flutamide and Phenobarbital in Male Sprague Dawley Rats and Methoxychlor, Ethinyl Estradiol and Phenobarbital in Female Sprague Dawley Rats When Administered in Corn Oil by Oral Gavage

Group Sex	Animal Number	Postnatal Day 33	34	35	36	37	38	39	40	41	42	43
5F	9221	131.1	138.9	144.7	154.1	161.4	167.0	170.2	178.4	180.8	189.4	.
	9222	135.3	147.0	148.6	161.0	168.1	172.9	177.9	186.4	191.1	196.6	.
	9223	122.9	133.5	138.8	147.8	156.0	160.9	167.8	175.1	177.4	177.4	.
	9224	122.0	129.8	135.4	141.2	144.4	154.6	156.6	164.4	165.0	162.2	.
	9225	127.2	134.7	142.4	146.9	154.5	161.4	166.1	167.3	175.9	175.5	.
	9226	125.0	134.2	141.6	147.9	154.8	161.0	165.2	170.6	174.7	183.1	.
	9227	129.1	136.6	138.0	150.6	155.5	162.6	162.5	171.1	173.2	180.1	.
	9228	121.0	128.8	134.4	140.0	149.8	153.3	155.6	163.0	166.6	172.4	.
	9229	119.4	124.7	127.6	135.3	142.8	148.4	150.5	149.5	157.3	161.2	166.4
	9230	105.3	112.1	118.0	124.1	129.3	136.9	141.5	144.9	147.0	150.6	156.2
	9231	126.8	133.5	139.6	148.0	154.0	159.0	163.3	164.4	170.4	176.7	177.0
	9232	118.6	125.5	130.8	135.9	142.7	148.6	150.6	152.5	158.2	162.2	165.8
	9233	112.7	109.9	122.1	110.1	127.4	137.0	140.8	145.2	149.7	151.7	159.2
	9234	119.4	108.7	133.7	119.2	141.1	143.5	151.6	154.6	158.9	161.7	160.8
	9235	114.6	106.6	123.8	113.4	136.0	142.8	143.3	153.5	160.7	164.6	165.9

Dose Level: Group 1 – 0 mg/kg/day; Group 2 – 0.0025 mg/kg/day Ethinyl Estradiol; Group 3 – 0.005 mg/kg/day Ethinyl Estradiol
Group 4 – 12.5 mg/kg/day Methoxychlor; Group 5 – 25 mg/kg/day Methoxychlor; Group 6 – 50 mg/kg/day Methoxychlor
Group 7 – 25 mg/kg/day Phenobarbital; Group 8 – 50 mg/kg/day Phenobarbital; Group 9 – 100 mg/kg/day Phenobarbital
F = Females
. = No Data

Appendix 8 (continued)
F_1 Individual Body Weights (Grams)
Pubertal Toxicity Study of Vinclozolin, Flutamide and Phenobarbital in Male Sprague Dawley Rats and Methoxychlor, Ethinyl Estradiol and Phenobarbital in Female Sprague Dawley Rats When Administered in Corn Oil by Oral Gavage

		Postnatal Day										
Group Sex	Animal Number	22	23	24	25	26	27	28	29	30	31	32
6F	9236	61.3	68.3	72.6	80.3	84.6	88.9	96.4	102.8	105.6	112.8	118.4
	9237	62.6	67.0	72.8	77.4	83.3	87.4	94.4	96.9	102.0	107.2	115.3
	9238	63.0	69.6	73.8	81.4	85.9	91.1	97.7	102.1	110.4	113.0	117.8
	9239	62.7	66.5	71.7	76.6	82.2	86.6	93.8	98.3	105.1	109.3	113.7
	9240	60.5	66.6	71.9	78.7	83.2	89.4	95.6	102.5	108.6	113.4	120.1
	9241	57.6	64.2	68.8	73.9	80.2	87.0	92.5	97.9	103.8	110.0	116.2
	9242	57.1	63.1	68.8	73.8	79.0	85.4	93.1	98.5	104.7	108.6	113.5
	9243	58.6	64.4	72.4	77.6	83.7	90.5	96.7	101.3	108.1	111.4	117.2
	9244	55.9	61.6	69.0	73.5	78.5	85.3	92.5	95.8	103.8	110.9	114.3
	9245	56.1	62.2	66.6	73.8	79.7	87.6	93.1	99.4	106.3	113.5	120.9
	9246	55.1	60.9	64.8	72.0	75.5	81.7	88.8	93.7	101.4	106.2	111.3
	9247	54.2	60.1	63.8	70.9	74.0	80.2	86.6	92.2	98.4	104.5	110.8
	9248	53.9	58.3	64.5	70.6	75.6	79.8	87.1	91.4	97.4	102.7	109.1
	9249	52.3	58.1	61.7	69.0	72.2	76.7	82.2	86.6	91.5	97.8	102.1
	9250	51.2	56.1	61.0	67.7	71.2	76.2	83.0	87.4	92.3	97.9	103.8

Dose Level: Group 1 - 0 mg/kg/day; Group 2 - 0.0025 mg/kg/day Ethinyl Estradiol; Group 3 - 0.005 mg/kg/day Ethinyl Estradiol
Group 4 - 12.5 mg/kg/day Methoxychlor; Group 5 - 25 mg/kg/day Methoxychlor; Group 6 - 50 mg/kg/day Methoxychlor
Group 7 - 25 mg/kg/day Phenobarbital; Group 8 - 50 mg/kg/day Phenobarbital; Group 9 - 100 mg/kg/day Phenobarbital
F = Females
. = No Data

Appendix 8 (continued)
F_1 Individual Body Weights (Grams)
Pubertal Toxicity Study of Vinclozolin, Flutamide and Phenobarbital in Male Sprague Dawley Rats and Methoxychlor, Ethinyl Estradiol and Phenobarbital in Female Sprague Dawley Rats When Administered in Corn Oil by Oral Gavage

		Postnatal Day										
Group Sex	Animal Number	33	34	35	36	37	38	39	40	41	42	43
6F	9236	121.0	127.5	134.0	135.5	138.4	146.6	148.6	153.3	155.6	159.5	.
	9237	118.2	127.6	134.1	135.8	143.1	148.9	151.8	158.3	160.4	165.9	.
	9238	123.7	133.3	137.8	141.9	151.8	157.4	162.4	168.7	172.1	174.8	.
	9239	119.9	126.4	131.1	133.5	143.4	147.3	151.4	154.1	159.1	164.0	.
	9240	123.3	130.0	134.0	139.7	145.8	151.1	154.0	157.8	160.6	163.9	.
	9241	119.3	129.6	135.7	140.4	142.0	147.0	154.8	156.1	161.0	165.4	.
	9242	121.9	129.3	133.4	136.6	144.9	146.1	152.7	154.3	159.3	163.0	.
	9243	125.2	132.9	138.3	144.8	151.1	153.5	153.0	161.9	166.1	168.8	.
	9244	119.7	127.0	133.0	141.0	147.4	152.7	155.6	162.7	165.5	170.4	170.6
	9245	126.6	131.9	139.6	144.6	151.6	157.7	161.3	164.3	170.5	174.2	179.9
	9246	116.9	122.3	130.0	136.4	143.5	151.1	150.1	154.0	155.5	160.7	166.6
	9247	116.7	120.2	127.3	131.8	138.5	143.9	144.9	150.3	151.1	157.9	161.6
	9248	116.2	120.7	125.7	133.0	136.9	146.6	151.4	152.2	158.7	162.2	169.8
	9249	108.7	114.1	119.7	122.8	130.6	135.1	141.4	146.2	149.8	153.5	158.0
	9250	108.5	116.7	119.3	127.5	128.9	134.9	138.5	143.6	147.2	148.9	152.0

Dose Level: Group 1 – 0 mg/kg/day; Group 2 – 0.0025 mg/kg/day Ethinyl Estradiol; Group 3 – 0.005 mg/kg/day Ethinyl Estradiol
Group 4 – 12.5 mg/kg/day Methoxychlor; Group 5 – 25 mg/kg/day Methoxychlor; Group 6 – 50 mg/kg/day Methoxychlor
Group 7 – 25 mg/kg/day Phenobarbital; Group 8 – 50 mg/kg/day Phenobarbital; Group 9 – 100 mg/kg/day Phenobarbital
F = Females
. = No Data

Appendix 8 (continued)
F_1 Individual Body Weights (Grams)
Pubertal Toxicity Study of Vinclozolin, Flutamide and Phenobarbital in Male Sprague Dawley Rats and Methoxychlor, Ethinyl Estradiol and Phenobarbital in Female Sprague Dawley Rats When Administered in Corn Oil by Oral Gavage

		Postnatal Day										
Group Sex	Animal Number	22	23	24	25	26	27	28	29	30	31	32
7F	9251	63.8	74.1	79.8	86.5	91.6	98.3	106.1	110.3	117.6	126.4	135.4
	9252	52.6	60.1	67.8	71.9	77.8	82.3	89.4	93.8	100.6	108.0	115.1
	9253	59.8	69.6	75.9	80.5	84.4	90.7	97.6	102.8	110.4	117.6	122.9
	9254	59.3	67.4	72.6	77.2	81.8	87.4	93.2	98.0	103.9	109.9	116.6
	9255	57.9	65.3	70.6	77.6	83.2	89.2	94.9	100.9	106.6	114.0	117.3
	9256	58.8	66.0	72.9	77.4	82.3	86.8	93.5	98.9	104.1	113.4	117.2
	9257	58.3	66.7	74.0	77.9	85.8	90.1	95.8	100.5	108.6	116.6	122.8
	9258	55.8	64.9	68.5	73.5	78.7	84.5	91.4	96.2	102.6	111.3	116.4
	9259	56.9	64.1	72.4	77.6	82.5	89.7	96.6	102.8	110.9	119.0	126.3
	9260	55.7	62.2	66.3	73.6	78.7	84.4	90.6	95.9	103.5	105.0	115.1
	9261	55.4	60.3	67.2	72.1	77.1	82.5	86.9	92.5	98.1	104.6	109.3
	9262	53.5	61.2	65.5	69.2	76.4	81.7	86.3	90.4	98.4	104.9	108.0
	9263	54.4	61.7	64.9	70.1	74.2	79.5	85.8	89.8	96.6	102.6	104.8
	9264	54.3	63.5	67.2	73.1	77.7	83.0	89.2	94.7	100.4	105.7	114.0
	9265	54.0	62.5	66.4	71.5	76.2	83.3	89.0	94.1	100.9	107.0	113.3

Dose Level: Group 1 – 0 mg/kg/day; Group 2 – 0.0025 mg/kg/day Ethinyl Estradiol; Group 3 – 0.005 mg/kg/day Ethinyl Estradiol
Group 4 – 12.5 mg/kg/day Methoxychlor; Group 5 – 25 mg/kg/day Methoxychlor; Group 6 – 50 mg/kg/day Methoxychlor
Group 7 – 25 mg/kg/day Phenobarbital; Group 8 – 50 mg/kg/day Phenobarbital; Group 9 – 100 mg/kg/day Phenobarbital
F = Females
. = No Data

Appendix 8 (continued)
F_1 Individual Body Weights (Grams)
Pubertal Toxicity Study of Vinclozolin, Flutamide and Phenobarbital in Male Sprague Dawley Rats and Methoxychlor, Ethinyl Estradiol and Phenobarbital in Female Sprague Dawley Rats When Administered in Corn Oil by Oral Gavage

		Postnatal Day										
Group Sex	Animal Number	33	34	35	36	37	38	39	40	41	42	43
7F	9251	125.2	147.8	155.9	160.5	169.5	175.3	181.0	188.6	194.0	204.0	.
	9252	106.0	125.3	131.2	133.9	140.2	148.0	151.0	157.0	157.9	166.8	.
	9253	113.3	134.9	137.9	142.7	146.2	148.5	158.0	160.6	163.8	163.7	.
	9254	121.7	129.1	134.8	141.8	143.2	150.9	157.8	160.2	161.6	163.2	.
	9255	126.1	134.3	142.6	146.0	150.5	158.9	166.1	168.1	171.3	181.9	.
	9256	124.9	131.4	139.9	145.0	153.4	160.1	160.2	167.9	170.9	180.2	.
	9257	128.2	134.6	136.4	151.0	158.5	161.1	168.3	174.0	181.0	181.3	.
	9258	120.3	128.5	131.6	141.9	145.1	146.4	152.7	157.2	161.0	163.7	.
	9259	133.7	140.4	138.7	152.3	159.9	167.1	166.3	173.6	178.7	185.3	186.1
	9260	121.1	128.2	133.9	140.4	141.3	149.8	156.2	161.2	159.2	170.9	174.1
	9261	117.7	126.4	131.3	138.2	144.9	150.1	156.4	162.3	162.0	161.3	169.4
	9262	116.3	126.0	130.0	138.0	137.3	146.2	153.8	158.4	158.9	167.9	170.8
	9263	114.2	120.4	123.8	132.5	130.9	138.0	141.0	145.2	147.7	142.7	150.9
	9264	119.2	127.1	131.6	136.9	143.4	146.7	154.5	158.3	163.2	164.0	160.8
	9265	120.6	117.8	122.9	132.3	139.9	144.1	144.3	151.4	154.9	159.0	153.5

Dose Level: Group 1 – 0 mg/kg/day; Group 2 – 0.0025 mg/kg/day Ethinyl Estradiol; Group 3 – 0.005 mg/kg/day Ethinyl Estradiol
Group 4 – 12.5 mg/kg/day Methoxychlor; Group 5 – 25 mg/kg/day Methoxychlor; Group 6 – 50 mg/kg/day Methoxychlor
Group 7 – 25 mg/kg/day Phenobarbital; Group 8 – 50 mg/kg/day Phenobarbital; Group 9 – 100 mg/kg/day Phenobarbital
F = Females
. = No Data

Appendix 8 (continued)
F_1 Individual Body Weights (Grams)
Pubertal Toxicity Study of Vinclozolin, Flutamide and Phenobarbital in Male Sprague Dawley Rats and Methoxychlor, Ethinyl Estradiol and Phenobarbital in Female Sprague Dawley Rats When Administered in Corn Oil by Oral Gavage

		Postnatal Day										
Group Sex	Animal Number	22	23	24	25	26	27	28	29	30	31	32
8F	9266	63.1	72.2	79.8	85.4	90.9	97.5	105.9	112.8	118.3	124.2	132.8
	9267	63.1	68.7	74.5	79.8	84.6	91.4	94.4	101.1	107.5	112.6	120.4
	9268	62.9	68.7	75.2	81.9	87.1	94.5	100.2	105.7	111.8	120.5	128.4
	9269	61.2	71.0	77.6	82.7	88.2	95.8	102.7	108.9	116.0	121.6	129.0
	9270	60.6	69.5	75.9	81.5	86.2	92.4	98.4	103.4	109.3	114.3	120.9
	9271	62.0	72.5	77.2	82.2	87.5	92.5	99.8	105.7	113.6	119.5	127.2
	9272	58.4	67.1	72.2	76.7	80.4	88.1	94.7	100.1	107.1	112.8	120.2
	9273	58.1	67.0	72.2	76.0	79.2	85.4	91.1	94.9	100.8	107.7	114.1
	9274	59.2	67.9	71.6	76.0	81.9	86.8	93.4	98.7	105.5	108.6	118.3
	9275	54.2	61.5	67.2	74.1	76.8	83.7	88.1	93.3	101.4	109.2	116.7
	9276	52.0	57.4	65.2	69.1	73.8	80.3	84.6	88.4	95.7	99.5	105.4
	9277	53.0	59.4	66.1	70.2	75.3	80.2	84.2	90.8	97.2	101.6	108.6
	9278	54.1	62.9	67.3	71.7	76.1	80.2	86.6	91.7	98.4	102.5	108.1
	9279	52.5	60.4	66.3	72.4	77.8	83.6	90.3	95.6	102.4	111.4	115.1
	9280	55.0	64.0	69.2	72.9	77.1	82.1	87.3	91.9	96.6	102.7	109.3

Dose Level: Group 1 – 0 mg/kg/day; Group 2 – 0.0025 mg/kg/day Ethinyl Estradiol; Group 3 – 0.005 mg/kg/day Ethinyl Estradiol
Group 4 – 12.5 mg/kg/day Methoxychlor; Group 5 – 25 mg/kg/day Methoxychlor; Group 6 – 50 mg/kg/day Methoxychlor
Group 7 – 25 mg/kg/day Phenobarbital; Group 8 – 50 mg/kg/day Phenobarbital; Group 9 – 100 mg/kg/day Phenobarbital
F = Females
. = No Data

Appendix 8 (continued)
F_1 Individual Body Weights (Grams)
Pubertal Toxicity Study of Vinclozolin, Flutamide and Phenobarbital in Male Sprague Dawley Rats and Methoxychlor, Ethinyl Estradiol and Phenobarbital in Female Sprague Dawley Rats When Administered in Corn Oil by Oral Gavage

		Postnatal Day										
Group Sex	Animal Number	33	34	35	36	37	38	39	40	41	42	43
8F	9266	141.6	149.9	156.9	157.2	165.7	172.4	179.4	182.0	186.9	195.0	.
	9267	127.7	134.0	137.6	140.8	148.0	152.6	156.3	161.0	161.8	172.1	.
	9268	135.1	144.9	149.2	156.3	163.5	169.2	175.0	178.8	185.0	184.2	.
	9269	136.8	147.1	148.6	151.6	161.4	164.4	170.2	174.4	170.4	181.1	.
	9270	129.2	136.5	141.2	147.5	154.0	162.7	166.3	175.3	176.1	187.4	.
	9271	135.4	145.9	149.0	148.8	160.5	168.2	172.4	170.6	181.0	188.3	.
	9272	125.3	132.3	139.6	145.2	147.1	155.5	162.9	166.6	167.4	177.9	.
	9273	119.0	127.2	133.9	137.3	143.9	150.0	157.4	160.9	167.2	171.5	.
	9274	123.1	130.0	130.2	138.0	142.6	149.9	145.4	154.3	156.6	159.6	157.4
	9275	104.8	126.4	132.9	138.6	145.0	151.7	156.9	164.6	167.7	173.6	175.4
	9276	91.4	99.0	109.3	115.6	125.5	129.1	135.9	142.7	148.4	155.7	159.0
	9277	102.0	122.7	128.5	134.0	143.8	150.5	157.8	158.8	165.6	170.7	177.8
	9278	114.0	115.9	125.0	126.2	134.7	139.3	145.3	147.0	151.4	154.3	159.6
	9279	124.6	130.0	138.3	146.2	155.5	156.9	165.1	171.3	178.4	171.9	185.6
	9280	113.4	120.5	124.6	128.6	136.0	139.7	144.7	146.6	149.5	153.3	158.2

Dose Level: Group 1 – 0 mg/kg/day; Group 2 – 0.0025 mg/kg/day Ethinyl Estradiol; Group 3 – 0.005 mg/kg/day Ethinyl Estradiol
Group 4 – 12.5 mg/kg/day Methoxychlor; Group 5 – 25 mg/kg/day Methoxychlor; Group 6 – 50 mg/kg/day Methoxychlor
Group 7 – 25 mg/kg/day Phenobarbital; Group 8 – 50 mg/kg/day Phenobarbital; Group 9 – 100 mg/kg/day Phenobarbital
F = Females
. = No Data

Appendix 8 (continued)
F_1 Individual Body Weights (Grams)
Pubertal Toxicity Study of Vinclozolin, Flutamide and Phenobarbital in Male Sprague Dawley Rats and Methoxychlor, Ethinyl Estradiol and Phenobarbital in Female Sprague Dawley Rats When Administered in Corn Oil by Oral Gavage

Group Sex	Animal Number	Postnatal Day 22	23	24	25	26	27	28	29	30	31	32
9F	9281	66.6	75.0	81.2	83.4	88.0	96.6	103.0	107.0	114.4	119.3	128.6
	9282	63.5	68.8	72.9	80.1	83.2	88.8	91.9	97.0	104.9	109.5	115.2
	9283	60.9	65.5	72.8	80.4	83.6	88.6	93.9	102.5	101.8	114.8	123.0
	9284	60.5	69.8	74.6	78.8	83.6	89.6	92.7	103.0	107.2	111.9	119.8
	9285	58.8	65.0	69.2	74.3	77.4	84.2	90.8	93.6	101.0	108.4	115.1
	9286	59.8	68.3	74.3	79.0	84.4	84.0	89.3	92.0	96.7	106.2	111.5
	9287	59.6	66.3	73.1	77.2	80.7	86.3	91.8	99.0	103.5	112.7	119.5
	9288	57.0	60.7	63.6	71.4	73.6	77.7	82.1	89.1	93.6	99.6	105.2
	9289	56.2	63.6	68.3	73.3	76.6	82.5	87.7	96.0	100.8	106.6	112.4
	9290	56.6	64.8	68.9	75.3	79.8	86.5	91.5	99.4	105.5	111.9	120.0
	9291	55.2	60.1	62.1	68.7	71.8	75.8	80.9	90.6	96.0	99.9	103.3
	9292	55.0	62.8	65.9	71.5	74.0	81.2	85.3	91.4	96.8	102.0	106.8
	9293	54.4	60.8	66.8	69.1	72.8	79.6	83.4	90.7	96.0	102.1	107.5
	9294	55.2	63.4	67.0	69.2	74.4	79.8	85.7	91.3	96.4	103.2	110.6
	9295	52.8	50.4	58.2	64.5	67.1	73.6	79.1	84.7	88.5	96.8	102.5

Dose Level: Group 1 - 0 mg/kg/day; Group 2 - 0.0025 mg/kg/day Ethinyl Estradiol; Group 3 - 0.005 mg/kg/day Ethinyl Estradiol
Group 4 - 12.5 mg/kg/day Methoxychlor; Group 5 - 25 mg/kg/day Methoxychlor; Group 6 - 50 mg/kg/day Methoxychlor
Group 7 - 25 mg/kg/day Phenobarbital; Group 8 - 50 mg/kg/day Phenobarbital; Group 9 - 100 mg/kg/day Phenobarbital
F = Females
. = No Data

Appendix 8 (continued)
F_1 Individual Body Weights (Grams)
Pubertal Toxicity Study of Vinclozolin, Flutamide and Phenobarbital in Male Sprague Dawley Rats and Methoxychlor, Ethinyl Estradiol and Phenobarbital in Female Sprague Dawley Rats When Administered in Corn Oil by Oral Gavage

		Postnatal Day										
Group Sex	Animal Number	33	34	35	36	37	38	39	40	41	42	43
9F	9281	136.7	146.4	154.6	156.0	164.8	171.9	183.5	188.6	194.5	184.9	.
	9282	121.7	130.8	135.4	141.2	141.5	151.3	158.4	162.1	163.5	156.6	.
	9283	129.8	137.9	141.4	148.6	156.5	163.4	170.7	175.9	178.2	180.7	.
	9284	122.9	132.8	135.7	139.5	146.7	156.1	159.7	165.8	170.0	179.7	.
	9285	119.1	128.4	135.3	139.6	148.9	151.7	159.8	162.5	168.0	173.9	.
	9286	118.0	125.8	129.6	135.6	145.3	149.4	154.5	157.0	162.9	171.0	.
	9287	124.8	134.0	138.4	145.8	154.5	160.7	168.2	172.6	179.0	184.9	.
	9288	113.3	120.4	126.0	130.9	140.4	147.7	153.9	148.9	156.1	160.3	.
	9289	118.5	127.4	131.1	136.4	143.7	147.4	156.0	157.4	159.9	162.3	159.2
	9290	126.4	136.5	141.1	148.0	153.8	160.8	168.1	174.2	179.2	185.2	190.1
	9291	107.9	115.8	118.2	128.7	133.0	131.8	133.3	139.3	140.5	150.5	160.0
	9292	113.0	118.4	120.1	127.3	132.8	136.0	144.3	149.2	152.2	159.9	163.8
	9293	111.3	118.3	123.6	129.6	134.9	136.6	146.6	150.2	158.1	151.5	165.6
	9294	114.3	122.4	126.4	131.8	139.9	146.4	147.8	154.0	158.7	167.8	169.7
	9295	109.9	117.9	121.8	126.7	137.6	143.5	147.9	154.4	162.3	168.1	171.5

Dose Level: Group 1 – 0 mg/kg/day; Group 2 – 0.0025 mg/kg/day Ethinyl Estradiol; Group 3 – 0.005 mg/kg/day Ethinyl Estradiol
Group 4 – 12.5 mg/kg/day Methoxychlor; Group 5 – 25 mg/kg/day Methoxychlor; Group 6 – 50 mg/kg/day Methoxychlor
Group 7 – 25 mg/kg/day Phenobarbital; Group 8 – 50 mg/kg/day Phenobarbital; Group 9 – 100 mg/kg/day Phenobarbital
F = Females
. = No Data

Appendix 9
F_1 Sexual Development Data – Preputial Separation
Pubertal Toxicity Study of Vinclozolin, Flutamide and Phenobarbital in Male Sprague Dawley Rats and Methoxychlor, Ethinyl Estradiol and Phenobarbital in Female Sprague Dawley Rats when Administered in Corn Oil by Oral Gavage

Appendix 9
F_1 Sexual Development Data – Preputial Separation
Pubertal Toxicity Study of Vinclozolin, Flutamide and Phenobarbital in Male Sprague Dawley Rats and Methoxychlor, Ethinyl Estradiol and Phenobarbital in Female Sprague Dawley Rats When Administered in Corn Oil by Oral Gavage

Group 1			Group 2		
Animal Number	Postnatal Day of Preputial Separation	Bodyweight at Preputial Separation (Grams)	Animal Number	Postnatal Day of Preputial Separation	Bodyweight at Preputial Separation (Grams)
9026	42	244.9	9041	41	229.4
9027	39	196.5	9042	42	235.8
9028	40	225.0	9043	42	206.1
9029	40	197.1	9044	40	207.5
9030	40	194.2	9045	42	218.8
9031	41	208.4	9046	42	213.0
9032	42	221.1	9047	40	202.4
9033	42	211.2	9048	39	185.0
9034	41	212.3	9049	42	201.4
9035	44	230.1	9050	45	230.9
9036	42	218.8	9051	40	201.3
9037	39	179.8	9052	42	207.8
9038	40	203.5	9053	42	229.5
9039	42	227.5	9054	42	220.1
9040	43	234.0	9055	42	212.6

Dose Level: Group 1 – 0 mg/kg/day; Group 2 – 25 mg/kg/day Phenobarbital; Group 3 – 50 mg/kg/day Phenobarbital
Group 4 – 100 mg/kg/day Phenobarbital; Group 5 – 10 mg/kg/day Vinclozolin; Group 6 – 30 mg/kg/day Vinclozolin
Group 7 – 100 mg/kg/day Vinclozolin; Group 8 – 25 mg/kg/day Flutamide; Group 9 – 50 mg/kg/day Flutamide
M = Males

Appendix 9 (continued)
F_1 Sexual Development Data – Preputial Separation
Pubertal Toxicity Study of Vinclozolin, Flutamide and Phenobarbital in Male Sprague Dawley Rats and Methoxychlor, Ethinyl Estradiol and Phenobarbital in Female Sprague Dawley Rats When Administered in Corn Oil by Oral Gavage

Group 3			Group 4		
Animal Number	Postnatal Day of Preputial Separation	Bodyweight at Preputial Separation (Grams)	Animal Number	Postnatal Day of Preputial Separation	Bodyweight at Preputial Separation (Grams)
9056	39	218.0	9071	42	217.1
9057	39	237.0	9072	42	244.1
9058	40	206.3	9073	42	198.2
9059	40	197.9	9074	42	225.4
9060	42	229.0	9075	45	246.4
9061	39	203.6	9076	41	195.4
9062	40	187.7	9077	a	a
9063	42	217.2	9078	45	235.1
9064	42	215.0	9079	42	203.7
9065	42	234.5	9080	45	223.3
9066	42	224.3	9081	44	209.6
9067	42	233.4	9082	47	238.1
9068	42	200.4	9083	45	219.8
9069	42	216.1	9084	47	231.3
9070	44	216.3	9085	42	189.8

Dose Level: Group 1 – 0 mg/kg/day; Group 2 – 25 mg/kg/day Phenobarbital; Group 3 – 50 mg/kg/day Phenobarbital
Group 4 – 100 mg/kg/day Phenobarbital; Group 5 – 10 mg/kg/day Vinclozolin; Group 6 – 30 mg/kg/day Vinclozolin
Group 7 – 100 mg/kg/day Vinclozolin; Group 8 – 25 mg/kg/day Flutamide; Group 9 – 50 mg/kg/day Flutamide
M = Males
a = Animal found dead on PND 4

Appendix 9 (continued)
F_1 Sexual Development Data – Preputial Separation
Pubertal Toxicity Study of Vinclozolin, Flutamide and Phenobarbital in Male Sprague Dawley Rats and Methoxychlor, Ethinyl Estradiol and Phenobarbital in Female Sprague Dawley Rats When Administered in Corn Oil by Oral Gavage

Group 5			Group 6		
Animal Number	Postnatal Day of Preputial Separation	Bodyweight at Preputial Separation (Grams)	Animal Number	Postnatal Day of Preputial Separation	Bodyweight at Preputial Separation (Grams)
9086	39	216.7	9101	43	252.5
9087	42	212.0	9102	43	263.6
9088	42	241.7	9103	44	247.3
9089	42	225.0	9104	45	260.5
9090	42	238.8	9105	45	242.9
9091	44	226.8	9106	44	251.4
9092	42	230.1	9107	42	217.4
9093	43	222.6	9108	42	213.6
9094	42	209.7	9109	45	269.5
9095	43	256.8	9110	45	278.3
9096	42	227.2	9111	46	263.1
9097	47	288.3	9112	43	221.5
9098	42	228.3	9113	42	244.3
9099	43	216.5	9114	44	259.4
9100	44	228.8	9115	45	243.5

Dose Level: Group 1 – 0 mg/kg/day; Group 2 – 25 mg/kg/day Phenobarbital; Group 3 – 50 mg/kg/day Phenobarbital
Group 4 – 100 mg/kg/day Phenobarbital; Group 5 – 10 mg/kg/day Vinclozolin; Group 6 – 30 mg/kg/day Vinclozolin
Group 7 – 100 mg/kg/day Vinclozolin; Group 8 – 25 mg/kg/day Flutamide; Group 9 – 50 mg/kg/day Flutamide
M = Males

Appendix 9 (continued)
F_1 Sexual Development Data – Preputial Separation
Pubertal Toxicity Study of Vinclozolin, Flutamide and Phenobarbital in Male Sprague Dawley Rats and Methoxychlor, Ethinyl Estradiol and Phenobarbital in Female Sprague Dawley Rats When Administered in Corn Oil by Oral Gavage

Group 7			Group 8		
Animal Number	Postnatal Day of Preputial Separation	Bodyweight at Preputial Separation (Grams)	Animal Number	Postnatal Day of Preputial Separation	Bodyweight at Preputial Separation (Grams)
9116	46	267.4	9131	53	334.7
9117	49	299.1	9132	.	.
9118	49	307.6	9133	52	308.1
9119	46	266.6	9134	.	.
9120	49	284.2	9135	.	.
9121	46	253.0	9136	.	.
9122	47	248.9	9137	.	.
9123	46	243.2	9138	.	.
9124	47	284.5	9139	54	300.2
9125	49	296.5	9140	54	304.5
9126	48	262.8	9141	54	351.6
9127	48	241.1	9142	53	312.3
9128	49	274.3	9143	.	.
9129	48	226.0	9144	.	.
9130	48	262.2	9145	.	.

Dose Level: Group 1 – 0 mg/kg/day; Group 2 – 25 mg/kg/day Phenobarbital; Group 3 – 50 mg/kg/day Phenobarbital
Group 4 – 100 mg/kg/day Phenobarbital; Group 5 – 10 mg/kg/day Vinclozolin; Group 6 – 30 mg/kg/day Vinclozolin
Group 7 – 100 mg/kg/day Vinclozolin; Group 8 – 25 mg/kg/day Flutamide; Group 9 – 50 mg/kg/day Flutamide
M = Males
. = Preputial separation not completed before scheduled termination.

Appendix 9 (continued)
F_1 Sexual Development Data – Preputial Separation
Pubertal Toxicity Study of Vinclozolin, Flutamide and Phenobarbital in Male Sprague Dawley Rats and Methoxychlor, Ethinyl Estradiol and Phenobarbital in Female Sprague Dawley Rats When Administered in Corn Oil by Oral Gavage

Group 9

Animal Number	Postnatal Day of Preputial Separation	Bodyweight at Preputial Separation (Grams)
9146	.	.
9147	.	.
9148	.	.
9149	.	.
9150	.	.
9151	.	.
9152	.	.
9153	.	.
9154	.	.
9155	.	.
9156	.	.
9157	.	.
9158	.	.
9159	54	311.0
9160	.	.

Dose Level: Group 1 – 0 mg/kg/day; Group 2 – 25 mg/kg/day Phenobarbital; Group 3 – 50 mg/kg/day Phenobarbital
Group 4 – 100 mg/kg/day Phenobarbital; Group 5 – 10 mg/kg/day Vinclozolin; Group 6 – 30 mg/kg/day Vinclozolin
Group 7 – 100 mg/kg/day Vinclozolin; Group 8 – 25 mg/kg/day Flutamide; Group 9 – 50 mg/kg/day Flutamide
M = Males
. = Preputial separation not completed before scheduled termination.

Appendix 10
F_1 Sexual Development Data – Vaginal Opening
Pubertal Toxicity Study of Vinclozolin, Flutamide and Phenobarbital in Male Sprague Dawley Rats and Methoxychlor, Ethinyl Estradiol and Phenobarbital in Female Sprague Dawley Rats when Administered in Corn Oil by Oral Gavage

Appendix 10
F_1 Sexual Development Data – Vaginal Opening
Pubertal Toxicity Study of Vinclozolin, Flutamide and Phenobarbital in Male Sprague Dawley Rats and Methoxychlor, Ethinyl Estradiol and Phenobarbital in Female Sprague Dawley Rats When Administered in Corn Oil by Oral Gavage

Group 1			Group 2		
Animal Number	Postnatal Day of Vaginal Opening	Bodyweight at Vaginal Opening (Grams)	Animal Number	Postnatal Day of Vaginal Opening	Bodyweight at Vaginal Opening (Grams)
9161	33	133.3	9176	30	122.2
9162	31	108.3	9177	32	122.5
9163	32	122.8	9178	32	120.7
9164	33	129.3	9179	30	111.8
9165	31	118.1	9180	38	149.6
9166	32	118.1	9181	33	118.1
9167	33	128.7	9182	29	99.4
9168	31	112.5	9183	29	100.3
9169	34	128.3	9184	33	125.3
9170	31	108.6	9185	29	97.4
9171	29	102.2	9186	29	93.7
9172	31	104.7	9187	30	105.4
9173	32	113.8	9188	30	97.2
9174	32	113.4	9189	33	119.9
9175	33	121.6	9190	30	97.2

Dose Level: Group 1 – 0 mg/kg/day; Group 2 – 0.0025 mg/kg/day Ethinyl Estradiol; Group 3 – 0.005 mg/kg/day Ethinyl Estradiol
Group 4 – 12.5 mg/kg/day Methoxychlor; Group 5 – 25 mg/kg/day Methoxychlor; Group 6 – 50 mg/kg/day Methoxychlor
Group 7 – 25 mg/kg/day Phenobarbital; Group 8 – 50 mg/kg/day Phenobarbital; Group 9 – 100 mg/kg/day Phenobarbital
F = Females

Appendix 10 (continued)
F_1 Sexual Development Data – Vaginal Opening
Pubertal Toxicity Study of Vinclozolin, Flutamide and Phenobarbital in Male Sprague Dawley Rats and Methoxychlor, Ethinyl Estradiol and Phenobarbital in Female Sprague Dawley Rats When Administered in Corn Oil by Oral Gavage

Group 3			Group 4		
Animal Number	Postnatal Day of Vaginal Opening	Bodyweight at Vaginal Opening (Grams)	Animal Number	Postnatal Day of Vaginal Opening	Bodyweight at Vaginal Opening (Grams)
9191	28	99.6	9206	29	107.0
9192	29	109.9	9207	28	106.9
9193	29	100.8	9208	29	102.4
9194	28	101.9	9209	29	105.6
9195	30	104.6	9210	28	92.1
9196	27	91.3	9211	28	93.0
9197	28	96.0	9212	27	86.4
9198	27	87.8	9213	27	83.7
9199	29	97.2	9214	27	88.0
9200	28	88.9	9215	27	86.7
9201	29	90.3	9216	28	84.1
9202	29	90.9	9217	28	92.6
9203	29	89.1	9218	27	85.4
9204	28	86.1	9219	27	83.9
9205	29	94.0	9220	29	88.4

Dose Level: Group 1 – 0 mg/kg/day; Group 2 – 0.0025 mg/kg/day Ethinyl Estradiol; Group 3 – 0.005 mg/kg/day Ethinyl Estradiol
Group 4 – 12.5 mg/kg/day Methoxychlor; Group 5 – 25 mg/kg/day Methoxychlor; Group 6 – 50 mg/kg/day Methoxychlor
Group 7 – 25 mg/kg/day Phenobarbital; Group 8 – 50 mg/kg/day Phenobarbital; Group 9 – 100 mg/kg/day Phenobarbital
F = Females

Appendix 10 (continued)
F_1 Sexual Development Data – Vaginal Opening
Pubertal Toxicity Study of Vinclozolin, Flutamide and Phenobarbital in Male Sprague Dawley Rats and Methoxychlor, Ethinyl Estradiol and Phenobarbital in Female Sprague Dawley Rats When Administered in Corn Oil by Oral Gavage

Group 5			Group 6		
Animal Number	Postnatal Day of Vaginal Opening	Bodyweight at Vaginal Opening (Grams)	Animal Number	Postnatal Day of Vaginal Opening	Bodyweight at Vaginal Opening (Grams)
9221	27	92.8	9236	26	84.6
9222	27	96.5	9237	26	83.3
9223	27	90.5	9238	27	91.1
9224	27	87.5	9239	26	82.2
9225	29	103.9	9240	26	83.2
9226	27	91.0	9241	27	87.0
9227	27	91.1	9242	26	79.0
9228	27	85.6	9243	26	83.7
9229	27	91.0	9244	26	78.5
9230	26	68.1	9245	27	87.6
9231	27	88.4	9246	27	81.7
9232	27	83.8	9247	27	80.2
9233	26	76.8	9248	27	79.8
9234	26	77.7	9249	26	72.2
9235	28	87.5	9250	27	76.2

Dose Level: Group 1 – 0 mg/kg/day; Group 2 – 0.0025 mg/kg/day Ethinyl Estradiol; Group 3 – 0.005 mg/kg/day Ethinyl Estradiol
Group 4 – 12.5 mg/kg/day Methoxychlor; Group 5 – 25 mg/kg/day Methoxychlor; Group 6 – 50 mg/kg/day Methoxychlor
Group 7 – 25 mg/kg/day Phenobarbital; Group 8 – 50 mg/kg/day Phenobarbital; Group 9 – 100 mg/kg/day Phenobarbital
F = Females

Appendix 10 (continued)
F_1 Sexual Development Data – Vaginal Opening
Pubertal Toxicity Study of Vinclozolin, Flutamide and Phenobarbital in Male Sprague Dawley Rats and Methoxychlor, Ethinyl Estradiol and Phenobarbital in Female Sprague Dawley Rats When Administered in Corn Oil by Oral Gavage

Group 7			Group 8		
Animal Number	Postnatal Day of Vaginal Opening	Bodyweight at Vaginal Opening (Grams)	Animal Number	Postnatal Day of Vaginal Opening	Bodyweight at Vaginal Opening (Grams)
9251	34	147.8	9266	31	124.2
9252	35	131.2	9267	32	120.4
9253	33	113.3	9268	37	163.5
9254	31	109.9	9269	31	121.6
9255	31	114.0	9270	32	120.9
9256	34	131.4	9271	31	119.5
9257	33	128.2	9272	32	120.2
9258	33	120.3	9273	33	119.0
9259	35	138.7	9274	33	123.1
9260	33	121.1	9275	38	151.7
9261	36	138.1	9276	33	91.4
9262	33	116.3	9277	33	102.0
9263	33	114.2	9278	32	108.1
9264	32	114.0	9279	32	115.1
9265	30	100.9	9280	32	109.3

Dose Level: Group 1 – 0 mg/kg/day; Group 2 – 0.0025 mg/kg/day Ethinyl Estradiol; Group 3 – 0.005 mg/kg/day Ethinyl Estradiol
Group 4 – 12.5 mg/kg/day Methoxychlor; Group 5 – 25 mg/kg/day Methoxychlor; Group 6 – 50 mg/kg/day Methoxychlor
Group 7 – 25 mg/kg/day Phenobarbital; Group 8 – 50 mg/kg/day Phenobarbital; Group 9 – 100 mg/kg/day Phenobarbital
F = Females

Appendix 10 (continued)
F_1 Sexual Development Data – Vaginal Opening
Pubertal Toxicity Study of Vinclozolin, Flutamide and Phenobarbital in Male Sprague Dawley Rats and Methoxychlor, Ethinyl Estradiol and Phenobarbital in Female Sprague Dawley Rats When Administered in Corn Oil by Oral Gavage

Group 9

Animal Number	Postnatal Day of Vaginal Opening	Bodyweight at Vaginal Opening (Grams)
9281	31	119.3
9282	32	115.2
9283	37	156.3
9284	33	122.9
9285	33	119.1
9286	35	129.6
9287	41	179.0
9288	34	120.4
9289	33	118.5
9290	37	153.8
9291	35	118.2
9292	37	132.8
9293	33	111.3
9294	33	114.3
9295	33	109.9

Dose Level: Group 1 – 0 mg/kg/day; Group 2 – 0.0025 mg/kg/day Ethinyl Estradiol; Group 3 – 0.005 mg/kg/day Ethinyl Estradiol
Group 4 – 12.5 mg/kg/day Methoxychlor; Group 5 – 25 mg/kg/day Methoxychlor; Group 6 – 50 mg/kg/day Methoxychlor
Group 7 – 25 mg/kg/day Phenobarbital; Group 8 – 50 mg/kg/day Phenobarbital; Group 9 – 100 mg/kg/day Phenobarbital
F = Females

Appendix 11
F_1 Generation – Vaginal Cytology Data
Pubertal Toxicity Study of Vinclozolin, Flutamide and Phenobarbital in Male Sprague Dawley Rats and Methoxychlor, Ethinyl Estradiol and Phenobarbital in Female Sprague Dawley Rats when Administered in Corn Oil by Oral Gavage

Appendix 11
Vaginal Cytology Data – F_1 Generation
Pubertal Toxicity Study of Vinclozolin, Flutamide and Phenobarbital in Male Sprague Dawley Rats and Methoxychlor, Ethinyl Estradiol and Phenobarbital in Female Sprague Dawley Rats When Administered in Corn Oil by Oral Gavage

		Postnatal Day																	
Group	Female Number	26	27	28	29	30	31	32	33	34	35	36	37	38	39	40	41	42	43
1	9161	.	.	.	.	.	.	.	E	M	D	D	P	E	D	D	D	E	.
	9162	.	.	.	.	.	M	D	D	D	E	E	D	D	D	E	E	D	.
	9163	.	.	.	.	.	.	D	D	D	E	E	E	M	D	D	E	M	.
	9164	.	.	.	.	.	.	.	P	M	D	D	D	P	E	M	D	D	.
	9165	.	.	.	.	.	E	M	D	D	P	E	D	D	P	E	D	D	.
	9166	.	.	.	.	.	.	E	D	D	D	P	E	M	D	P	E	D	.
	9167	.	.	.	.	.	.	.	P	E	M	D	D	D	P	E	M	D	.
	9168	.	.	.	.	.	E	M	D	D	D	E	E	D	D	D	P	E	.
	9169	.	.	.	.	.	.	.	.	E	M	D	D	P	E	M	D	D	E
	9170	.	.	.	.	.	IC	P	E	D	D	D	D	P	E	M	D	D	E
	9171	.	.	.	E	M	D	D	P	E	M	D	D	P	E	D	D	D	P
	9172	.	.	.	.	.	M	D	D	P	E	M	D	D	E	M	D	P	E
	9173	.	.	.	.	.	.	E	M	D	D	D	E	D	D	P	E	D	D
	9174	.	.	.	.	.	.	E	M	D	D	D	D	E	E	M	D	D	P
	9175	.	.	.	.	.	.	.	M	D	D	E	M	D	D	E	E	M	D

E = Estrous M = Metestrous D = Diestrous P = Proestrous . = No Data
IC = Insufficient number of cells
Dose Level: Group 1 – 0 mg/kg/day; Group 2 – 0.0025 mg/kg/day Ethinyl Estradiol; Group 3 – 0.005 mg/kg/day Ethinyl Estradiol
Group 4 – 12.5 mg/kg/day Methoxychlor; Group 5 – 25 mg/kg/day Methoxychlor; Group 6 – 50 mg/kg/day Methoxychlor
Group 7 – 25 mg/kg/day Phenobarbital; Group 8 – 50 mg/kg/day Phenobarbital; Group 9 – 100 mg/kg/day Phenobarbital

Appendix 11 (continued)
Vaginal Cytology Data – F_1 Generation
Pubertal Toxicity Study of Vinclozolin, Flutamide and Phenobarbital in Male Sprague Dawley Rats and Methoxychlor, Ethinyl Estradiol and Phenobarbital in Female Sprague Dawley Rats When Administered in Corn Oil by Oral Gavage

Group	Female Number	Postnatal Day of First Estrous	Cycling	Nature of Cycle	Extended Estrous	Extended Diestrous	Number of Cycles	Cycle Length (Days)
1	9161	33	Y	R	N	N	2	4.5
	9162	35	Y	R	N	N	1	5.0
	9163	35	Y	I	Y	N	1	4.0
	9164	39	Y	R	N	N	1	5.0
	9165	31	Y	R	N	N	2	4.5
	9166	32	Y	R	N	N	2	4.5
	9167	34	Y	R	N	N	1	6.0
	9168	31	Y	R	N	N	2	5.5
	9169	34	Y	R	N	N	2	4.5
	9170	33	Y	I	N	Y	2	5.0
	9171	29	Y	R	N	N	2	5.0
	9172	35	Y	R	N	N	2	4.0
	9173	32	Y	R	N	N	2	4.5
	9174	32	Y	I	N	Y	1	7.0
	9175	36	Y	R	N	N	2	4.5

Y = Yes N = No U = Unclear R = Regular I = Irregular . = No Data
Dose Level: Group 1 – 0 mg/kg/day; Group 2 – 0.0025 mg/kg/day Ethinyl Estradiol; Group 3 – 0.005 mg/kg/day Ethinyl Estradiol
Group 4 – 12.5 mg/kg/day Methoxychlor; Group 5 – 25 mg/kg/day Methoxychlor; Group 6 – 50 mg/kg/day Methoxychlor
Group 7 – 25 mg/kg/day Phenobarbital; Group 8 – 50 mg/kg/day Phenobarbital; Group 9 – 100 mg/kg/day Phenobarbital

Appendix 11 (continued)
Vaginal Cytology Data – F_1 Generation
Pubertal Toxicity Study of Vinclozolin, Flutamide and Phenobarbital in Male Sprague Dawley Rats and Methoxychlor, Ethinyl Estradiol and Phenobarbital in Female Sprague Dawley Rats When Administered in Corn Oil by Oral Gavage

		Postnatal Day																
Group	Female Number	26	27	28	29	30	31	32	33	34	35	36	37	38	39	40	41	42	43
2	9176	.	.	.	.	D	P	E	M	D	D	P	E	M	D	P	E	D	.
	9177	.	.	.	.	.	.	E	M	D	D	D	E	E	M	D	D	E	.
	9178	.	.	.	.	.	.	E	M	D	D	D	P	E	E	M	D	D	.
	9179	.	.	.	.	D	D	P	E	E	D	D	D	D	P	E	D	D	.
	9180	.	.	.	.	.	.	.	.	.	.	.	.	E	M	D	D	D	.
	9181	.	.	.	.	.	.	.	E	M	D	D	P	E	M	D	D	P	.
	9182	.	.	.	E	E	D	D	P	E	M	D	D	E	M	D	P	E	.
	9183	.	.	.	P	E	E	M	D	D	P	E	D	D	P	E	E	D	.
	9184	.	.	.	.	.	.	.	E	M	D	D	P	E	M	D	D	E	E
	9185	.	.	.	D	D	E	E	D	D	D	D	D	E	E	M	D	D	E
	9186	.	.	.	D	P	E	M	D	D	D	D	D	D	D	D	D	D	P
	9187	.	.	.	.	D	P	E	E	M	D	D	P	E	M	D	D	P	E
	9188	.	.	.	.	E	M	D	D	P	E	M	D	D	E	M	D	P	E
	9189	.	.	.	.	.	.	.	M	D	D	P	E	M	D	D	D	E	M
	9190	.	.	.	.	P	D	D	D	D	E	D	D	D	E	M	D	P	E

E = Estrous M = Metestrous D = Diestrous P = Proestrous . = No Data
IC = Insufficient number of cells
Dose Level: Group 1 – 0 mg/kg/day; Group 2 – 0.0025 mg/kg/day Ethinyl Estradiol; Group 3 – 0.005 mg/kg/day Ethinyl Estradiol
Group 4 – 12.5 mg/kg/day Methoxychlor; Group 5 – 25 mg/kg/day Methoxychlor; Group 6 – 50 mg/kg/day Methoxychlor
Group 7 – 25 mg/kg/day Phenobarbital; Group 8 – 50 mg/kg/day Phenobarbital; Group 9 – 100 mg/kg/day Phenobarbital

Appendix 11 (continued)
Vaginal Cytology Data – F_1 Generation
Pubertal Toxicity Study of Vinclozolin, Flutamide and Phenobarbital in Male Sprague Dawley Rats and Methoxychlor, Ethinyl Estradiol and Phenobarbital in Female Sprague Dawley Rats When Administered in Corn Oil by Oral Gavage

Group	Female Number	Postnatal Day of First Estrous	Cycling	Nature of Cycle	Extended Estrous	Extended Diestrous	Number of Cycles	Cycle Length (Days)
2	9176	32	Y	R	N	N	2	4.5
	9177	32	Y	R	N	N	2	5.0
	9178	32	Y	R	N	N	1	7.0
	9179	33	Y	I	N	Y	1	7.0
	9180	38	U	.	N	N	.	.
	9181	33	Y	R	N	N	1	5.0
	9182	29	Y	R	N	N	3	4.0
	9183	30	Y	R	N	N	2	5.0
	9184	33	Y	R	N	N	2	5.0
	9185	31	Y	I	N	Y	2	5.5
	9186	31	Y	I	N	Y	a	a
	9187	32	Y	R	N	N	2	5.0
	9188	30	Y	R	N	N	3	4.3
	9189	37	Y	R	N	N	2	5.0
	9190	35	Y	I	N	Y	2	4.0

Y = Yes N = No U = Unclear R = Regular I = Irregular . = No Data
Dose Level: Group 1 – 0 mg/kg/day; Group 2 – 0.0025 mg/kg/day Ethinyl Estradiol; Group 3 – 0.005 mg/kg/day Ethinyl Estradiol
Group 4 – 12.5 mg/kg/day Methoxychlor; Group 5 – 25 mg/kg/day Methoxychlor; Group 6 – 50 mg/kg/day Methoxychlor
Group 7 – 25 mg/kg/day Phenobarbital; Group 8 – 50 mg/kg/day Phenobarbital; Group 9 – 100 mg/kg/day Phenobarbital
a = Cycle length greater than 9 days, values excluded from calculations

Appendix 11 (continued)
Vaginal Cytology Data – F_1 Generation
Pubertal Toxicity Study of Vinclozolin, Flutamide and Phenobarbital in Male Sprague Dawley Rats and Methoxychlor, Ethinyl Estradiol and Phenobarbital in Female Sprague Dawley Rats When Administered in Corn Oil by Oral Gavage

		Postnatal Day																	
Group	Female Number	26	27	28	29	30	31	32	33	34	35	36	37	38	39	40	41	42	43
3	9191	.	.	E	E	E	E	D	D	D	D	P	E	D	D	D	P	E	.
	9192	.	.	.	E	E	E	M	D	D	D	D	D	P	E	M	D	E	.
	9193	.	.	.	E	E	E	M	D	D	D	P	E	E	D	D	D	P	.
	9194	.	.	P	E	E	E	M	D	D	D	P	E	E	M	D	P	E	.
	9195	.	.	.	.	E	E	E	E	E	M	E	E	D	D	D	E	M	.
	9196	.	P	E	E	E	E	E	E	E	E	M	D	D	P	E	D	D	.
	9197	.	.	E	E	E	E	E	M	D	D	P	E	M	D	D	P	E	.
	9198	.	P	M	E	E	M	D	D	E	E	E	M	D	D	P	E	M	.
	9199	.	.	.	E	E	M	D	D	D	D	E	E	M	D	D	E	M	D
	9200	.	.	E	E	M	D	D	D	P	E	M	D	D	P	E	M	D	D
	9201	.	.	.	E	E	E	M	D	D	D	P	E	D	D	D	P	E	M
	9202	.	.	.	E	E	E	E	M	D	P	E	D	D	P	E	M	D	P
	9203	.	.	.	E	E	E	M	D	D	D	P	E	M	D	D	E	D	D
	9204	.	.	E	M	D	D	P	E	M	D	D	P	E	M	D	P	E	M
	9205	.	.	.	E	E	E	E	E	M	D	D	P	E	M	D	D	P	E

E = Estrous M = Metestrous D = Diestrous P = Proestrous . = No Data
IC = Insufficient number of cells
Dose Level: Group 1 – 0 mg/kg/day; Group 2 – 0.0025 mg/kg/day Ethinyl Estradiol; Group 3 – 0.005 mg/kg/day Ethinyl Estradiol
Group 4 – 12.5 mg/kg/day Methoxychlor; Group 5 – 25 mg/kg/day Methoxychlor; Group 6 – 50 mg/kg/day Methoxychlor
Group 7 – 25 mg/kg/day Phenobarbital; Group 8 – 50 mg/kg/day Phenobarbital; Group 9 – 100 mg/kg/day Phenobarbital

Appendix 11 (continued)
Vaginal Cytology Data – F_1 Generation
Pubertal Toxicity Study of Vinclozolin, Flutamide and Phenobarbital in Male Sprague Dawley Rats and Methoxychlor, Ethinyl Estradiol and Phenobarbital in Female Sprague Dawley Rats When Administered in Corn Oil by Oral Gavage

Group	Female Number	Postnatal Day of First Estrous	Cycling	Nature of Cycle	Extended Estrous	Extended Diestrous	Number of Cycles	Cycle Length (Days)
3	9191	28	Y	I	Y	Y	2	5.5
	9192	29	Y	I	Y	Y	2	5.5
	9193	29	Y	I	Y	N	1	6.0
	9194	29	Y	I	Y	N	2	5.5
	9195	30	Y	I	Y	N	2	3.5
	9196	28	Y	I	Y	N	1	5.0
	9197	28	Y	I	Y	N	2	5.0
	9198	29	Y	I	Y	N	2	5.5
	9199	29	Y	I	N	Y	2	5.5
	9200	28	Y	R	N	N	2	5.5
	9201	29	Y	I	Y	N	2	5.5
	9202	29	Y	I	Y	N	2	4.0
	9203	29	Y	R	Y	N	2	5.0
	9204	28	Y	R	N	N	3	4.7
	9205	29	Y	I	Y	N	2	5.0

Y = Yes N = No U = Unclear R = Regular I = Irregular . = No Data
Dose Level: Group 1 – 0 mg/kg/day; Group 2 – 0.0025 mg/kg/day Ethinyl Estradiol; Group 3 – 0.005 mg/kg/day Ethinyl Estradiol
Group 4 – 12.5 mg/kg/day Methoxychlor; Group 5 – 25 mg/kg/day Methoxychlor; Group 6 – 50 mg/kg/day Methoxychlor
Group 7 – 25 mg/kg/day Phenobarbital; Group 8 – 50 mg/kg/day Phenobarbital; Group 9 – 100 mg/kg/day Phenobarbital

Appendix 11 (continued)
Vaginal Cytology Data – F_1 Generation
Pubertal Toxicity Study of Vinclozolin, Flutamide and Phenobarbital in Male Sprague Dawley Rats and Methoxychlor, Ethinyl Estradiol and Phenobarbital in Female Sprague Dawley Rats When Administered in Corn Oil by Oral Gavage

		Postnatal Day																
Group	Female Number	26	27	28	29	30	31	32	33	34	35	36	37	38	39	40	41	42	43
4	9206	.	.	.	D	D	P	E	M	D	D	P	E	D	D	D	P	E	.
	9207	.	.	D	D	P	E	M	D	D	P	E	E	D	D	D	P	E	.
	9208	.	.	.	D	P	E	M	D	D	P	E	M	D	D	P	E	M	.
	9209	.	.	.	D	IC	P	E	M	D	D	P	E	M	D	D	P	E	.
	9210	.	.	D	D	P	E	M	D	D	P	E	M	D	D	P	E	D	.
	9211	.	.	D	D	E	E	M	D	D	P	E	D	D	D	P	E	M	.
	9212	.	D	D	P	E	D	D	D	D	P	E	M	D	D	D	E	E	.
	9213	.	M	D	D	D	P	E	M	D	D	D	D	P	E	M	D	D	.
	9214	.	M	D	D	P	E	M	D	D	D	E	E	M	D	D	P	E	M
	9215	.	E	D	D	P	E	M	D	D	P	E	D	D	D	P	E	M	D
	9216	.	.	M	D	D	P	E	M	D	D	D	D	E	E	M	D	D	D
	9217	.	.	M	D	P	E	M	D	D	P	E	D	D	D	E	E	D	D
	9218	.	M	D	E	E	M	D	D	P	E	M	D	D	E	M	D	D	P
	9219	.	M	D	D	P	E	M	D	D	D	P	E	M	D	D	D	E	M
	9220	.	.	.	D	D	D	P	E	M	D	D	P	E	D	D	D	P	E

E = Estrous M = Metestrous D = Diestrous P = Proestrous . = No Data
IC = Insufficient number of cells
Dose Level: Group 1 – 0 mg/kg/day; Group 2 – 0.0025 mg/kg/day Ethinyl Estradiol; Group 3 – 0.005 mg/kg/day Ethinyl Estradiol
Group 4 – 12.5 mg/kg/day Methoxychlor; Group 5 – 25 mg/kg/day Methoxychlor; Group 6 – 50 mg/kg/day Methoxychlor
Group 7 – 25 mg/kg/day Phenobarbital; Group 8 – 50 mg/kg/day Phenobarbital; Group 9 – 100 mg/kg/day Phenobarbital

Appendix 11 (continued)
Vaginal Cytology Data – F_1 Generation
Pubertal Toxicity Study of Vinclozolin, Flutamide and Phenobarbital in Male Sprague Dawley Rats and Methoxychlor, Ethinyl Estradiol and Phenobarbital in Female Sprague Dawley Rats When Administered in Corn Oil by Oral Gavage

Group	Female Number	Postnatal Day of First Estrous	Cycling	Nature of Cycle	Extended Estrous	Extended Diestrous	Number of Cycles	Cycle Length (Days)
4	9206	32	Y	R	N	N	2	5.0
	9207	31	Y	R	N	N	2	5.5
	9208	31	Y	R	N	N	2	5.0
	9209	32	Y	R	N	N	2	5.0
	9210	31	Y	R	N	N	2	5.0
	9211	30	Y	R	N	N	2	5.0
	9212	30	Y	I	N	Y	2	6.0
	9213	32	Y	I	N	Y	2	6.5
	9214	31	Y	R	N	N	3	5.3
	9215	27	Y	R	N	N	3	4.7
	9216	32	Y	I	N	Y	2	6.0
	9217	31	Y	R	N	N	2	5.0
	9218	29	Y	R	N	N	3	4.3
	9219	31	Y	R	N	N	3	5.3
	9220	33	Y	R	N	N	2	5.0

Y = Yes N = No U = Unclear R = Regular I = Irregular . = No Data
Dose Level: Group 1 – 0 mg/kg/day; Group 2 – 0.0025 mg/kg/day Ethinyl Estradiol; Group 3 – 0.005 mg/kg/day Ethinyl Estradiol
Group 4 – 12.5 mg/kg/day Methoxychlor; Group 5 – 25 mg/kg/day Methoxychlor; Group 6 – 50 mg/kg/day Methoxychlor
Group 7 – 25 mg/kg/day Phenobarbital; Group 8 – 50 mg/kg/day Phenobarbital; Group 9 – 100 mg/kg/day Phenobarbital

Appendix 11 (continued)
Vaginal Cytology Data – F_1 Generation
Pubertal Toxicity Study of Vinclozolin, Flutamide and Phenobarbital in Male Sprague Dawley Rats and Methoxychlor, Ethinyl Estradiol and Phenobarbital in Female Sprague Dawley Rats When Administered in Corn Oil by Oral Gavage

		Postnatal Day																
Group	Female Number	26	27	28	29	30	31	32	33	34	35	36	37	38	39	40	41	42	43
5	9221	.	M	D	D	P	E	E	M	D	D	D	D	D	D	D	D	D	.
5	9222	.	D	P	E	E	D	D	D	P	E	D	D	P	E	E	D	D	.
5	9223	.	M	D	D	P	E	M	D	D	D	P	E	M	D	D	P	E	.
5	9224	.	M	D	D	D	D	D	D	D	D	P	E	D	D	D	P	E	.
5	9225	.	.	.	D	D	D	P	E	D	D	D	D	D	P	E	D	D	.
5	9226	.	D	D	D	D	P	E	E	D	D	D	D	D	P	E	M	D	.
5	9227	.	M	D	D	D	P	E	M	D	D	D	P	P	E	M	D	D	.
5	9228	.	D	D	D	P	E	M	D	D	D	D	D	P	E	M	D	D	.
5	9229	.	E	E	M	D	D	D	D	E	E	M	D	D	D	E	M	D	D
5	9230	M	D	D	D	P	E	M	D	D	D	P	E	M	D	D	P	E	M
5	9231	.	D	D	E	E	D	D	D	P	E	M	D	D	P	E	M	D	D
5	9232	.	M	D	D	D	P	E	E	M	D	D	D	D	E	E	M	D	D
5	9233	E	M	D	D	D	P	E	M	D	D	D	D	D	P	E	M	D	D
5	9234	P	E	M	D	D	E	M	D	D	D	D	P	E	M	D	D	D	E
5	9235	.	.	D	D	D	P	E	M	D	D	D	D	E	E	M	D	D	D

E = Estrous M = Metestrous D = Diestrous P = Proestrous . = No Data
IC = Insufficient number of cells
Dose Level: Group 1 – 0 mg/kg/day; Group 2 – 0.0025 mg/kg/day Ethinyl Estradiol; Group 3 – 0.005 mg/kg/day Ethinyl Estradiol
Group 4 – 12.5 mg/kg/day Methoxychlor; Group 5 – 25 mg/kg/day Methoxychlor; Group 6 – 50 mg/kg/day Methoxychlor
Group 7 – 25 mg/kg/day Phenobarbital; Group 8 – 50 mg/kg/day Phenobarbital; Group 9 – 100 mg/kg/day Phenobarbital

Appendix 11 (continued)
Vaginal Cytology Data – F_1 Generation
Pubertal Toxicity Study of Vinclozolin, Flutamide and Phenobarbital in Male Sprague Dawley Rats and Methoxychlor, Ethinyl Estradiol and Phenobarbital in Female Sprague Dawley Rats When Administered in Corn Oil by Oral Gavage

Group	Female Number	Postnatal Day of First Estrous	Cycling	Nature of Cycle	Extended Estrous	Extended Diestrous	Number of Cycles	Cycle Length (Days)
5	9221	31	Y	I	N	Y	1	6.0
	9222	29	Y	R	N	N	2	5.0
	9223	31	Y	R	N	N	2	5.5
	9224	37	Y	I	N	Y	1	5.0
	9225	33	Y	I	N	Y	1	7.0
	9226	32	Y	I	N	Y	1	7.0
	9227	32	Y	I	N	N	2	6.5
	9228	31	Y	I	N	Y	1	8.0
	9229	27	Y	I	N	Y	2	6.0
	9230	31	Y	R	N	N	3	5.7
	9231	29	Y	R	N	N	2	5.0
	9232	32	Y	I	N	Y	2	7.0
	9233	26	Y	I	N	Y	2	7.0
	9234	27	Y	I	N	Y	3	5.3
	9235	32	Y	I	N	Y	1	7.0

Y = Yes N = No U = Unclear R = Regular I = Irregular . = No Data
Dose Level: Group 1 – 0 mg/kg/day; Group 2 – 0.0025 mg/kg/day Ethinyl Estradiol; Group 3 – 0.005 mg/kg/day Ethinyl Estradiol
Group 4 – 12.5 mg/kg/day Methoxychlor; Group 5 – 25 mg/kg/day Methoxychlor; Group 6 – 50 mg/kg/day Methoxychlor
Group 7 – 25 mg/kg/day Phenobarbital; Group 8 – 50 mg/kg/day Phenobarbital; Group 9 – 100 mg/kg/day Phenobarbital

Appendix 11 (continued)
Vaginal Cytology Data – F_1 Generation
Pubertal Toxicity Study of Vinclozolin, Flutamide and Phenobarbital in Male Sprague Dawley Rats and Methoxychlor, Ethinyl Estradiol and Phenobarbital in Female Sprague Dawley Rats When Administered in Corn Oil by Oral Gavage

		Postnatal Day																	
Group	Female Number	26	27	28	29	30	31	32	33	34	35	36	37	38	39	40	41	42	43
6	9236	E	E	E	E	E	D	D	D	D	P	P	E	M	D	D	D	P	.
	9237	E	E	E	E	E	E	D	D	D	P	E	E	M	D	D	D	P	.
	9238	.	M	D	D	P	E	M	D	D	D	D	D	D	D	D	P	E	.
	9239	.	E	M	D	D	E	E	E	E	D	E	M	D	D	P	E	M	.
	9240	E	M	M	M	D	D	P	E	E	M	M	M	D	D	P	E	M	.
	9241	.	M	D	D	D	P	E	M	D	D	D	P	E	M	D	D	P	.
	9242	M	D	D	D	P	E	M	D	D	D	D	P	E	D	D	D	P	.
	9243	E	E	M	M	E	E	E	M	M	M	M	M	P	E	M	D	D	.
	9244	M	M	M	P	E	E	D	D	D	D	D	P	E	D	D	D	P	E
	9245	.	P	E	E	E	M	D	D	D	P	E	E	M	D	D	P	E	M
	9246	.	M	D	D	D	P	E	E	M	M	D	D	P	E	M	M	D	D
	9247	.	M	D	E	E	D	D	D	D	P	E	D	D	D	P	E	D	D
	9248	.	D	P	E	E	M	D	D	P	E	M	D	D	P	E	M	D	D
	9249	E	E	E	E	E	E	M	D	D	P	E	E	M	D	D	P	E	M
	9250	.	D	D	D	E	E	M	D	D	P	E	E	M	D	D	P	E	M

E = Estrous M = Metestrous D = Diestrous P = Proestrous . = No Data
IC = Insufficient number of cells
Dose Level: Group 1 – 0 mg/kg/day; Group 2 – 0.0025 mg/kg/day Ethinyl Estradiol; Group 3 – 0.005 mg/kg/day Ethinyl Estradiol
Group 4 – 12.5 mg/kg/day Methoxychlor; Group 5 – 25 mg/kg/day Methoxychlor; Group 6 – 50 mg/kg/day Methoxychlor
Group 7 – 25 mg/kg/day Phenobarbital; Group 8 – 50 mg/kg/day Phenobarbital; Group 9 – 100 mg/kg/day Phenobarbital

Appendix 11 (continued)
Vaginal Cytology Data – F_1 Generation
Pubertal Toxicity Study of Vinclozolin, Flutamide and Phenobarbital in Male Sprague Dawley Rats and Methoxychlor, Ethinyl Estradiol and Phenobarbital in Female Sprague Dawley Rats When Administered in Corn Oil by Oral Gavage

Group	Female Number	Postnatal Day of First Estrous	Cycling	Nature of Cycle	Extended Estrous	Extended Diestrous	Number of Cycles	Cycle Length (Days)
6	9236	26	Y	I	Y	Y	1	7.0
	9237	26	Y	I	Y	N	1	6.0
	9238	31	Y	I	N	Y	a	a
	9239	27	Y	I	Y	N	3	4.7
	9240	26	Y	I	N	N	2	6.5
	9241	32	Y	R	N	N	2	5.5
	9242	31	Y	I	N	Y	2	6.0
	9243	26	Y	I	Y	N	2	5.5
	9244	30	Y	I	N	Y	2	6.5
	9245	28	Y	I	Y	N	2	6.0
	9246	32	Y	I	N	N	2	7.0
	9247	29	Y	I	N	Y	2	5.5
	9248	29	Y	R	N	N	2	5.0
	9249	26	Y	I	Y	N	2	5.5
	9250	30	Y	R	N	N	2	5.5

Y = Yes N = No U = Unclear R = Regular I = Irregular . = No Data
Dose Level: Group 1 – 0 mg/kg/day; Group 2 – 0.0025 mg/kg/day Ethinyl Estradiol; Group 3 – 0.005 mg/kg/day Ethinyl Estradiol
Group 4 – 12.5 mg/kg/day Methoxychlor; Group 5 – 25 mg/kg/day Methoxychlor; Group 6 – 50 mg/kg/day Methoxychlor
Group 7 – 25 mg/kg/day Phenobarbital; Group 8 – 50 mg/kg/day Phenobarbital; Group 9 – 100 mg/kg/day Phenobarbital
a = Cycle length greater than 9 days, values excluded from calculations

Appendix 11 (continued)
Vaginal Cytology Data – F_1 Generation
Pubertal Toxicity Study of Vinclozolin, Flutamide and Phenobarbital in Male Sprague Dawley Rats and Methoxychlor, Ethinyl Estradiol and Phenobarbital in Female Sprague Dawley Rats When Administered in Corn Oil by Oral Gavage

		Postnatal Day																	
Group	Female Number	26	27	28	29	30	31	32	33	34	35	36	37	38	39	40	41	42	43
7	9251	.	.	.	.	.	.	.	.	M	D	D	D	E	E	M	D	D	.
	9252	.	.	.	.	.	.	.	.	.	P	E	D	D	D	P	E	D	.
	9253	.	.	.	.	.	.	.	M	D	D	P	E	E	M	D	D	P	.
	9254	.	.	.	.	.	E	M	D	D	D	E	D	D	D	D	E	E	.
	9255	.	.	.	.	.	P	E	M	D	D	P	E	M	D	D	E	M	.
	9256	.	.	.	.	.	.	.	.	E	D	D	D	P	E	D	P	E	.
	9257	.	.	.	.	.	.	.	E	M	D	D	P	E	M	D	P	E	.
	9258	.	.	.	.	.	.	.	D	D	D	D	P	E	D	D	D	E	.
	9259	.	.	.	.	.	.	.	.	.	M	D	D	P	E	M	D	D	P
	9260	.	.	.	.	.	.	.	M	D	D	P	E	D	D	P	E	M	D
	9261	.	.	.	.	.	.	.	.	.	.	P	E	M	D	D	P	E	M
	9262	.	.	.	.	.	.	.	M	D	D	P	E	M	D	P	E	M	D
	9263	.	.	.	.	.	.	.	M	D	D	P	E	M	D	D	E	E	M
	9264	.	.	.	.	.	.	M	D	D	D	D	P	E	M	D	D	P	E
	9265	.	.	.	.	E	M	D	D	E	E	M	D	D	E	M	D	D	E

E = Estrous M = Metestrous D = Diestrous P = Proestrous . = No Data
IC = Insufficient number of cells
Dose Level: Group 1 – 0 mg/kg/day; Group 2 – 0.0025 mg/kg/day Ethinyl Estradiol; Group 3 – 0.005 mg/kg/day Ethinyl Estradiol
Group 4 – 12.5 mg/kg/day Methoxychlor; Group 5 – 25 mg/kg/day Methoxychlor; Group 6 – 50 mg/kg/day Methoxychlor
Group 7 – 25 mg/kg/day Phenobarbital; Group 8 – 50 mg/kg/day Phenobarbital; Group 9 – 100 mg/kg/day Phenobarbital

Appendix 11 (continued)
Vaginal Cytology Data – F_1 Generation
Pubertal Toxicity Study of Vinclozolin, Flutamide and Phenobarbital in Male Sprague Dawley Rats and Methoxychlor, Ethinyl Estradiol and Phenobarbital in Female Sprague Dawley Rats When Administered in Corn Oil by Oral Gavage

Group	Female Number	Postnatal Day of First Estrous	Cycling	Nature of Cycle	Extended Estrous	Extended Diestrous	Number of Cycles	Cycle Length (Days)
7	9251	38	Y	R	N	N	1	6.0
	9252	36	Y	R	N	N	1	5.0
	9253	37	Y	R	N	N	1	6.0
	9254	31	Y	I	N	Y	2	5.5
	9255	32	Y	R	N	N	2	4.5
	9256	34	Y	R	N	N	2	4.0
	9257	33	Y	R	N	N	2	4.5
	9258	38	Y	I	N	Y	1	4.0
	9259	39	Y	R	N	N	1	5.0
	9260	37	Y	R	N	N	2	4.5
	9261	37	Y	R	N	N	1	5.0
	9262	37	Y	R	N	N	2	4.5
	9263	37	Y	R	N	N	2	5.0
	9264	38	Y	I	N	Y	1	5.0
	9265	30	Y	R	N	N	3	4.3

Y = Yes N = No U = Unclear R = Regular I = Irregular . = No Data
Dose Level: Group 1 – 0 mg/kg/day; Group 2 – 0.0025 mg/kg/day Ethinyl Estradiol; Group 3 – 0.005 mg/kg/day Ethinyl Estradiol
Group 4 – 12.5 mg/kg/day Methoxychlor; Group 5 – 25 mg/kg/day Methoxychlor; Group 6 – 50 mg/kg/day Methoxychlor
Group 7 – 25 mg/kg/day Phenobarbital; Group 8 – 50 mg/kg/day Phenobarbital; Group 9 – 100 mg/kg/day Phenobarbital

Appendix 11 (continued)
Vaginal Cytology Data – F_1 Generation
Pubertal Toxicity Study of Vinclozolin, Flutamide and Phenobarbital in Male Sprague Dawley Rats and Methoxychlor, Ethinyl Estradiol and Phenobarbital in Female Sprague Dawley Rats When Administered in Corn Oil by Oral Gavage

		Postnatal Day																
Group	Female Number	26	27	28	29	30	31	32	33	34	35	36	37	38	39	40	41	42	43
8	9266	.	.	.	.	.	E	M	D	D	E	E	M	D	D	E	M	D	.
	9267	.	.	.	.	.	.	M	D	D	P	E	D	D	D	P	E	D	.
	9268	.	.	.	.	.	.	.	.	.	.	.	E	M	D	D	E	E	.
	9269	.	.	.	.	.	E	M	D	D	P	E	M	D	D	E	E	D	.
	9270	.	.	.	.	.	.	E	M	D	D	P	E	M	D	D	E	D	.
	9271	.	.	.	.	.	E	M	D	D	P	E	D	D	P	E	M	D	.
	9272	.	.	.	.	.	.	M	D	D	P	E	E	M	D	E	E	M	.
	9273	.	.	.	.	.	.	.	E	D	D	D	D	E	E	M	D	D	.
	9274	.	.	.	.	.	.	.	M	D	E	D	D	D	E	D	D	P	E
	9275	.	.	.	.	.	.	.	.	.	.	.	.	E	D	D	D	P	E
	9276	.	.	.	.	.	.	.	E	M	D	D	D	D	D	D	D	D	P
	9277	.	.	.	.	.	.	.	E	D	D	D	D	D	E	E	M	D	D
	9278	.	.	.	.	.	.	M	D	D	P	E	M	D	D	E	D	D	P
	9279	.	.	.	.	.	.	E	M	D	D	D	P	E	M	D	D	E	M
	9280	.	.	.	.	.	.	M	D	D	P	E	M	D	D	D	E	E	M

E = Estrous M = Metestrous D = Diestrous P = Proestrous . = No Data
IC = Insufficient number of cells
Dose Level: Group 1 – 0 mg/kg/day; Group 2 – 0.0025 mg/kg/day Ethinyl Estradiol; Group 3 – 0.005 mg/kg/day Ethinyl Estradiol
Group 4 – 12.5 mg/kg/day Methoxychlor; Group 5 – 25 mg/kg/day Methoxychlor; Group 6 – 50 mg/kg/day Methoxychlor
Group 7 – 25 mg/kg/day Phenobarbital; Group 8 – 50 mg/kg/day Phenobarbital; Group 9 – 100 mg/kg/day Phenobarbital

Appendix 11 (continued)
Vaginal Cytology Data – F_1 Generation
Pubertal Toxicity Study of Vinclozolin, Flutamide and Phenobarbital in Male Sprague Dawley Rats and Methoxychlor, Ethinyl Estradiol and Phenobarbital in Female Sprague Dawley Rats When Administered in Corn Oil by Oral Gavage

Group	Female Number	Postnatal Day of First Estrous	Cycling	Nature of Cycle	Extended Estrous	Extended Diestrous	Number of Cycles	Cycle Length (Days)
8	9266	31	Y	R	N	N	2	4.5
	9267	36	Y	R	N	N	1	5.0
	9268	37	Y	R	N	N	1	5.0
	9269	31	Y	R	N	N	2	5.0
	9270	32	Y	R	N	N	2	4.5
	9271	31	Y	R	N	N	2	4.5
	9272	36	Y	R	N	N	2	5.0
	9273	33	Y	I	N	Y	1	6.0
	9274	35	Y	R	N	N	2	4.0
	9275	38	Y	R	N	N	1	5.0
	9276	33	N	I	N	Y	.	.
	9277	33	Y	I	N	Y	1	7.0
	9278	36	Y	R	N	N	2	4.0
	9279	32	Y	R	N	N	2	5.0
	9280	36	Y	R	N	N	2	5.5

Y = Yes N = No U = Unclear R = Regular I = Irregular . = No Data
Dose Level: Group 1 – 0 mg/kg/day; Group 2 – 0.0025 mg/kg/day Ethinyl Estradiol; Group 3 – 0.005 mg/kg/day Ethinyl Estradiol
Group 4 – 12.5 mg/kg/day Methoxychlor; Group 5 – 25 mg/kg/day Methoxychlor; Group 6 – 50 mg/kg/day Methoxychlor
Group 7 – 25 mg/kg/day Phenobarbital; Group 8 – 50 mg/kg/day Phenobarbital; Group 9 – 100 mg/kg/day Phenobarbital

Appendix 11 (continued)
Vaginal Cytology Data – F_1 Generation
Pubertal Toxicity Study of Vinclozolin, Flutamide and Phenobarbital in Male Sprague Dawley Rats and Methoxychlor, Ethinyl Estradiol and Phenobarbital in Female Sprague Dawley Rats When Administered in Corn Oil by Oral Gavage

		Postnatal Day																	
Group	Female Number	26	27	28	29	30	31	32	33	34	35	36	37	38	39	40	41	42	43
9	9281	.	.	.	.	.	E	D	D	D	E	E	M	D	D	D	E	E	.
	9282	.	.	.	.	.	.	E	M	D	D	D	E	M	D	P	E	E	.
	9283	.	.	.	.	.	.	.	.	.	.	.	E	M	D	D	P	E	.
	9284	.	.	.	.	.	.	.	E	M	D	D	D	P	E	M	D	D	.
	9285	.	.	.	.	.	.	.	E	M	D	D	P	E	M	D	D	P	.
	9286	.	.	.	.	.	.	.	.	.	E	M	D	D	D	E	E	E	.
	9287	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	E	D	.
	9288	.	.	.	.	.	.	.	.	E	M	D	D	D	P	E	M	D	.
	9289	.	.	.	.	.	.	.	E	D	D	D	P	E	D	D	D	P	E
	9290	.	.	.	.	.	.	.	.	.	.	.	E	D	D	D	D	D	D
	9291	.	.	.	.	.	.	.	.	.	E	M	D	D	D	D	D	D	P
	9292	.	.	.	.	.	.	.	.	.	.	.	D	E	M	D	D	D	D
	9293	.	.	.	.	.	.	.	E	M	D	D	P	E	M	D	D	E	M
	9294	.	.	.	.	.	.	.	E	M	D	D	D	P	E	M	D	D	P
	9295	.	.	.	.	.	.	.	E	M	D	D	D	E	E	M	D	D	E

E = Estrous M = Metestrous D = Diestrous P = Proestrous . = No Data
IC = Insufficient number of cells
Dose Level: Group 1 – 0 mg/kg/day; Group 2 – 0.0025 mg/kg/day Ethinyl Estradiol; Group 3 – 0.005 mg/kg/day Ethinyl Estradiol
Group 4 – 12.5 mg/kg/day Methoxychlor; Group 5 – 25 mg/kg/day Methoxychlor; Group 6 – 50 mg/kg/day Methoxychlor
Group 7 – 25 mg/kg/day Phenobarbital; Group 8 – 50 mg/kg/day Phenobarbital; Group 9 – 100 mg/kg/day Phenobarbital

Appendix 11 (continued)
Vaginal Cytology Data – F_1 Generation
Pubertal Toxicity Study of Vinclozolin, Flutamide and Phenobarbital in Male Sprague Dawley Rats and Methoxychlor, Ethinyl Estradiol and Phenobarbital in Female Sprague Dawley Rats When Administered in Corn Oil by Oral Gavage

Group	Female Number	Postnatal Day of First Estrous	Cycling	Nature of Cycle	Extended Estrous	Extended Diestrous	Number of Cycles	Cycle Length (Days)
9	9281	31	Y	R	N	N	2	5.5
	9282	32	Y	R	N	N	2	5.0
	9283	37	Y	R	N	N	1	5.0
	9284	33	Y	R	N	N	1	5.0
	9285	33	Y	R	N	N	1	5.0
	9286	35	Y	I	Y	N	1	7.0
	9287	41	U	.	.	.	.	.
	9288	34	Y	R	N	N	1	6.0
	9289	33	Y	R	N	N	2	5.0
	9290	37	U	I	N	Y	.	.
	9291	35	N	I	N	Y	.	.
	9292	38	U	I	N	Y	.	.
	9293	33	Y	R	N	N	2	4.5
	9294	33	Y	R	N	N	1	5.0
	9295	33	Y	R	N	N	2	5.0

Y = Yes N = No U = Unclear R = Regular I = Irregular . = No Data
Dose Level: Group 1 – 0 mg/kg/day; Group 2 – 0.0025 mg/kg/day Ethinyl Estradiol; Group 3 – 0.005 mg/kg/day Ethinyl Estradiol
Group 4 – 12.5 mg/kg/day Methoxychlor; Group 5 – 25 mg/kg/day Methoxychlor; Group 6 – 50 mg/kg/day Methoxychlor
Group 7 – 25 mg/kg/day Phenobarbital; Group 8 – 50 mg/kg/day Phenobarbital; Group 9 – 100 mg/kg/day Phenobarbital

Appendix 12
F_1 Individual Gross Pathology
Pubertal Toxicity Study of Vinclozolin, Flutamide and Phenobarbital in Male Sprague Dawley Rats and Methoxychlor, Ethinyl Estradiol and Phenobarbital in Female Sprague Dawley Rats when Administered in Corn Oil by Oral Gavage

Appendix 12
F_1 Individual Gross Pathology
Pubertal Toxicity Study of Vinclozolin, Flutamide and Phenobarbital in Male Sprague Dawley Rats and Methoxychlor, Ethinyl Estradiol and Phenobarbital in Female Sprague Dawley Rats when Administered in Corn Oil by Oral Gavage

Group: 1 Dose Level: 0 mg/kg/day Sex: Male

Animal Number	Mode Of Death	Death Day	Death (Week)	Observation(s)
9026	TERMINAL KILL	53	(7)	No Visible Lesions
9027	TERMINAL KILL	53	(7)	No Visible Lesions
9028	TERMINAL KILL	53	(7)	No Visible Lesions
9029	TERMINAL KILL	53	(7)	No Visible Lesions
9030	TERMINAL KILL	53	(7)	No Visible Lesions
9031	TERMINAL KILL	53	(7)	Majority of fluid from left seminal vesicle lost at necropsy (prior to weighing) kidneys; dilation; right; moderate (TGL): renal pelvis Any remaining protocol required tissues have no visible lesions
9032	TERMINAL KILL	53	(7)	No Visible Lesions
9033	TERMINAL KILL	53	(7)	No Visible Lesions
9034	TERMINAL KILL	54	(7)	No Visible Lesions
9035	TERMINAL KILL	54	(7)	No Visible Lesions
9036	TERMINAL KILL	54	(7)	No Visible Lesions
9037	TERMINAL KILL	54	(7)	thymus; discoloration; red; bilateral; diffuse (TGL) Any remaining protocol required tissues have no visible lesions
9038	TERMINAL KILL	54	(7)	No Visible Lesions

TGL = Traceable gross lesion

Appendix 12 (continued)
F_1 Individual Gross Pathology
Pubertal Toxicity Study of Vinclozolin, Flutamide and Phenobarbital in Male Sprague Dawley Rats and Methoxychlor, Ethinyl Estradiol and Phenobarbital in Female Sprague Dawley Rats when Administered in Corn Oil by Oral Gavage

Group: 1 Dose Level: 0 mg/kg/day Sex: Male

Animal Number	Mode Of Death	Death Day	Death (Week)	Observation(s)
9039	TERMINAL KILL	54	(7)	epididymides; reduction in size; bilateral; moderate (TGL) spleen; enlargement (TGL): 55x15x10mm spleen; rough testes; reduction in size; bilateral; minimal (TGL) Any remaining protocol required tissues have no visible lesions
9040	TERMINAL KILL	54	(7)	spleen; enlargement (TGL): 55x20x10mm spleen; nodule(s); white (TGL): single; 4x4x4mm thymus; discoloration; red; bilateral (TGL) Any remaining protocol required tissues have no visible lesions

TGL = Traceable gross lesion

Appendix 12 (continued)
F_1 Individual Gross Pathology
Pubertal Toxicity Study of Vinclozolin, Flutamide and Phenobarbital in Male Sprague Dawley Rats and Methoxychlor, Ethinyl Estradiol and Phenobarbital in Female Sprague Dawley Rats when Administered in Corn Oil by Oral Gavage

Group: 2 Dose Level: 25 mg/kg/day Phenobarbital Sex: Male

Animal Number	Mode Of Death	Death Day	Death (Week)	Observation(s)
9041	TERMINAL KILL	53	(7)	Small amount of fluid lost from left seminal vesicle at necropsy (prior to weighing) kidneys; discoloration; dark; left; focal (TGL) Any remaining protocol required tissues have no visible lesions
9042	TERMINAL KILL	53	(7)	liver; nodule(s); white (TGL): focal; caudate lobe Any remaining protocol required tissues have no visible lesions
9043	TERMINAL KILL	53	(7)	No Visible Lesions
9044	TERMINAL KILL	53	(7)	No Visible Lesions
9045	TERMINAL KILL	53	(7)	No Visible Lesions
9046	TERMINAL KILL	53	(7)	No Visible Lesions
9047	TERMINAL KILL	53	(7)	kidneys; dilation; right; minimal (TGL): renal pelvis Any remaining protocol required tissues have no visible lesions
9048	TERMINAL KILL	53	(7)	No Visible Lesions
9049	TERMINAL KILL	54	(7)	No Visible Lesions
9050	TERMINAL KILL	54	(7)	No Visible Lesions
9051	TERMINAL KILL	54	(7)	No Visible Lesions
9052	TERMINAL KILL	54	(7)	No Visible Lesions

TGL = Traceable gross lesion

Appendix 12 (continued)
F_1 Individual Gross Pathology
Pubertal Toxicity Study of Vinclozolin, Flutamide and Phenobarbital in Male Sprague Dawley Rats and Methoxychlor, Ethinyl Estradiol and Phenobarbital in Female Sprague Dawley Rats when Administered in Corn Oil by Oral Gavage

Group: 2 Dose Level: 25 mg/kg/day Phenobarbital Sex: Male

Animal Number	Mode Of Death	Death Day	Death (Week)	Observation(s)
9053	TERMINAL KILL	54	(7)	kidneys; dilation; right; minimal (TGL): renal pelvis Any remaining protocol required tissues have no visible lesions
9054	TERMINAL KILL	54	(7)	thymus; discoloration; dark; bilateral (TGL) Any remaining protocol required tissues have no visible lesions
9055	TERMINAL KILL	54	(7)	No Visible Lesions

TGL = Traceable gross lesion

Appendix 12 (continued)
F_1 Individual Gross Pathology
Pubertal Toxicity Study of Vinclozolin, Flutamide and Phenobarbital in Male Sprague Dawley Rats and Methoxychlor, Ethinyl Estradiol and Phenobarbital in Female Sprague Dawley Rats when Administered in Corn Oil by Oral Gavage

Group: 3 Dose Level: 50 mg/kg/day Phenobarbital Sex: Male

Animal Number	Mode Of Death	Death Day	Death (Week)	Observation(s)
9056	TERMINAL KILL	53	(7)	adrenal glands: Left Damaged At Necropsy Any remaining protocol required tissues have no visible lesions
9057	TERMINAL KILL	53	(7)	No Visible Lesions
9058	TERMINAL KILL	53	(7)	No Visible Lesions
9059	TERMINAL KILL	53	(7)	kidneys; discoloration; dark; left; single (TGL): focal Any remaining protocol required tissues have no visible lesions
9060	TERMINAL KILL	53	(7)	seminal vesicles and coagulating glands with fluid; reduction in size; bilateral; minimal (TGL) Any remaining protocol required tissues have no visible lesions
9061	TERMINAL KILL	53	(7)	No Visible Lesions
9062	TERMINAL KILL	53	(7)	kidneys; dilation; right; moderate (TGL): renal pelvis Any remaining protocol required tissues have no visible lesions
9063	TERMINAL KILL	53	(7)	kidneys; dilation; right; moderate (TGL): renal pelvis Any remaining protocol required tissues have no visible lesions
9064	TERMINAL KILL	54	(7)	No Visible Lesions
9065	TERMINAL KILL	54	(7)	thymus; discoloration; dark; left (TGL) Any remaining protocol required tissues have no visible lesions
9066	TERMINAL KILL	54	(7)	kidneys; dilation; bilateral; moderate (TGL): renal pelvis Any remaining protocol required tissues have no visible lesions

TGL = Traceable gross lesion

Appendix 12 (continued)
F_1 Individual Gross Pathology
Pubertal Toxicity Study of Vinclozolin, Flutamide and Phenobarbital in Male Sprague Dawley Rats and Methoxychlor, Ethinyl Estradiol and Phenobarbital in Female Sprague Dawley Rats when Administered in Corn Oil by Oral Gavage

Group: 3 Dose Level: 50 mg/kg/day Phenobarbital Sex: Male

Animal Number	Mode Of Death	Death Day	Death (Week)	Observation(s)
9067	TERMINAL KILL	54	(7)	thyroid glands; discoloration; bilateral (TGL): dark red Any remaining protocol required tissues have no visible lesions
9068	TERMINAL KILL	54	(7)	No Visible Lesions
9069	TERMINAL KILL	54	(7)	No Visible Lesions
9070	TERMINAL KILL	54	(7)	No Visible Lesions

TGL = Traceable gross lesion

Appendix 12 (continued)
F_1 Individual Gross Pathology
Pubertal Toxicity Study of Vinclozolin, Flutamide and Phenobarbital in Male Sprague Dawley Rats and Methoxychlor, Ethinyl Estradiol and Phenobarbital in Female Sprague Dawley Rats when Administered in Corn Oil by Oral Gavage

Group: 4 Dose Level: 100 mg/kg/day Phenobarbital Sex: Male

Animal Number	Mode Of Death	Death Day	Death (Week)	Observation(s)
9071	TERMINAL KILL	53	(7)	dorsolateral prostate: Lost At Necropsy Any remaining protocol required tissues have no visible lesions
9072	TERMINAL KILL	53	(7)	No Visible Lesions
9073	TERMINAL KILL	53	(7)	testes; reduction in size; bilateral; moderate (TGL) seminal vesicles and coagulating glands with fluid; reduction in size; bilateral; moderate (TGL) Any remaining protocol required tissues have no visible lesions
9074	TERMINAL KILL	53	(7)	No Visible Lesions
9075	TERMINAL KILL	53	(7)	kidneys; dilation; bilateral; moderate (TGL): renal pelvis Any remaining protocol required tissues have no visible lesions
9076	TERMINAL KILL	53	(7)	No Visible Lesions
9077	Found Dead	24	(3)	esophagus: No Evidence Of Gavage Error Seen At Necropsy skin; abrasion(s); multiple; present, no grade assigned (TGL): green and pink; abdominal area urinary bladder; distension; moderate (TGL): fluid-filled Any remaining protocol required tissues have no visible lesions
9078	TERMINAL KILL	53	(7)	kidneys; dilation; right; moderate (TGL): renal pelvis Any remaining protocol required tissues have no visible lesions
9079	TERMINAL KILL	54	(7)	No Visible Lesions

TGL = Traceable gross lesion

Appendix 12 (continued)
F_1 Individual Gross Pathology
Pubertal Toxicity Study of Vinclozolin, Flutamide and Phenobarbital in Male Sprague Dawley Rats and Methoxychlor, Ethinyl Estradiol and Phenobarbital in Female Sprague Dawley Rats when Administered in Corn Oil by Oral Gavage

Group: 4 Dose Level: 100 mg/kg/day Phenobarbital Sex: Male

Animal Number	Mode Of Death	Death Day	Death (Week)	Observation(s)
9080	TERMINAL KILL	54	(7)	No Visible Lesions
9081	TERMINAL KILL	54	(7)	No Visible Lesions
9082	TERMINAL KILL	54	(7)	No Visible Lesions
9083	TERMINAL KILL	54	(7)	kidneys; dilation; right; moderate (TGL): renal pelvis Any remaining protocol required tissues have no visible lesions
9084	TERMINAL KILL	54	(7)	epididymides; reduction in size; bilateral; minimal (TGL) testes; reduction in size; bilateral; minimal (TGL) thymus; discoloration; left (TGL): dark red Any remaining protocol required tissues have no visible lesions
9085	TERMINAL KILL	54	(7)	epididymides; enlargement; bilateral; minimal (TGL) Any remaining protocol required tissues have no visible lesions

TGL = Traceable gross lesion

Appendix 12 (continued)
F_1 Individual Gross Pathology
Pubertal Toxicity Study of Vinclozolin, Flutamide and Phenobarbital in Male Sprague Dawley Rats and Methoxychlor, Ethinyl Estradiol and Phenobarbital in Female Sprague Dawley Rats when Administered in Corn Oil by Oral Gavage

Group: 5 Dose Level: 10 mg/kg/day Vinclozolin Sex: Male

Animal Number	Mode Of Death	Death Day	(Week)	Observation(s)
9086	TERMINAL KILL	53	(7)	pituitary: Lost At Necropsy Any remaining protocol required tissues have no visible lesions
9087	TERMINAL KILL	53	(7)	No Visible Lesions
9088	TERMINAL KILL	53	(7)	kidneys; dilation; right; moderate (TGL): renal pelvis Any remaining protocol required tissues have no visible lesions
9089	TERMINAL KILL	53	(7)	No Visible Lesions
9090	TERMINAL KILL	53	(7)	No Visible Lesions
9091	TERMINAL KILL	53	(7)	No Visible Lesions
9092	TERMINAL KILL	53	(7)	No Visible Lesions
9093	TERMINAL KILL	53	(7)	No Visible Lesions
9094	TERMINAL KILL	54	(7)	No Visible Lesions
9095	TERMINAL KILL	54	(7)	No Visible Lesions
9096	TERMINAL KILL	54	(7)	No Visible Lesions
9097	TERMINAL KILL	54	(7)	dorsolateral prostate; reduction in size; minimal (TGL) spleen; enlargement (TGL): 52x10x6mm ventral prostate; reduction in size; minimal (TGL) Any remaining protocol required tissues have no visible lesions

TGL = Traceable gross lesion

Appendix 12 (continued)
F_1 Individual Gross Pathology
Pubertal Toxicity Study of Vinclozolin, Flutamide and Phenobarbital in Male Sprague Dawley Rats and Methoxychlor, Ethinyl Estradiol and Phenobarbital in Female Sprague Dawley Rats when Administered in Corn Oil by Oral Gavage

Group: 5 Dose Level: 10 mg/kg/day Vinclozolin Sex: Male

Animal Number	Mode Of Death	Death Day	Death (Week)	Observation(s)
9098	TERMINAL KILL	54	(7)	No Visible Lesions
9099	TERMINAL KILL	54	(7)	kidneys; dilation; right; minimal (TGL): renal pelvis seminal vesicles and coagulating glands with fluid; reduction in size; left; moderate (TGL) Any remaining protocol required tissues have no visible lesions
9100	TERMINAL KILL	54	(7)	kidneys; dilation; right; moderate (TGL): renal pelvis Any remaining protocol required tissues have no visible lesions

TGL = Traceable gross lesion

Appendix 12 (continued)
F_1 Individual Gross Pathology
Pubertal Toxicity Study of Vinclozolin, Flutamide and Phenobarbital in Male Sprague Dawley Rats and Methoxychlor, Ethinyl Estradiol and Phenobarbital in Female Sprague Dawley Rats when Administered in Corn Oil by Oral Gavage

Group: 6 Dose Level: 30 mg/kg/day Vinclozolin Sex: Male

Animal Number	Mode Of Death	Death Day	Death (Week)	Observation(s)
9101	TERMINAL KILL	53	(7)	No Visible Lesions
9102	TERMINAL KILL	53	(7)	No Visible Lesions
9103	TERMINAL KILL	53	(7)	No Visible Lesions
9104	TERMINAL KILL	53	(7)	kidneys; dilation; right; minimal (TGL): renal pelvis Any remaining protocol required tissues have no visible lesions
9105	TERMINAL KILL	53	(7)	No Visible Lesions
9106	TERMINAL KILL	53	(7)	spleen; nodule(s); red (TGL): single; 7x6x3mm Any remaining protocol required tissues have no visible lesions
9107	TERMINAL KILL	53	(7)	No Visible Lesions
9108	TERMINAL KILL	53	(7)	No Visible Lesions
9109	TERMINAL KILL	54	(7)	kidneys; dilation; right; minimal (TGL): renal pelvis Any remaining protocol required tissues have no visible lesions
9110	TERMINAL KILL	54	(7)	kidneys; dilation; right; moderate (TGL): renal pelvis Any remaining protocol required tissues have no visible lesions
9111	TERMINAL KILL	54	(7)	skin; abrasion(s); red; single; present, no grade assigned (TGL): 10x5mm; adjacent to right ear Any remaining protocol required tissues have no visible lesions

TGL = Traceable gross lesion

Appendix 12 (continued)
F_1 Individual Gross Pathology
Pubertal Toxicity Study of Vinclozolin, Flutamide and Phenobarbital in Male Sprague Dawley Rats and Methoxychlor, Ethinyl Estradiol and Phenobarbital in Female Sprague Dawley Rats when Administered in Corn Oil by Oral Gavage

Group: 6 Dose Level: 30 mg/kg/day Vinclozolin Sex: Male

Animal Number	Mode Of Death	Death Day	Death (Week)	Observation(s)
9112	TERMINAL KILL	54	(7)	Fluids lost from both seminal vesicles at necropsy (prior to weighing) kidneys; dilation; right; minimal (TGL): renal pelvis Any remaining protocol required tissues have no visible lesions
9113	TERMINAL KILL	54	(7)	No Visible Lesions
9114	TERMINAL KILL	54	(7)	No Visible Lesions
9115	TERMINAL KILL	54	(7)	No Visible Lesions

TGL = Traceable gross lesion

Appendix 12 (continued)
F_1 Individual Gross Pathology
Pubertal Toxicity Study of Vinclozolin, Flutamide and Phenobarbital in Male Sprague Dawley Rats and Methoxychlor, Ethinyl Estradiol and Phenobarbital in Female Sprague Dawley Rats when Administered in Corn Oil by Oral Gavage

Group: 7 Dose Level: 100 mg/kg/day Vinclozolin Sex: Male

Animal Number	Mode Of Death	Death Day	Death (Week)	Observation(s)
9116	TERMINAL KILL	53	(7)	No Visible Lesions
9117	TERMINAL KILL	53	(7)	No Visible Lesions
9118	TERMINAL KILL	53	(7)	dorsolateral prostate; reduction in size; moderate (TGL) epididymides; reduction in size; bilateral; moderate (TGL) ventral prostate; reduction in size; moderate (TGL) seminal vesicles and coagulating glands with fluid; reduction in size; bilateral; moderate (TGL) Any remaining protocol required tissues have no visible lesions
9119	TERMINAL KILL	53	(7)	kidneys; dilation; right; moderate (TGL): renal pelvis liver; discoloration; pale (TGL): all lobes Any remaining protocol required tissues have no visible lesions
9120	TERMINAL KILL	53	(7)	kidneys; dilation; left; moderate (TGL): renal pelvis Any remaining protocol required tissues have no visible lesions
9121	TERMINAL KILL	53	(7)	kidneys; dilation; right; moderate (TGL): renal pelvis seminal vesicles and coagulating glands with fluid; reduction in size; right; moderate (TGL) Any remaining protocol required tissues have no visible lesions
9122	TERMINAL KILL	53	(7)	kidneys; dilation; right; moderate (TGL): renal pelvis Any remaining protocol required tissues have no visible lesions
9123	TERMINAL KILL	53	(7)	No Visible Lesions

TGL = Traceable gross lesion

Appendix 12 (continued)
F_1 Individual Gross Pathology
Pubertal Toxicity Study of Vinclozolin, Flutamide and Phenobarbital in Male Sprague Dawley Rats and Methoxychlor, Ethinyl Estradiol and Phenobarbital in Female Sprague Dawley Rats when Administered in Corn Oil by Oral Gavage

Group: 7 Dose Level: 100 mg/kg/day Vinclozolin Sex: Male

Animal Number	Mode Of Death	Death Day	Death (Week)	Observation(s)
9124	TERMINAL KILL	54	(7)	No Visible Lesions
9125	TERMINAL KILL	54	(7)	No Visible Lesions
9126	TERMINAL KILL	54	(7)	No Visible Lesions
9127	TERMINAL KILL	54	(7)	kidneys; dilation; right; moderate (TGL): renal pelvis Any remaining protocol required tissues have no visible lesions
9128	TERMINAL KILL	54	(7)	kidneys; discoloration; dark; right; single (TGL): focal kidneys; dilation; right; moderate (TGL): renal pelvis Any remaining protocol required tissues have no visible lesions
9129	TERMINAL KILL	54	(7)	No Visible Lesions
9130	TERMINAL KILL	54	(7)	No Visible Lesions

TGL = Traceable gross lesion

Appendix 12 (continued)
F_1 Individual Gross Pathology
Pubertal Toxicity Study of Vinclozolin, Flutamide and Phenobarbital in Male Sprague Dawley Rats and Methoxychlor, Ethinyl Estradiol and Phenobarbital in Female Sprague Dawley Rats when Administered in Corn Oil by Oral Gavage

Group: 8 Dose Level: 25 mg/kg/day Flutamide Sex: Male

Animal Number	Mode Of Death	Death Day	(Week)	Observation(s)
9131	TERMINAL KILL	53	(7)	dorsolateral prostate; reduction in size; moderate (TGL) ventral prostate; reduction in size; moderate (TGL) seminal vesicles and coagulating glands with fluid; reduction in size; bilateral; moderate (TGL) Any remaining protocol required tissues have no visible lesions
9132	TERMINAL KILL	53	(7)	seminal vesicles and coagulating glands with fluid; reduction in size; bilateral; severe (TGL) Any remaining protocol required tissues have no visible lesions
9133	TERMINAL KILL	53	(7)	seminal vesicles and coagulating glands with fluid; reduction in size; bilateral; moderate (TGL) Any remaining protocol required tissues have no visible lesions
9134	TERMINAL KILL	53	(7)	dorsolateral prostate; reduction in size; moderate (TGL) epididymides; reduction in size; bilateral; moderate (TGL) ventral prostate; reduction in size; moderate (TGL) seminal vesicles and coagulating glands with fluid; reduction in size; bilateral; severe (TGL) Any remaining protocol required tissues have no visible lesions
9135	TERMINAL KILL	53	(7)	seminal vesicles and coagulating glands with fluid; reduction in size; bilateral; moderate (TGL) Any remaining protocol required tissues have no visible lesions
9136	TERMINAL KILL	53	(7)	dorsolateral prostate; reduction in size; moderate (TGL) epididymides; reduction in size; bilateral; moderate (TGL) ventral prostate; reduction in size; moderate (TGL) seminal vesicles and coagulating glands with fluid; reduction in size; bilateral; severe (TGL) Any remaining protocol required tissues have no visible lesions

TGL = Traceable gross lesion

Appendix 12 (continued)
F_1 Individual Gross Pathology
Pubertal Toxicity Study of Vinclozolin, Flutamide and Phenobarbital in Male Sprague Dawley Rats and Methoxychlor, Ethinyl Estradiol and Phenobarbital in Female Sprague Dawley Rats when Administered in Corn Oil by Oral Gavage

Group: 8 Dose Level: 25 mg/kg/day Flutamide Sex: Male

Animal Number	Mode Of Death	Death Day	Death (Week)	Observation(s)
9137	TERMINAL KILL	53	(7)	dorsolateral prostate; reduction in size; moderate (TGL) ventral prostate; reduction in size; moderate (TGL) seminal vesicles and coagulating glands with fluid; reduction in size; bilateral; moderate (TGL) Any remaining protocol required tissues have no visible lesions
9138	TERMINAL KILL	53	(7)	dorsolateral prostate; reduction in size; moderate (TGL) ventral prostate; reduction in size; moderate (TGL) seminal vesicles and coagulating glands with fluid; reduction in size; bilateral; moderate (TGL) Any remaining protocol required tissues have no visible lesions
9139	TERMINAL KILL	54	(7)	epididymides; reduction in size; bilateral; moderate (TGL) seminal vesicles and coagulating glands with fluid; reduction in size; bilateral; moderate (TGL) Any remaining protocol required tissues have no visible lesions
9140	TERMINAL KILL	54	(7)	dorsolateral prostate; reduction in size; moderate (TGL) epididymides; reduction in size; bilateral; moderate (TGL) ventral prostate; reduction in size; moderate (TGL) seminal vesicles and coagulating glands with fluid; reduction in size; bilateral; moderate (TGL) Any remaining protocol required tissues have no visible lesions
9141	TERMINAL KILL	54	(7)	dorsolateral prostate; reduction in size; moderate (TGL) epididymides; reduction in size; bilateral; moderate (TGL) ventral prostate; reduction in size; moderate (TGL) seminal vesicles and coagulating glands with fluid; reduction in size; bilateral; moderate (TGL) Any remaining protocol required tissues have no visible lesions

TGL = Traceable gross lesion

Appendix 12 (continued)
F_1 Individual Gross Pathology
Pubertal Toxicity Study of Vinclozolin, Flutamide and Phenobarbital in Male Sprague Dawley Rats and Methoxychlor, Ethinyl Estradiol and Phenobarbital in Female Sprague Dawley Rats when Administered in Corn Oil by Oral Gavage

Group: 8 Dose Level: 25 mg/kg/day Flutamide Sex: Male

Animal Number	Mode Of Death	Death Day	Death (Week)	Observation(s)
9142	TERMINAL KILL	54	(7)	No Visible Lesions
9143	TERMINAL KILL	54	(7)	dorsolateral prostate; reduction in size; moderate (TGL) ventral prostate; reduction in size; moderate (TGL) seminal vesicles and coagulating glands with fluid; reduction in size; bilateral; moderate (TGL) Any remaining protocol required tissues have no visible lesions
9144	TERMINAL KILL	54	(7)	dorsolateral prostate; reduction in size; moderate (TGL) ventral prostate; reduction in size; moderate (TGL) seminal vesicles and coagulating glands with fluid; reduction in size; bilateral; moderate (TGL) Any remaining protocol required tissues have no visible lesions
9145	TERMINAL KILL	54	(7)	dorsolateral prostate; reduction in size; minimal (TGL) ventral prostate; reduction in size; minimal (TGL) seminal vesicles and coagulating glands with fluid; reduction in size; bilateral; minimal (TGL) Any remaining protocol required tissues have no visible lesions

TGL = Traceable gross lesion

Appendix 12 (continued)
F_1 Individual Gross Pathology
Pubertal Toxicity Study of Vinclozolin, Flutamide and Phenobarbital in Male Sprague Dawley Rats and Methoxychlor, Ethinyl Estradiol and Phenobarbital in Female Sprague Dawley Rats when Administered in Corn Oil by Oral Gavage

Group: 9 Dose Level: 50 mg/kg/day Flutamide Sex: Male

Animal Number	Mode Of Death	Death Day	Death (Week)	Observation(s)
9146	TERMINAL KILL	53	(7)	seminal vesicles and coagulating glands with fluid; reduction in size; bilateral; severe (TGL) Any remaining protocol required tissues have no visible lesions
9147	TERMINAL KILL	53	(7)	dorsolateral prostate; reduction in size; moderate (TGL) epididymides; reduction in size; bilateral; moderate (TGL) levator ani plus bulbocavernosus; reduction in size; minimal (TGL) ventral prostate; reduction in size; moderate (TGL) seminal vesicles and coagulating glands with fluid; reduction in size; bilateral; severe (TGL) Any remaining protocol required tissues have no visible lesions
9148	TERMINAL KILL	53	(7)	seminal vesicles and coagulating glands with fluid; reduction in size; bilateral; severe (TGL) Any remaining protocol required tissues have no visible lesions
9149	TERMINAL KILL	53	(7)	dorsolateral prostate; reduction in size; severe (TGL) epididymides; reduction in size; bilateral; moderate (TGL) kidneys; dilation; right; moderate (TGL): renal pelvis ventral prostate; reduction in size; severe (TGL) seminal vesicles and coagulating glands with fluid; reduction in size; bilateral; severe (TGL) Any remaining protocol required tissues have no visible lesions
9150	TERMINAL KILL	53	(7)	dorsolateral prostate; reduction in size; moderate (TGL) epididymides; reduction in size; bilateral; minimal (TGL) ventral prostate; reduction in size; moderate (TGL) seminal vesicles and coagulating glands with fluid; reduction in size; bilateral; severe (TGL) Any remaining protocol required tissues have no visible lesions

TGL = Traceable gross lesion

Appendix 12 (continued)
F_1 Individual Gross Pathology
Pubertal Toxicity Study of Vinclozolin, Flutamide and Phenobarbital in Male Sprague Dawley Rats and Methoxychlor, Ethinyl Estradiol and Phenobarbital in Female Sprague Dawley Rats when Administered in Corn Oil by Oral Gavage

Group: 9 Dose Level: 50 mg/kg/day Flutamide Sex: Male

Animal Number	Mode Of Death	Death Day	Death (Week)	Observation(s)
9151	TERMINAL KILL	53	(7)	dorsolateral prostate; reduction in size; moderate (TGL) ventral prostate; reduction in size; moderate (TGL) seminal vesicles and coagulating glands with fluid; reduction in size; bilateral; moderate (TGL) Any remaining protocol required tissues have no visible lesions
9152	TERMINAL KILL	53	(7)	epididymides; reduction in size; bilateral; moderate (TGL) testes; enlargement; bilateral; minimal (TGL) seminal vesicles and coagulating glands with fluid; reduction in size; bilateral; moderate (TGL) Any remaining protocol required tissues have no visible lesions
9153	TERMINAL KILL	53	(7)	dorsolateral prostate; reduction in size; moderate (TGL) ventral prostate; reduction in size; moderate (TGL) seminal vesicles and coagulating glands with fluid; reduction in size; bilateral moderate (TGL) Any remaining protocol required tissues have no visible lesions
9154	TERMINAL KILL	54	(7)	dorsolateral prostate; reduction in size; moderate (TGL) epididymides; reduction in size; right; minimal (TGL) testes; enlargement; bilateral; minimal (TGL) levator ani plus bulbocavernosus; reduction in size; bilateral; moderate (TGL) ventral prostate; reduction in size; moderate (TGL) seminal vesicles and coagulating glands with fluid; reduction in size; bilateral; moderate (TGL) Any remaining protocol required tissues have no visible lesions

TGL = Traceable gross lesion

Appendix 12 (continued)
F_1 Individual Gross Pathology
Pubertal Toxicity Study of Vinclozolin, Flutamide and Phenobarbital in Male Sprague Dawley Rats and Methoxychlor, Ethinyl Estradiol and Phenobarbital in Female Sprague Dawley Rats when Administered in Corn Oil by Oral Gavage

Group: 9 Dose Level: 50 mg/kg/day Flutamide Sex: Male

Animal Number	Mode Of Death	Death Day	Death (Week)	Observation(s)
9155	TERMINAL KILL	54	(7)	dorsolateral prostate; reduction in size; moderate (TGL) ventral prostate; reduction in size; moderate (TGL) seminal vesicles and coagulating glands with fluid; reduction in size; bilateral; moderate (TGL) Any remaining protocol required tissues have no visible lesions
9156	TERMINAL KILL	54	(7)	dorsolateral prostate; reduction in size; moderate (TGL) epididymides; reduction in size; bilateral; moderate (TGL) levator ani plus bulbocavernosus; reduction in size; bilateral; minimal (TGL) ventral prostate; reduction in size; moderate (TGL) seminal vesicles and coagulating glands with fluid; reduction in size; bilateral; moderate (TGL) Any remaining protocol required tissues have no visible lesions
9157	TERMINAL KILL	54	(7)	dorsolateral prostate; reduction in size; moderate (TGL) ventral prostate; reduction in size; moderate (TGL) seminal vesicles and coagulating glands with fluid; reduction in size; bilateral; moderate (TGL) Any remaining protocol required tissues have no visible lesions
9158	TERMINAL KILL	54	(7)	dorsolateral prostate; reduction in size; moderate (TGL) ventral prostate; reduction in size; moderate (TGL) seminal vesicles and coagulating glands with fluid; reduction in size; bilateral; moderate (TGL) Any remaining protocol required tissues have no visible lesions

TGL = Traceable gross lesion

Appendix 12 (continued)
F_1 Individual Gross Pathology
Pubertal Toxicity Study of Vinclozolin, Flutamide and Phenobarbital in Male Sprague Dawley Rats and Methoxychlor, Ethinyl Estradiol and Phenobarbital in Female Sprague Dawley Rats when Administered in Corn Oil by Oral Gavage

Group: 9 Dose Level: 50 mg/kg/day Flutamide Sex: Male

Animal Number	Mode Of Death	Death Day	Death (Week)	Observation(s)
9159	TERMINAL KILL	54	(7)	dorsolateral prostate; reduction in size; moderate (TGL) kidneys; dilation; right; minimal (TGL): renal pelvis testes; enlargement; left (TGL): 27x13x12mm ventral prostate; reduction in size; moderate (TGL) seminal vesicles and coagulating glands with fluid; reduction in size; bilateral; moderate (TGL) Any remaining protocol required tissues have no visible lesions
9160	TERMINAL KILL	54	(7)	dorsolateral prostate; reduction in size; moderate (TGL) epididymides; reduction in size; bilateral; moderate (TGL) levator ani plus bulbocavernosus; reduction in size; bilateral; moderate (TGL) ventral prostate; reduction in size; moderate (TGL) seminal vesicles and coagulating glands with fluid; reduction in size; bilateral; moderate (TGL) Any remaining protocol required tissues have no visible lesions

TGL = Traceable gross lesion

Appendix 12 (continued)
F_1 Individual Gross Pathology
Pubertal Toxicity Study of Vinclozolin, Flutamide and Phenobarbital in Male Sprague Dawley Rats and Methoxychlor, Ethinyl Estradiol and Phenobarbital in Female Sprague Dawley Rats when Administered in Corn Oil by Oral Gavage

Group: 1 Dose Level: 0 mg/kg/day Sex: Female

Animal Number	Mode Of Death	Death Day	(Week)	Observation(s)
9161	TERMINAL KILL	42	(6)	No Visible Lesions
9162	TERMINAL KILL	42	(6)	No Visible Lesions
9163	TERMINAL KILL	42	(6)	No Visible Lesions
9164	TERMINAL KILL	42	(6)	uterus and cervix without fluid; distension; bilateral; moderate (TGL): uterus Any remaining protocol required tissues have no visible lesions
9165	TERMINAL KILL	42	(6)	No Visible Lesions
9166	TERMINAL KILL	42	(6)	No Visible Lesions
9167	TERMINAL KILL	42	(6)	kidneys; dilation; right; minimal (TGL): renal pelvis Any remaining protocol required tissueshave no visible lesions
9168	TERMINAL KILL	42	(6)	No Visible Lesions
9169	TERMINAL KILL	43	(6)	kidneys; discoloration; dark; bilateral; multifocal (TGL) Any remaining protocol required tissues have no visible lesions
9170	TERMINAL KILL	43	(6)	uterus and cervix without fluid; distension; bilateral; moderate (TGL): uterus Any remaining protocol required tissues have no visible lesions
9171	TERMINAL KILL	43	(6)	liver; discoloration; pale (TGL): all lobes uterus and cervix without fluid; distension; bilateral; moderate (TGL): uterus Any remaining protocol required tissues have no visible lesions
9172	TERMINAL KILL	43	(6)	pituitary: Damaged At Necropsy Any remaining protocol required tissues have no visible lesions

TGL = Traceable gross lesion

Appendix 12 (continued)
F_1 Individual Gross Pathology
Pubertal Toxicity Study of Vinclozolin, Flutamide and Phenobarbital in Male Sprague Dawley Rats and Methoxychlor, Ethinyl Estradiol and Phenobarbital in Female Sprague Dawley Rats when Administered in Corn Oil by Oral Gavage

Group: 1 Dose Level: 0 mg/kg/day Sex: Female

Animal Number	Mode Of Death	Death Day	Death (Week)	Observation(s)
9173	TERMINAL KILL	43	(6)	No Visible Lesions
9174	TERMINAL KILL	43	(6)	uterus and cervix without fluid; distension; bilateral; minimal (TGL): uterus Any remaining protocol required tissues have no visible lesions
9175	TERMINAL KILL	43	(6)	No Visible Lesions

TGL = Traceable gross lesion

Appendix 12 (continued)
F_1 Individual Gross Pathology
Pubertal Toxicity Study of Vinclozolin, Flutamide and Phenobarbital in Male Sprague Dawley Rats and Methoxychlor, Ethinyl Estradiol and Phenobarbital in Female Sprague Dawley Rats when Administered in Corn Oil by Oral Gavage

Group: 2 Dose Level: 0.0025 mg/kg/day Ethinyl Estradiol Sex: Female

Animal Number	Mode Of Death	Death Day	Death (Week)	Observation(s)
9176	TERMINAL KILL	42	(6)	No Visible Lesions
9177	TERMINAL KILL	42	(6)	pituitary: Damaged At Necropsy Any remaining protocol required tissues have no visible lesions
9178	TERMINAL KILL	42	(6)	No Visible Lesions
9179	TERMINAL KILL	42	(6)	No Visible Lesions
9180	TERMINAL KILL	42	(6)	No Visible Lesions
9181	TERMINAL KILL	42	(6)	kidneys; dilation; right; minimal (TGL): renal pelvis pituitary: Damaged At Necropsy uterus and cervix without fluid: Left Damaged At Necropsy uterus and cervix without fluid; distension; bilateral; moderate (TGL): uterus Any remaining protocol required tissues have no visible lesions
9182	TERMINAL KILL	42	(6)	No Visible Lesions
9183	TERMINAL KILL	42	(6)	No Visible Lesions
9184	TERMINAL KILL	43	(6)	No Visible Lesions
9185	TERMINAL KILL	43	(6)	No Visible Lesions
9186	TERMINAL KILL	43	(6)	ovaries; discoloration; red; right; single (TGL) Any remaining protocol required tissues have no visible lesions
9187	TERMINAL KILL	43	(6)	pituitary: Damaged At Necropsy Any remaining protocol required tissues have no visible lesions

TGL = Traceable gross lesion

Appendix 12 (continued)
F_1 Individual Gross Pathology
Pubertal Toxicity Study of Vinclozolin, Flutamide and Phenobarbital in Male Sprague Dawley Rats and Methoxychlor, Ethinyl Estradiol and Phenobarbital in Female Sprague Dawley Rats when Administered in Corn Oil by Oral Gavage

Group: 2 Dose Level: 0.0025 mg/kg/day Ethinyl Estradiol Sex: Female

Animal Number	Mode Of Death	Death Day	Death (Week)	Observation(s)
9188	TERMINAL KILL	43	(6)	uterus and cervix without fluid; distension; left; moderate (TGL): uterus Any remaining protocol required tissues have no visible lesions
9189	TERMINAL KILL	43	(6)	No Visible Lesions
9190	TERMINAL KILL	43	(6)	No Visible Lesions

TGL = Traceable gross lesion

Appendix 12 (continued)
F_1 Individual Gross Pathology
Pubertal Toxicity Study of Vinclozolin, Flutamide and Phenobarbital in Male Sprague Dawley Rats and Methoxychlor, Ethinyl Estradiol and Phenobarbital in Female Sprague Dawley Rats when Administered in Corn Oil by Oral Gavage

Group: 3 Dose Level: 0.005 mg/kg/day Ethinyl Estradiol Sex: Female

Animal Number	Mode Of Death	Death Day	Death (Week)	Observation(s)
9191	TERMINAL KILL	42	(6)	No Visible Lesions
9192	TERMINAL KILL	42	(6)	No Visible Lesions
9193	TERMINAL KILL	42	(6)	uterus and cervix without fluid; distension; bilateral; moderate (TGL): uterus Any remaining protocol required tissues have no visible lesions
9194	TERMINAL KILL	42	(6)	No Visible Lesions
9195	TERMINAL KILL	42	(6)	No Visible Lesions
9196	TERMINAL KILL	42	(6)	No Visible Lesions
9197	TERMINAL KILL	42	(6)	kidneys; dilation; right; minimal (TGL): renal pelvis tail; irregularly shaped: multiple kinks Any remaining protocol required tissues have no visible lesions
9198	TERMINAL KILL	42	(6)	No Visible Lesions
9199	TERMINAL KILL	43	(6)	kidneys; dilation; right; minimal (TGL): renal pelvis Any remaining protocol required tissues have no visible lesions
9200	TERMINAL KILL	43	(6)	No Visible Lesions
9201	TERMINAL KILL	43	(6)	No Visible Lesions
9202	TERMINAL KILL	43	(6)	No Visible Lesions

TGL = Traceable gross lesion

Appendix 12 (continued)
F_1 Individual Gross Pathology
Pubertal Toxicity Study of Vinclozolin, Flutamide and Phenobarbital in Male Sprague Dawley Rats and Methoxychlor, Ethinyl Estradiol and Phenobarbital in Female Sprague Dawley Rats when Administered in Corn Oil by Oral Gavage

Group: 3 Dose Level: 0.005 mg/kg/day Ethinyl Estradiol Sex: Female

Animal Number	Mode Of Death	Death Day	Death (Week)	Observation(s)
9203	TERMINAL KILL	43	(6)	No Visible Lesions
9204	TERMINAL KILL	43	(6)	No Visible Lesions
9205	TERMINAL KILL	43	(6)	No Visible Lesions

TGL = Traceable gross lesion

Appendix 12 (continued)
F_1 Individual Gross Pathology
Pubertal Toxicity Study of Vinclozolin, Flutamide and Phenobarbital in Male Sprague Dawley Rats and Methoxychlor, Ethinyl Estradiol and Phenobarbital in Female Sprague Dawley Rats when Administered in Corn Oil by Oral Gavage

Group: 4 Dose Level: 12.5 mg/kg/day Methoxychlor Sex: Female

Animal Number	Mode Of Death	Death Day	Death (Week)	Observation(s)
9206	TERMINAL KILL	42	(6)	No Visible Lesions
9207	TERMINAL KILL	42	(6)	No Visible Lesions
9208	TERMINAL KILL	42	(6)	No Visible Lesions
9209	TERMINAL KILL	42	(6)	kidneys; discoloration; dark; bilateral; multifocal (TGL) Any remaining protocol required tissues have no visible lesions
9210	TERMINAL KILL	42	(6)	No Visible Lesions
9211	TERMINAL KILL	42	(6)	kidneys; dilation; right; moderate (TGL): renal pelvis Any remaining protocol required tissues have no visible lesions
9212	TERMINAL KILL	42	(6)	No Visible Lesions
9213	TERMINAL KILL	42	(6)	No Visible Lesions
9214	TERMINAL KILL	43	(6)	kidneys; cyst(s); clear; bilateral; focal (TGL): left - one; right - one Any remaining protocol required tissues have no visible lesions
9215	TERMINAL KILL	43	(6)	No Visible Lesions
9216	TERMINAL KILL	43	(6)	No Visible Lesions
9217	TERMINAL KILL	43	(6)	No Visible Lesions
9218	TERMINAL KILL	43	(6)	uterus and cervix without fluid; distension; bilateral; moderate (TGL): uterus Any remaining protocol required tissues have no visible lesions

TGL = Traceable gross lesion

Appendix 12 (continued)
F_1 Individual Gross Pathology
Pubertal Toxicity Study of Vinclozolin, Flutamide and Phenobarbital in Male Sprague Dawley Rats and Methoxychlor, Ethinyl Estradiol and Phenobarbital in Female Sprague Dawley Rats when Administered in Corn Oil by Oral Gavage

Group: 4 Dose Level: 12.5 mg/kg/day Methoxychlor Sex: Female

Animal Number	Mode Of Death	Death Day	Death (Week)	Observation(s)
9219	TERMINAL KILL	43	(6)	No Visible Lesions
9220	TERMINAL KILL	43	(6)	kidneys; discoloration; dark; bilateral; multifocal (TGL) kidneys; dilation; right; minimal (TGL): renal pelvis uterus and cervix without fluid; distension; bilateral; minimal (TGL): uterus Any remaining protocol required tissues have no visible lesions

TGL = Traceable gross lesion

Appendix 12 (continued)
F_1 Individual Gross Pathology
Pubertal Toxicity Study of Vinclozolin, Flutamide and Phenobarbital in Male Sprague Dawley Rats and Methoxychlor, Ethinyl Estradiol and Phenobarbital in Female Sprague Dawley Rats when Administered in Corn Oil by Oral Gavage

Group: 5 Dose Level: 25 mg/kg/day Methoxychlor Sex: Female

Animal Number	Mode Of Death	Death Day	Death (Week)	Observation(s)
9221	TERMINAL KILL	42	(6)	No Visible Lesions
9222	TERMINAL KILL	42	(6)	No Visible Lesions
9223	TERMINAL KILL	42	(6)	No Visible Lesions
9224	TERMINAL KILL	42	(6)	No Visible Lesions
9225	TERMINAL KILL	42	(6)	No Visible Lesions
9226	TERMINAL KILL	42	(6)	No Visible Lesions
9227	TERMINAL KILL	42	(6)	spleen; enlargement (TGL): 47x12x7mm Any remaining protocol required tissues have no visible lesions
9228	TERMINAL KILL	42	(6)	No Visible Lesions
9229	TERMINAL KILL	43	(6)	kidneys; dilation; right; moderate (TGL): renal pelvis Any remaining protocol required tissues have no visible lesions
9230	TERMINAL KILL	43	(6)	No Visible Lesions
9231	TERMINAL KILL	43	(6)	spleen; enlargement (TGL): 46x12x5mm Any remaining protocol required tissues have no visible lesions
9232	TERMINAL KILL	43	(6)	adrenal glands; discoloration; pale; bilateral (TGL) Any remaining protocol required tissues have no visible lesions

TGL = Traceable gross lesion

Appendix 12 (continued)
F_1 Individual Gross Pathology
Pubertal Toxicity Study of Vinclozolin, Flutamide and Phenobarbital in Male Sprague Dawley Rats and Methoxychlor, Ethinyl Estradiol and Phenobarbital in Female Sprague Dawley Rats when Administered in Corn Oil by Oral Gavage

Group: 5 Dose Level: 25 mg/kg/day Methoxychlor Sex: Female

Animal Number	Mode Of Death	Death Day	Death (Week)	Observation(s)
9233	TERMINAL KILL	43	(6)	No Visible Lesions
9234	TERMINAL KILL	43	(6)	No Visible Lesions
9235	TERMINAL KILL	43	(6)	uterus and cervix without fluid; distension; bilateral; moderate (TGL): uterus Any remaining protocol required tissues have no visible lesions

TGL = Traceable gross lesion

Appendix 12 (continued)
F_1 Individual Gross Pathology
Pubertal Toxicity Study of Vinclozolin, Flutamide and Phenobarbital in Male Sprague Dawley Rats and Methoxychlor, Ethinyl Estradiol and Phenobarbital in Female Sprague Dawley Rats when Administered in Corn Oil by Oral Gavage

Group: 6 Dose Level: 50 mg/kg/day Methoxychlor Sex: Female

Animal Number	Mode Of Death	Death Day	Death (Week)	Observation(s)
9236	TERMINAL KILL	42	(6)	No Visible Lesions
9237	TERMINAL KILL	42	(6)	No Visible Lesions
9238	TERMINAL KILL	42	(6)	No Visible Lesions
9239	TERMINAL KILL	42	(6)	No Visible Lesions
9240	TERMINAL KILL	42	(6)	No Visible Lesions
9241	TERMINAL KILL	42	(6)	uterus and cervix without fluid; distension; bilateral; moderate (TGL): uterus Any remaining protocol required tissues have no visible lesions
9242	TERMINAL KILL	42	(6)	uterus and cervix without fluid; distension; bilateral; moderate (TGL): uterus Any remaining protocol required tissues have no visible lesions
9243	TERMINAL KILL	42	(6)	kidneys; dilation; right; severe (TGL): renal pelvis Any remaining protocol required tissues have no visible lesions
9244	TERMINAL KILL	43	(6)	No Visible Lesions
9245	TERMINAL KILL	43	(6)	No Visible Lesions
9246	TERMINAL KILL	43	(6)	ovaries; discoloration; red; left; focal (TGL) Any remaining protocol required tissues have no visible lesions
9247	TERMINAL KILL	43	(6)	No Visible Lesions

TGL = Traceable gross lesion

Appendix 12 (continued)
F_1 Individual Gross Pathology
Pubertal Toxicity Study of Vinclozolin, Flutamide and Phenobarbital in Male Sprague Dawley Rats and Methoxychlor, Ethinyl Estradiol and Phenobarbital in Female Sprague Dawley Rats when Administered in Corn Oil by Oral Gavage

Group: 6 Dose Level: 50 mg/kg/day Methoxychlor Sex: Female

Animal Number	Mode Of Death	Death Day	Death (Week)	Observation(s)
9248	TERMINAL KILL	43	(6)	uterus and cervix without fluid; distension; bilateral; moderate (TGL): uterus Any remaining protocol required tissues have no visible lesions
9249	TERMINAL KILL	43	(6)	No Visible Lesions
9250	TERMINAL KILL	43	(6)	No Visible Lesions

TGL = Traceable gross lesion

Appendix 12 (continued)
F_1 Individual Gross Pathology
Pubertal Toxicity Study of Vinclozolin, Flutamide and Phenobarbital in Male Sprague Dawley Rats and Methoxychlor, Ethinyl Estradiol and Phenobarbital in Female Sprague Dawley Rats when Administered in Corn Oil by Oral Gavage

Group: 7 Dose Level: 25 mg/kg/day Phenobarbital Sex: Female

Animal Number	Mode Of Death	Death Day	Death (Week)	Observation(s)
9251	TERMINAL KILL	42	(6)	kidneys; discoloration; dark; left; single (TGL): pinpoint Any remaining protocol required tissues have no visible lesions
9252	TERMINAL KILL	42	(6)	kidneys; dilation; right; present, no grade assigned (TGL): renal pelvis Any remaining protocol required tissues have no visible lesions
9253	TERMINAL KILL	42	(6)	tail; abrasion(s); single; present, no grade assigned (TGL): tip uterus and cervix without fluid; distension; bilateral; moderate (TGL): uterus Any remaining protocol required tissues have no visible lesions
9254	TERMINAL KILL	42	(6)	No Visible Lesions
9255	TERMINAL KILL	42	(6)	No Visible Lesions
9256	TERMINAL KILL	42	(6)	uterus and cervix without fluid; distension; bilateral; moderate (TGL): uterus Any remaining protocol required tissues have no visible lesions
9257	TERMINAL KILL	42	(6)	No Visible Lesions
9258	TERMINAL KILL	42	(6)	No Visible Lesions
9259	TERMINAL KILL	43	(6)	uterus and cervix without fluid; distension; bilateral; minimal (TGL): uterus Any remaining protocol required tissues have no visible lesions
9260	TERMINAL KILL	43	(6)	kidneys; discoloration; dark; bilateral; multifocal (TGL) Any remaining protocol required tissues have no visible lesions
9261	TERMINAL KILL	43	(6)	No Visible Lesions

TGL = Traceable gross lesion

Appendix 12 (continued)
F_1 Individual Gross Pathology
Pubertal Toxicity Study of Vinclozolin, Flutamide and Phenobarbital in Male Sprague Dawley Rats and Methoxychlor, Ethinyl Estradiol and Phenobarbital in Female Sprague Dawley Rats when Administered in Corn Oil by Oral Gavage

Group: 7 Dose Level: 25 mg/kg/day Phenobarbital Sex: Female

Animal Number	Mode Of Death	Death Day	Death (Week)	Observation(s)
9262	TERMINAL KILL	43	(6)	adrenal glands; enlargement; right (TGL): 5x5x5mm kidneys; cyst(s); clear; right; single (TGL): 40x25x20mm kidneys; dilation; left; minimal (TGL) ovaries; discoloration; red; bilateral; multifocal (TGL) ureter(s); dilation; right; severe (TGL) Any remaining protocol required tissues have no visible lesions
9263	TERMINAL KILL	43	(6)	No Visible Lesions
9264	TERMINAL KILL	43	(6)	pituitary: Damaged At Necropsy Any remaining protocol required tissues have no visible lesions
9265	TERMINAL KILL	43	(6)	No Visible Lesions

TGL = Traceable gross lesion

Appendix 12 (continued)
F_1 Individual Gross Pathology
Pubertal Toxicity Study of Vinclozolin, Flutamide and Phenobarbital in Male Sprague Dawley Rats and Methoxychlor, Ethinyl Estradiol and Phenobarbital in Female Sprague Dawley Rats when Administered in Corn Oil by Oral Gavage

Group: 8 Dose Level: 50 mg/kg/day Phenobarbital Sex: Female

Animal Number	Mode Of Death	Death Day	Death (Week)	Observation(s)
9266	TERMINAL KILL	42	(6)	No Visible Lesions
9267	TERMINAL KILL	42	(6)	No Visible Lesions
9268	TERMINAL KILL	42	(6)	No Visible Lesions
9269	TERMINAL KILL	42	(6)	ovaries; cyst(s); clear; right; single; minimal (TGL): 3x3x3mm Any remaining protocol required tissues, which have been examined, have no visible lesions
9270	TERMINAL KILL	42	(6)	No Visible Lesions
9271	TERMINAL KILL	42	(6)	No Visible Lesions
9272	TERMINAL KILL	42	(6)	kidneys; dilation; right; moderate (TGL): renal pelvis Any remaining protocol required tissues have no visible lesions
9273	TERMINAL KILL	42	(6)	pituitary: Damaged At Necropsy Any remaining protocol required tissues have no visible lesions
9274	TERMINAL KILL	43	(6)	kidneys; discoloration; dark; bilateral; multifocal (TGL) pituitary: Damaged At Necropsy Any remaining protocol required tissues, which have been examined, have no visible lesions
9275	TERMINAL KILL	43	(6)	kidneys; dilation; right; moderate (TGL): renal pelvis uterus and cervix without fluid; distension; bilateral; minimal (TGL): uterus Any remaining protocol required tissues have no visible lesions

TGL = Traceable gross lesion

Appendix 12 (continued)
F_1 Individual Gross Pathology
Pubertal Toxicity Study of Vinclozolin, Flutamide and Phenobarbital in Male Sprague Dawley Rats and Methoxychlor, Ethinyl Estradiol and Phenobarbital in Female Sprague Dawley Rats when Administered in Corn Oil by Oral Gavage

Group: 8 Dose Level: 50 mg/kg/day Phenobarbital Sex: Female

Animal Number	Mode Of Death	Death Day	Death (Week)	Observation(s)
9276	TERMINAL KILL	43	(6)	kidneys; discoloration; white; bilateral; multifocal (TGL) spleen; enlargement (TGL): 40x14x5mm urinary bladder; calculus(i); white; multiple; present, no grade assigned (TGL) uterus and cervix without fluid; distension; bilateral; minimal (TGL): uterus Any remaining protocol required tissues have no visible lesions
9277	TERMINAL KILL	43	(6)	No Visible Lesions
9278	TERMINAL KILL	43	(6)	uterus and cervix without fluid; distension; bilateral; moderate (TGL): uterus Any remaining protocol required tissues have no visible lesions
9279	TERMINAL KILL	43	(6)	No Visible Lesions
9280	TERMINAL KILL	43	(6)	No Visible Lesions

TGL = Traceable gross lesion

Appendix 12 (continued)
F_1 Individual Gross Pathology
Pubertal Toxicity Study of Vinclozolin, Flutamide and Phenobarbital in Male Sprague Dawley Rats and Methoxychlor, Ethinyl Estradiol and Phenobarbital in Female Sprague Dawley Rats when Administered in Corn Oil by Oral Gavage

Group: 9 Dose: 100 mg/kg/day Phenobarbital Sex: Female

Animal Number	Mode Of Death	Death Day	Death (Week)	Observation(s)
9281	TERMINAL KILL	42	(6)	No Visible Lesions
9282	TERMINAL KILL	42	(6)	No Visible Lesions
9283	TERMINAL KILL	42	(6)	kidneys; dilation; right; minimal (TGL): renal pelvis Any remaining protocol required tissues have no visible lesions
9284	TERMINAL KILL	42	(6)	No Visible Lesions
9285	TERMINAL KILL	42	(6)	uterus and cervix without fluid; distension; bilateral; moderate (TGL): uterus Any remaining protocol required tissues have no visible lesions
9286	TERMINAL KILL	42	(6)	uterus and cervix without fluid; distension; bilateral; moderate (TGL): uterus Any remaining protocol required tissues have no visible lesions
9287	TERMINAL KILL	42	(6)	No Visible Lesions
9288	TERMINAL KILL	42	(6)	No Visible Lesions
9289	TERMINAL KILL	43	(6)	No Visible Lesions
9290	TERMINAL KILL	43	(6)	No Visible Lesions
9291	TERMINAL KILL	43	(6)	kidneys; enlargement; bilateral; moderate (TGL) kidneys; dilation; bilateral; severe (TGL) pituitary: Lost At Necropsy urinary bladder; thickening; severe (TGL) urinary bladder; calculus(i); white; multiple; present, no grade assigned (TGL) Any remaining protocol required tissues have no visible lesions

TGL = Traceable gross lesion

Appendix 12 (continued)
F_1 Individual Gross Pathology
Pubertal Toxicity Study of Vinclozolin, Flutamide and Phenobarbital in Male Sprague Dawley Rats and Methoxychlor, Ethinyl Estradiol and Phenobarbital in Female Sprague Dawley Rats when Administered in Corn Oil by Oral Gavage

Group: 9 Dose: 100 mg/kg/day Phenobarbital Sex: Female

Animal Number	Mode Of Death	Death Day	Death (Week)	Observation(s)
9292	TERMINAL KILL	43	(6)	ovaries; discoloration; red; bilateral; multifocal (TGL) Any remaining protocol required tissues have no visible lesions
9293	TERMINAL KILL	43	(6)	kidneys; dilation; right; minimal (TGL): renal pelvis Any remaining protocol required tissues have no visible lesions
9294	TERMINAL KILL	43	(6)	uterus and cervix without fluid; distension; bilateral; moderate (TGL): uterus Any remaining protocol required tissues have no visible lesions
9295	TERMINAL KILL	43	(6)	No Visible Lesions

TGL = Traceable gross lesion

Appendix 13
Individual Organ Weights
Pubertal Toxicity Study of Vinclozolin, Flutamide and Phenobarbital in Male Sprague Dawley Rats and Methoxychlor, Ethinyl Estradiol and Phenobarbital in Female Sprague Dawley Rats when Administered in Corn Oil by Oral Gavage

Appendix 13
Individual Organ Weights (Grams) – F_1 Males
Pubertal Toxicity Study of Vinclozolin, Flutamide and Phenobarbital in Male Sprague Dawley Rats and Methoxychlor, Ethinyl Estradiol and Phenobarbital in Female Sprague Dawley Rats when Administered in Corn Oil by Oral Gavage

Group 1 Dose Level: 0 mg/kg/day

Animal Number	Adrenal Glands (paired)	Right Epididy-mis	Epididy-mides (paired)	Kidneys (paired)	Levator Ani plus B-C	Liver	Pituita-ry	Seminal Ves. 1	Seminal Ves. 2	Testes (paired)	Ventral Prostate	Dorso-Lateral Prostate	Thyroid /Para
9026	0.0441	0.2813	0.6057	3.3434	0.6767	17.4701	0.0101	0.6935	0.4075	3.0150	0.3140	0.2471	0.0154
9027	0.0630	0.2502	0.5635	3.0861	0.6824	15.6929	0.0122	0.5295	0.3476	2.7329	0.1854	0.2514	0.0182
9028	0.0398	0.2152	0.4279	3.0780	0.3421	16.5660	0.0109	0.6274	0.3486	2.9420	0.2118	0.2395	0.0185
9029	0.0455	0.2626	0.5546	2.8932	0.5236	14.4125	0.0103	0.6354	0.3722	2.6643	0.2317	0.2490	0.0234
9030	0.0462	0.2245	0.4565	3.0071	0.7802	15.0086	0.0112	0.6941	0.3605	2.5333	0.2262	0.3415	0.0266
9031	0.0519	0.2911	0.6125	3.0392	0.5630	17.5130	0.0102	0.7673	0.5944	2.7692	0.2067	0.2794	0.0241
9032	0.0530	0.2436	0.5175	3.0263	0.4618	17.9140	0.0119	0.5795	0.4011	2.7645	0.1794	0.1579	0.0260
9033	0.0543	0.1975	0.4068	3.0884	0.6638	14.1542	0.0082	0.3624	0.2120	2.7231	0.2574	0.2785	0.0216
9034	0.0519	0.2807	0.5413	2.7682	0.3408	17.3192	0.0111	0.5652	0.4137	2.5154	0.2552	0.2875	0.0186
9035	0.0450	0.2522	0.5433	3.1702	0.6770	18.3111	0.0077	0.6046	0.3119	2.7021	0.3193	0.2768	0.0137
9036	0.0674	0.2777	0.5955	3.0208	0.6828	17.5587	0.0120	0.8088	0.4940	2.7233	0.2728	0.3139	0.0208
9037	0.0345	0.2942	0.5727	3.1367	0.6858	14.7177	0.0112	1.0544	0.5864	2.5901	0.2756	0.2310	0.0196
9038	0.0475	0.2368	0.4593	3.1639	0.7836	16.0926	0.0086	0.9156	0.4748	2.7978	0.2027	0.2269	0.0190
9039	0.0482	0.2346	0.5135	3.4854	0.5552	16.7382	0.0092	0.7211	0.3818	2.6963	0.2986	0.2721	0.0315
9040	0.0467	0.2361	0.4764	3.4019	0.5605	19.8283	0.0114	0.9119	0.4567	2.3835	0.1396	0.3337	0.0277

B-C = Bulbocavernosus muscle
Seminal Ves. 1 = Seminal vesicles and coagulating glands, with fluid
Seminal Ves. 2 = Seminal vesicles and coagulating glands, without fluid
Thyroid/Para = Thyroid and parathyroid, post-fixation

Appendix 13 (continued)
Individual Organ Weights (Grams) – F_1 Males
Pubertal Toxicity Study of Vinclozolin, Flutamide and Phenobarbital in Male Sprague Dawley Rats and Methoxychlor, Ethinyl Estradiol and Phenobarbital in Female Sprague Dawley Rats when Administered in Corn Oil by Oral Gavage

Group 2 Dose Level: 25 mg/kg/day Phenobarbital

Animal Number	Adrenal Glands (paired)	Right Epididy-mis	Epididy-mides (paired)	Kidneys (paired)	Levator Ani plus B-C	Liver	Pituita-ry	Seminal Ves. 1	Seminal Ves. 2	Testes (paired)	Ventral Prostate	Dorso-Lateral Prostate	Thyroid /Para
9041	0.0403	0.1881	0.3953	3.0425	0.7294	18.9745	0.0057	0.6218	0.4188	2.7112	0.2137	0.2127	0.0201
9042	0.0722	0.2103	0.4402	3.3681	0.9644	21.1274	0.0093	0.7215	0.3990	2.7742	0.2574	0.2767	0.0289
9043	0.0610	0.2964	0.5574	2.9315	0.5439	16.5039	0.0101	0.6891	0.4397	2.8776	0.1350	0.1988	0.0225
9044	0.0505	0.3570	0.7152	2.9954	0.6450	19.4180	0.0106	0.7144	0.5381	2.7198	0.1926	0.1915	0.0172
9045	0.0557	0.2605	0.5260	3.0340	0.4969	19.2596	0.0114	0.6210	0.3268	2.6184	0.2208	0.2005	0.0215
9046	0.0404	0.1941	0.4347	2.7949	0.8187	16.8834	0.0079	0.7158	0.3242	2.8788	0.2553	0.2499	0.0236
9047	0.0460	0.2018	0.4039	2.5810	0.7910	18.8777	0.0108	0.7311	0.3432	2.7439	0.2014	0.1764	0.0168
9048	0.0495	0.2149	0.4904	2.7936	0.2846	15.8481	0.0107	0.7199	0.4710	2.5514	0.2390	0.2254	0.0168
9049	0.0511	0.2811	0.5476	2.6741	0.5046	16.0646	0.0073	0.7136	0.4187	2.8606	0.1163	0.2687	0.0225
9050	0.0624	0.3324	0.6385	2.9329	0.4767	16.6149	0.0097	0.6961	0.4342	2.7453	0.1447	0.1868	0.0187
9051	0.0490	0.2496	0.5360	2.6777	0.6016	16.5414	0.0105	0.9866	0.5777	2.6295	0.1627	0.2499	0.0166
9052	0.0523	0.2610	0.5150	2.5346	0.7808	15.9885	0.0072	0.7943	0.4447	2.6152	0.1696	0.2813	0.0196
9053	0.0513	0.2689	0.5829	3.2355	0.6083	21.5094	0.0110	0.8692	0.4744	2.9727	0.1900	0.3221	0.0196
9054	0.0528	0.2248	0.4733	2.8588	0.6751	18.2524	0.0092	0.9964	0.6125	2.5648	0.3148	0.2091	0.0189
9055	0.0639	0.3415	0.6400	3.1096	0.6017	20.1696	0.0119	1.0517	0.5726	2.9055	0.1841	0.0954	0.0234

B-C = Bulbocavernosus muscle
Seminal Ves. 1 = Seminal vesicles and coagulating glands, with fluid
Seminal Ves. 2 = Seminal vesicles and coagulating glands, without fluid
Thyroid/Para = Thyroid and parathyroid, post-fixation

Appendix 13 (continued)
Individual Organ Weights (Grams) – F_1 Males
Pubertal Toxicity Study of Vinclozolin, Flutamide and Phenobarbital in Male Sprague Dawley Rats and Methoxychlor, Ethinyl Estradiol and Phenobarbital in Female Sprague Dawley Rats when Administered in Corn Oil by Oral Gavage

Group 3 Dose Level: 50 mg/kg/day Phenobarbital

Animal Number	Adrenal Glands (paired)	Right Epididy-mis	Epididy-mides (paired)	Kidneys (paired)	Levator Ani plus B-C	Liver	Pituita-ry	Seminal Ves. 1	Seminal Ves. 2	Testes (paired)	Ventral Prostate	Dorso-Lateral Prostate	Thyroid /Para
9056	0.0185	0.2445	0.5611	3.0266	0.5700	20.1527	0.0084	0.8585	0.4512	3.1153	0.2877	0.2627	0.0247
9057	0.0556	0.2670	0.5212	3.4668	0.6499	25.1508	0.0091	0.4978	0.3946	3.3218	0.2387	0.1794	0.0206
9058	0.0715	0.2774	0.5448	3.3123	0.5405	21.2298	0.0113	0.7007	0.3964	2.8874	0.1758	0.2215	0.0233
9059	0.0341	0.1806	0.3871	2.8727	0.6023	18.9378	0.0083	0.3626	0.2281	2.8991	0.1583	0.1986	0.0206
9060	0.0472	0.2289	0.5025	2.9048	0.2893	18.0150	0.0088	0.3087	0.2123	2.6972	0.1892	0.1549	0.0222
9061	0.0442	0.2998	0.6287	3.1076	0.4864	18.2536	0.0117	0.2891	0.2294	2.7435	0.2895	0.4767	0.0191
9062	0.0474	0.2462	0.5064	2.6692	0.2696	16.8281	0.0100	0.8416	0.3942	2.5171	0.2239	0.2494	0.0244
9063	0.0414	0.2841	0.5823	3.1621	0.5089	18.7608	0.0099	0.8443	0.4529	2.6764	0.1891	0.2586	0.0197
9064	0.0479	0.3819	0.6926	3.0180	0.9218	17.7559	0.0116	0.6490	0.4773	2.8745	0.1295	0.3524	0.0145
9065	0.0545	0.2903	0.6055	3.4653	0.6520	20.5742	0.0108	1.0708	0.4655	2.8790	0.2745	0.3009	0.0229
9066	0.0481	0.2015	0.4351	3.1943	0.7126	19.9212	0.0075	0.8177	0.3716	2.8622	0.2494	0.2037	0.0276
9067	0.0494	0.2663	0.5505	3.2741	0.6560	23.2196	0.0082	0.8711	0.5379	2.8583	0.2287	0.2880	0.0239
9068	0.0489	0.2310	0.4906	2.6211	0.5507	18.0477	0.0118	0.8607	0.4100	2.6218	0.1430	0.1944	0.0236
9069	0.0551	0.3373	0.6155	2.9717	0.5458	19.6885	0.0129	0.6651	0.5433	2.9175	0.2227	0.2564	0.0197
9070	0.0542	0.2037	0.4463	2.9359	0.8144	17.3480	0.0101	0.6571	0.3261	2.3537	0.1586	0.1864	0.0215

B-C = Bulbocavernosus muscle
Seminal Ves. 1 = Seminal vesicles and coagulating glands, with fluid
Seminal Ves. 2 = Seminal vesicles and coagulating glands, without fluid
Thyroid/Para = Thyroid and parathyroid, post-fixation

Appendix 13 (continued)
Individual Organ Weights (Grams) – F_1 Males
Pubertal Toxicity Study of Vinclozolin, Flutamide and Phenobarbital in Male Sprague Dawley Rats and Methoxychlor, Ethinyl Estradiol and Phenobarbital in Female Sprague Dawley Rats when Administered in Corn Oil by Oral Gavage

Group 4 Dose Level: 100 mg/kg/day Phenobarbital

Animal Number	Adrenal Glands (paired)	Right Epididy-mis	Epididy-mides (paired)	Kidneys (paired)	Levator Ani plus B-C	Liver	Pituita-ry	Seminal Ves. 1	Seminal Ves. 2	Testes (paired)	Ventral Prostate	Dorso-Lateral Prostate	Thyroid /Para
9071	0.0533	0.3294	0.6041	3.1365	0.5733	25.6183	0.0097	0.8292	0.5380	2.7817	0.2223	.	0.0315
9072	0.0542	0.2273	0.4562	3.3827	0.4700	22.6226	0.0099	0.4248	0.2953	2.8352	0.2326	0.2654	0.0186
9073	0.0418	0.1733	0.3466	2.1015	0.5729	17.0234	0.0071	0.3511	0.2251	1.8097	0.1198	0.1636	0.0213
9074	0.0581	0.2780	0.5133	2.8361	0.7311	23.6374	0.0093	0.6479	0.3742	2.9013	0.1946	0.1926	0.0244
9075	0.0584	0.1714	0.3585	2.9307	0.6787	19.8737	0.0086	0.4859	0.2550	2.7019	0.2063	0.2123	0.0228
9076	0.0445	0.2468	0.5185	2.5779	0.7491	18.4444	0.0088	0.6267	0.3253	2.7198	0.1888	0.2905	0.0226
9078	0.0640	0.2372	0.5468	3.0333	0.6255	18.2473	0.0105	0.7646	0.5572	2.8245	0.1741	0.2152	0.0215
9079	0.0372	0.1915	0.4282	2.9066	0.6591	19.4247	0.0093	0.6964	0.3909	2.4293	0.1804	0.2622	0.0290
9080	0.0758	0.3422	0.6756	2.8207	0.5221	20.0875	0.0087	0.7003	0.5006	2.7559	0.0866	0.1685	0.0184
9081	0.0394	0.2444	0.4628	2.5560	0.6992	18.6620	0.0071	0.4256	0.3055	2.6851	0.2336	0.1537	0.0220
9082	0.0533	0.3362	0.6258	2.4863	0.4902	18.5511	0.0065	0.6898	0.4374	2.7719	0.1459	0.1592	0.0214
9083	0.0598	0.2556	0.5628	2.7802	0.4954	19.1527	0.0104	0.5104	0.3386	2.7225	0.1600	0.2842	0.0176
9084	0.0555	0.2243	0.4660	2.7908	0.4394	19.6787	0.0083	0.4775	0.3144	2.5872	0.1553	0.2015	0.0203
9085	0.0405	0.3152	0.5547	3.0277	0.4842	16.4148	0.0080	0.5701	0.2301	2.6281	0.2086	0.1861	0.0338

B-C = Bulbocavernosus muscle
Seminal Ves. 1 = Seminal vesicles and coagulating glands, with fluid
Seminal Ves. 2 = Seminal vesicles and coagulating glands, without fluid
Thyroid/Para = Thyroid and parathyroid, post-fixation
. = No Data

Appendix 13 (continued)
Individual Organ Weights (Grams) – F_1 Males
Pubertal Toxicity Study of Vinclozolin, Flutamide and Phenobarbital in Male Sprague Dawley Rats and Methoxychlor, Ethinyl Estradiol and Phenobarbital in Female Sprague Dawley Rats when Administered in Corn Oil by Oral Gavage

Group 5 Dose Level: 10 mg/kg/day Vinclozolin

Animal Number	Adrenal Glands (paired)	Right Epididy-mis	Epididy-mides (paired)	Kidneys (paired)	Levator Ani plus B-C	Liver	Pituita-ry	Seminal Ves. 1	Seminal Ves. 2	Testes (paired)	Ventral Prostate	Dorso-Lateral Prostate	Thyroid /Para
9086	0.0517	0.3682	0.7935	3.2585	0.7113	19.6796	0.0136	0.7710	0.3913	2.9280	0.2644	0.3023	0.0203
9087	0.0435	0.3052	0.5621	2.9902	0.5914	17.1986	0.0085	0.6065	0.3999	2.8859	0.2518	0.2471	0.0165
9088	0.0473	0.2636	0.5353	3.3335	0.4144	15.7675	0.0109	0.6231	0.3397	2.9523	0.2717	0.2153	0.0163
9089	0.0536	0.2837	0.5685	2.7394	0.6351	15.9423	0.0084	0.7046	0.3466	2.8414	0.2215	0.2528	0.0187
9090	0.0718	0.2901	0.5595	2.9452	0.6206	18.0441	0.0094	0.6378	0.4196	2.7773	0.2112	0.1710	0.0159
9091	0.0623	0.2916	0.5929	2.6581	0.5716	14.2852	0.0095	0.3836	0.3155	2.5391	0.1173	0.2822	0.0203
9092	0.0507	0.2204	0.4831	3.2931	0.6491	17.9285	0.0111	0.9238	0.5015	3.0086	0.1132	0.2701	0.0224
9093	0.0525	0.3667	0.6865	2.6696	0.6772	15.4899	0.0098	0.7132	0.5270	2.8626	0.1491	0.1732	0.0211
9094	0.0495	0.2637	0.5614	3.0429	0.5588	17.2444	0.0099	0.7379	0.4165	2.8860	0.1630	0.2103	0.0182
9095	0.0549	0.2887	0.5984	3.4780	0.5928	20.4017	0.0129	0.8700	0.4940	2.8611	0.2014	0.3619	0.0197
9096	0.0683	0.3018	0.6261	2.8530	0.6729	16.5964	0.0098	0.7864	0.5381	2.8544	0.1473	0.3334	0.0177
9097	0.0568	0.2101	0.4779	3.2794	0.5067	19.7734	0.0110	0.6589	0.4216	2.6593	0.1765	0.2090	0.0180
9098	0.0576	0.2999	0.5813	3.1081	0.6003	17.7885	0.0108	0.4129	0.2869	2.7208	0.3334	0.2580	0.0162
9099	0.0334	0.2676	0.5965	2.6639	0.9884	12.9802	0.0086	0.4724	0.3386	2.7581	0.2729	0.2536	0.0285
9100	0.0429	0.2734	0.5269	3.0629	0.5196	17.1689	0.0082	0.7832	0.4087	2.7268	0.1599	0.1996	0.0186

B-C = Bulbocavernosus muscle
Seminal Ves. 1 = Seminal vesicles and coagulating glands, with fluid
Seminal Ves. 2 = Seminal vesicles and coagulating glands, without fluid
Thyroid/Para = Thyroid and parathyroid, post-fixation

Appendix 13 (continued)
Individual Organ Weights (Grams) – F_1 Males
Pubertal Toxicity Study of Vinclozolin, Flutamide and Phenobarbital in Male Sprague Dawley Rats and Methoxychlor, Ethinyl Estradiol and Phenobarbital in Female Sprague Dawley Rats when Administered in Corn Oil by Oral Gavage

Group 6 Dose Level: 30 mg/kg/day Vinclozolin

Animal Number	Adrenal Glands (paired)	Right Epididy-mis	Epididy-mides (paired)	Kidneys (paired)	Levator Ani plus B-C	Liver	Pituita-ry	Seminal Ves. 1	Seminal Ves. 2	Testes (paired)	Ventral Prostate	Dorso-Lateral Prostate	Thyroid /Para
9101	0.0472	0.3479	0.6871	3.2409	0.7194	18.7986	0.0103	0.7322	0.4148	2.9939	0.2445	0.2521	0.0211
9102	0.0517	0.2384	0.4844	3.4338	0.7686	20.1691	0.0094	0.7214	0.3690	3.2054	0.2495	0.2620	0.0232
9103	0.0646	0.2392	0.4934	3.0744	0.6379	17.1939	0.0080	0.6525	0.4226	2.8739	0.1084	0.3018	0.0207
9104	0.0474	0.2180	0.4235	2.8362	0.6832	17.6372	0.0109	0.5792	0.3586	2.9942	0.2425	0.2210	0.0160
9105	0.0451	0.2382	0.4875	2.8801	0.4764	16.2613	0.0114	0.5982	0.3302	2.7033	0.1592	0.2354	0.0266
9106	0.0424	0.1903	0.3885	3.0102	0.6601	17.6288	0.0105	0.4692	0.2994	2.9042	0.2284	0.2234	0.0263
9107	0.0385	0.2000	0.4307	3.2151	0.6143	16.1988	0.0091	0.7074	0.3674	2.9431	0.2391	0.2177	0.0184
9108	0.0462	0.1887	0.3775	2.8945	0.4907	15.9719	0.0099	0.3948	0.2670	3.0180	0.1806	0.1903	0.0218
9109	0.0543	0.2897	0.5563	3.4224	0.5986	20.3759	0.0103	0.6391	0.4350	3.0560	0.2549	0.1990	0.0185
9110	0.0511	0.2723	0.5336	2.9058	0.6469	18.2015	0.0101	0.6898	0.4366	3.3451	0.3070	0.2646	0.0178
9111	0.0650	0.2882	0.6148	3.2146	0.4883	17.0536	0.0105	0.7126	0.3689	2.9837	0.1326	0.2430	0.0260
9112	0.0420	0.2268	0.4807	3.2218	0.7040	17.0699	0.0100	0.4177	0.3590	3.0868	0.1967	0.2891	0.0212
9113	0.0635	0.3263	0.6111	3.4715	0.5750	21.0611	0.0127	0.7321	0.4259	3.0248	0.2274	0.2466	0.0225
9114	0.0538	0.2948	0.6271	3.3376	0.5987	20.9125	0.0111	0.6940	0.3856	2.9924	0.2017	0.2557	0.0233
9115	0.0638	0.2698	0.5501	3.0141	0.6391	16.5613	0.0089	0.6462	0.3725	3.0158	0.1806	0.2946	0.0250

B-C = Bulbocavernosus muscle
Seminal Ves. 1 = Seminal vesicles and coagulating glands, with fluid
Seminal Ves. 2 = Seminal vesicles and coagulating glands, without fluid
Thyroid/Para = Thyroid and parathyroid, post-fixation

Appendix 13 (continued)
Individual Organ Weights (Grams) – F_1 Males
Pubertal Toxicity Study of Vinclozolin, Flutamide and Phenobarbital in Male Sprague Dawley Rats and Methoxychlor, Ethinyl Estradiol and Phenobarbital in Female Sprague Dawley Rats when Administered in Corn Oil by Oral Gavage

Group 7 Dose Level: 100 mg/kg/day Vinclozolin

Animal Number	Adrenal Glands (paired)	Right Epididy-mis	Epididy-mides (paired)	Kidneys (paired)	Levator Ani plus B-C	Liver	Pituita-ry	Seminal Ves. 1	Seminal Ves. 2	Testes (paired)	Ventral Prostate	Dorso-Lateral Prostate	Thyroid /Para
9116	0.0419	0.1640	0.3571	2.9484	0.6778	16.7103	0.0061	0.3818	0.2201	2.9046	0.1188	0.1843	0.0155
9117	0.0621	0.2688	0.4841	3.1388	0.4961	18.6204	0.0091	0.4942	0.3195	3.2129	0.1365	0.1803	0.0214
9118	0.0533	0.2205	0.4663	3.1592	0.4490	16.7786	0.0085	0.4251	0.2730	2.9203	0.1504	0.2160	0.0251
9119	0.0544	0.2149	0.4472	2.9552	0.3257	17.8756	0.0103	0.6117	0.3762	3.0593	0.2709	0.2037	0.0172
9120	0.0614	0.1765	0.4022	3.0822	0.2165	16.6203	0.0109	0.2279	0.1878	2.6102	0.1507	0.1665	0.0195
9121	0.0577	0.2231	0.4764	3.2084	0.3084	15.7084	0.0117	0.3949	0.2738	2.8887	0.2324	0.1835	0.0211
9122	0.0473	0.2125	0.4987	2.8759	0.2395	13.9855	0.0110	0.3490	0.2560	2.7755	0.2210	0.1282	0.0226
9123	0.0477	0.2198	0.4772	2.9041	0.5625	16.5104	0.0088	0.6172	0.3706	2.9768	0.2149	0.2297	0.0226
9124	0.0710	0.2811	0.5433	3.1258	0.5288	19.0156	0.0068	0.5809	0.3859	3.1743	0.0967	0.1604	0.0247
9125	0.0658	0.2912	0.5785	3.0298	0.5224	19.7786	0.0100	0.4320	0.3367	3.1025	0.1861	0.1743	0.0224
9126	0.0564	0.2139	0.4177	2.8117	0.5608	16.0420	0.0086	0.4327	0.2767	3.2718	0.1887	0.1782	0.0209
9127	0.0503	0.2007	0.4172	2.8047	0.3502	14.6823	0.0092	0.5496	0.2886	2.8006	0.1745	0.1980	0.0241
9128	0.0609	0.1765	0.3587	2.9018	0.5355	17.2407	0.0093	0.4413	0.2824	3.1677	0.1940	0.1837	0.0240
9129	0.0535	0.2836	0.5008	2.7485	0.4384	11.7910	0.0103	0.4235	0.2785	2.7824	0.1317	0.2354	0.0140
9130	0.0603	0.2129	0.4523	2.6650	0.6730	14.4040	0.0115	0.4836	0.3446	2.9281	0.2834	0.2120	0.0200

B-C = Bulbocavernosus muscle
Seminal Ves. 1 = Seminal vesicles and coagulating glands, with fluid
Seminal Ves. 2 = Seminal vesicles and coagulating glands, without fluid
Thyroid/Para = Thyroid and parathyroid, post-fixation

Appendix 13 (continued)
Individual Organ Weights (Grams) – F_1 Males
Pubertal Toxicity Study of Vinclozolin, Flutamide and Phenobarbital in Male Sprague Dawley Rats and Methoxychlor, Ethinyl Estradiol and Phenobarbital in Female Sprague Dawley Rats when Administered in Corn Oil by Oral Gavage

Group 8 Dose Level: 25 mg/kg/day Flutamide

Animal Number	Adrenal Glands (paired)	Right Epididy-mis	Epididy-mides (paired)	Kidneys (paired)	Levator Ani plus B-C	Liver	Pituita-ry	Seminal Ves. 1	Seminal Ves. 2	Testes (paired)	Ventral Prostate	Dorso-Lateral Prostate	Thyroid /Para
9131	0.0612	0.1801	0.3470	3.0575	0.4094	18.2995	0.0120	0.2640	0.2299	3.0661	0.0721	0.1656	0.0182
9132	0.0436	0.1574	0.3553	2.7795	0.2636	15.7426	0.0120	0.1749	0.1366	2.8432	0.1332	0.1581	0.0179
9133	0.0577	0.1638	0.3226	2.4672	0.5321	16.3615	0.0105	0.2088	0.1481	3.0341	0.1377	0.1309	0.0280
9134	0.0459	0.1573	0.3080	2.6332	0.3363	19.1136	0.0099	0.1026	0.0909	2.9501	0.0961	0.0742	0.0176
9135	0.0439	0.1036	0.2156	2.7868	0.3837	18.3032	0.0088	0.1159	0.0881	2.9155	0.0686	0.1237	0.0216
9136	0.0445	0.1553	0.3575	2.2706	0.4102	12.7105	0.0111	0.1401	0.1295	2.8867	0.0769	0.0832	0.0124
9137	0.0567	0.1150	0.2607	2.8498	0.3893	16.0439	0.0090	0.0982	0.0904	2.4634	0.1007	0.1021	0.0206
9138	0.0488	0.1518	0.3219	2.4985	0.2594	13.7668	0.0109	0.1098	0.0944	3.3466	0.1457	0.1326	0.0178
9139	0.0524	0.1701	0.3680	2.5936	0.3849	16.5807	0.0070	0.2321	0.1710	3.0758	0.1003	0.1080	0.0233
9140	0.0524	0.1787	0.3491	2.8937	0.4216	16.2358	0.0122	0.1810	0.1553	2.9959	0.1133	0.1446	0.0236
9141	0.0505	0.2071	0.3938	2.9157	0.4058	19.5452	0.0150	0.2148	0.1765	3.1417	0.1144	0.1175	0.0243
9142	0.0595	0.2135	0.4293	2.7073	0.3718	17.9648	0.0137	0.3408	0.2853	3.2951	0.1293	0.1696	0.0227
9143	0.0519	0.2251	0.4426	2.6115	0.3740	16.0642	0.0126	0.1182	0.1060	2.8496	0.1331	0.0945	0.0205
9144	0.0482	0.2255	0.4122	2.8152	0.4954	18.5253	0.0121	0.1540	0.1309	3.1164	0.0963	0.1189	0.0204
9145	0.0598	0.1270	0.2806	2.8018	0.3793	15.7510	0.0087	0.1673	0.1405	2.9941	0.0747	0.1339	0.0284

B-C = Bulbocavernosus muscle
Seminal Ves. 1 = Seminal vesicles and coagulating glands, with fluid
Seminal Ves. 2 = Seminal vesicles and coagulating glands, without fluid
Thyroid/Para = Thyroid and parathyroid, post-fixation

Appendix 13 (continued)
Individual Organ Weights (Grams) – F_1 Males
Pubertal Toxicity Study of Vinclozolin, Flutamide and Phenobarbital in Male Sprague Dawley Rats and Methoxychlor, Ethinyl Estradiol and Phenobarbital in Female Sprague Dawley Rats when Administered in Corn Oil by Oral Gavage

Group 9 Dose Level: 50 mg/kg/day Flutamide

Animal Number	Adrenal Glands (paired)	Right Epididy-mis	Epididy-mides (paired)	Kidneys (paired)	Levator Ani plus B-C	Liver	Pituita-ry	Seminal Ves. 1	Seminal Ves. 2	Testes (paired)	Ventral Prostate	Dorso-Lateral Prostate	Thyroid /Para
9146	0.0541	0.1453	0.3785	3.0147	0.2802	18.2855	0.0068	0.1769	0.1441	2.9311	0.1289	0.1345	0.0207
9147	0.0532	0.1916	0.3577	2.9884	0.3321	18.2466	0.0090	0.1190	0.1051	4.5962	0.0724	0.1022	0.0160
9148	0.0484	0.1741	0.3053	3.2444	0.3614	21.3796	0.0128	0.0677	0.0612	3.7120	0.1567	0.1615	0.0188
9149	0.0610	0.1915	0.4555	2.6936	0.4553	16.5544	0.0121	0.2336	0.2061	3.0799	0.0613	0.1015	0.0254
9150	0.0746	0.1622	0.3640	2.5208	0.3509	18.1311	0.0114	0.1324	0.1189	3.8388	0.0851	0.0787	0.0221
9151	0.0658	0.1646	0.3182	2.6073	0.3807	18.5716	0.0092	0.1144	0.1038	3.4328	0.0742	0.0923	0.0308
9152	0.0506	0.1212	0.2854	2.2156	0.3559	13.7036	0.0099	0.1361	0.1171	3.5910	0.0716	0.0680	0.0148
9153	0.0766	0.1929	0.3480	2.5362	0.4843	16.4932	0.0091	0.1462	0.1366	2.8881	0.0358	0.0755	0.0173
9154	0.0549	0.0925	0.2881	2.8454	0.2738	15.7888	0.0121	0.0595	0.0506	4.2763	0.0136	0.0794	0.0259
9155	0.0659	0.2515	0.4227	2.9368	0.5600	17.5166	0.0131	0.1171	0.0940	3.3468	0.1222	0.0953	0.0203
9156	0.0599	0.1332	0.2828	3.0926	0.2650	17.8604	0.0130	0.1175	0.1032	3.4935	0.0630	0.0696	0.0223
9157	0.0609	0.1722	0.4376	2.7026	0.5521	18.2992	0.0124	0.1771	0.1405	3.1200	0.1098	0.1341	0.0191
9158	0.0580	0.1658	0.3421	2.3208	0.3881	15.2773	0.0133	0.1463	0.1186	3.2838	0.1023	0.1142	0.0150
9159	0.0580	0.1158	0.2495	2.8737	0.4008	17.6677	0.0101	0.1264	0.1100	3.9216	0.0938	0.0952	0.0160
9160	0.0455	0.1975	0.4213	2.8735	0.3333	16.2731	0.0108	0.1610	0.1372	2.9166	0.0614	0.1263	0.0211

B-C = Bulbocavernosus muscle
Seminal Ves. 1 = Seminal vesicles and coagulating glands, with fluid
Seminal Ves. 2 = Seminal vesicles and coagulating glands, without fluid
Thyroid/Para = Thyroid and parathyroid, post-fixation

Appendix 13 (continued)
Individual Organ Weights (Grams) – F_1 Females
Pubertal Toxicity Study of Vinclozolin, Flutamide and Phenobarbital in Male Sprague Dawley Rats and Methoxychlor, Ethinyl Estradiol and Phenobarbital in Female Sprague Dawley Rats when Administered in Corn Oil by Oral Gavage

Group 1 Dose Level: 0 mg/kg/day

Animal Number	Adrenal Glands (paired)	Kidneys (paired)	Liver	Ovaries (paired)	Pituitary	Thyroid /Parathyroid (post-fixation)	Uterus and Cervix (with fluid)	Uterus and Cervix (without fluid)
9161	0.0449	1.7815	9.4552	0.1260	0.0044	0.0112	0.5543	0.5405
9162	0.0382	1.7380	9.1648	0.0952	0.0066	0.0171	0.2782	0.2692
9163	0.0359	1.6476	8.8375	0.0847	0.0076	0.0179	0.2538	0.2476
9164	0.0357	1.7709	8.8142	0.0890	0.0089	0.0165	0.6048	0.4419
9165	0.0514	1.7098	8.0924	0.0977	0.0098	0.0107	0.3064	0.2962
9166	0.0317	1.6108	8.3375	0.0763	0.0070	0.0197	0.2271	0.2154
9167	0.0431	1.6820	8.7578	0.0779	0.0081	0.0142	0.2824	0.2669
9168	0.0442	1.6265	8.4857	0.0966	0.0118	0.0167	0.3022	0.2873
9169	0.0317	1.7718	8.2876	0.0868	0.0102	0.0142	0.3701	0.3518
9170	0.0438	1.6910	8.8336	0.0836	0.0079	0.0164	0.8769	0.5035
9171	0.0371	1.9592	9.1088	0.1252	0.0105	0.0194	0.6426	0.4791
9172	0.0458	1.5003	7.2869	0.0722	0.0066	0.0115	0.3984	0.3842
9173	0.0355	1.7518	9.1600	0.0805	0.0086	0.0222	0.2967	0.2811
9174	0.0522	1.8261	9.2464	0.0998	0.0118	0.0252	0.6017	0.4797
9175	0.0492	1.8677	8.1359	0.1033	0.0094	0.0145	0.2958	0.2856

Appendix 13 (continued)
Individual Organ Weights (Grams) – F_1 Females
Pubertal Toxicity Study of Vinclozolin, Flutamide and Phenobarbital in Male Sprague Dawley Rats and Methoxychlor, Ethinyl Estradiol and Phenobarbital in Female Sprague Dawley Rats when Administered in Corn Oil by Oral Gavage

Group 2 Dose Level: 0.0025 mg/kg/day Ethinyl Estradiol

Animal Number	Adrenal Glands (paired)	Kidneys (paired)	Liver	Ovaries (paired)	Pituitary	Thyroid /Parathyroid (post-fixation)	Uterus and Cervix (with fluid)	Uterus and Cervix (without fluid)
9176	0.0521	1.8373	9.1631	0.0993	0.0094	0.0186	0.2679	0.2617
9177	0.0411	1.7640	8.5883	0.0807	0.0052	0.0188	0.6831	0.5248
9178	0.0380	1.8795	8.4642	0.0771	0.0093	0.0183	0.3472	0.3367
9179	0.0481	1.7621	8.7576	0.0763	0.0077	0.0183	0.2601	0.2479
9180	0.0381	1.6822	7.6345	0.0854	0.0062	0.0135	0.3362	0.3213
9181	0.0448	1.6923	8.2038	0.0760	0.0074	0.0105	0.6494	0.4751
9182	0.0447	1.6152	8.2925	0.0882	0.0081	0.0203	0.4290	0.4102
9183	0.0576	1.4693	8.3341	0.0822	0.0066	0.0147	0.3305	0.3222
9184	0.0484	1.8241	9.5411	0.1028	0.0135	0.0195	0.5348	0.4830
9185	0.0623	1.5256	7.2405	0.1105	0.0090	0.0129	0.4890	0.4714
9186	0.0520	1.7097	9.0017	0.0958	0.0113	0.0173	0.3955	0.3758
9187	0.0535	1.6804	8.9671	0.1024	0.0062	0.0176	0.4260	0.4125
9188	0.0536	1.5309	8.1599	0.1151	0.0077	0.0164	0.6692	0.4762
9189	0.0529	1.9174	8.3071	0.0901	0.0088	0.0155	0.2706	0.2567
9190	0.0646	1.8455	7.5124	0.1334	0.0060	0.0209	0.4465	0.4316

Appendix 13 (continued)
Individual Organ Weights (Grams) – F_1 Females
Pubertal Toxicity Study of Vinclozolin, Flutamide and Phenobarbital in Male Sprague Dawley Rats and Methoxychlor, Ethinyl Estradiol and Phenobarbital in Female Sprague Dawley Rats when Administered in Corn Oil by Oral Gavage

Group 3 Dose Level: 0.005 mg/kg/day Ethinyl Estradiol

Animal Number	Adrenal Glands (paired)	Kidneys (paired)	Liver	Ovaries (paired)	Pituitary	Thyroid /Parathyroid (post-fixation)	Uterus and Cervix (with fluid)	Uterus and Cervix (without fluid)
9191	0.0506	1.7605	9.3358	0.1337	0.0089	0.0151	0.4474	0.4025
9192	0.0505	1.8737	9.9400	0.0860	0.0115	0.0237	0.3435	0.3325
9193	0.0519	1.7599	9.3631	0.1047	0.0100	0.0247	0.7735	0.4761
9194	0.0565	1.7667	8.3998	0.0855	0.0096	0.0118	0.4567	0.4415
9195	0.0373	1.5616	8.1008	0.0721	0.0057	0.0164	0.2777	0.2687
9196	0.0467	1.6759	8.3001	0.0860	0.0111	0.0108	0.3638	0.3559
9197	0.0514	1.6061	9.2438	0.1078	0.0102	0.0116	0.3642	0.3540
9198	0.0433	1.7875	8.5989	0.0893	0.0088	0.0153	0.3116	0.2977
9199	0.0498	1.7429	8.7057	0.1231	0.0087	0.0152	0.4154	0.3927
9200	0.0444	1.7404	9.4895	0.0941	0.0100	0.0206	0.4365	0.4162
9201	0.0441	1.7647	8.4966	0.1055	0.0086	0.0150	0.2676	0.2585
9202	0.0478	1.5840	8.5053	0.1028	0.0065	0.0209	0.5426	0.4924
9203	0.0550	1.4312	7.5851	0.0862	0.0096	0.0166	0.2502	0.2322
9204	0.0499	1.6307	8.7918	0.1232	0.0084	0.0176	0.3286	0.3228
9205	0.0515	1.8016	8.4818	0.0804	0.0125	0.0165	0.3788	0.3602

Appendix 13 (continued)
Individual Organ Weights (Grams) – F_1 Females
Pubertal Toxicity Study of Vinclozolin, Flutamide and Phenobarbital in Male Sprague Dawley Rats and Methoxychlor, Ethinyl Estradiol and Phenobarbital in Female Sprague Dawley Rats when Administered in Corn Oil by Oral Gavage

Group 4 Dose Level: 12.5 mg/kg/day Methoxychlor

Animal Number	Adrenal Glands (paired)	Kidneys (paired)	Liver	Ovaries (paired)	Pituitary	Thyroid /Parathyroid (post-fixation)	Uterus and Cervix (with fluid)	Uterus and Cervix (without fluid)
9206	0.0551	1.6270	8.2284	0.1233	0.0047	0.0223	0.4502	0.4403
9207	0.0532	1.8486	9.3722	0.1036	0.0072	0.0190	0.3931	0.3835
9208	0.0455	1.6316	7.7676	0.0759	0.0098	0.0158	0.2595	0.2488
9209	0.0522	1.7242	8.7324	0.0997	0.0107	0.0191	0.3680	0.3517
9210	0.0442	1.6518	7.9122	0.0707	0.0101	0.0171	0.2816	0.2750
9211	0.0397	1.6696	8.8583	0.1084	0.0091	0.0135	0.2819	0.2734
9212	0.0515	1.7593	8.2745	0.1134	0.0048	0.0197	0.3983	0.3835
9213	0.0531	1.6261	6.9512	0.1110	0.0080	0.0188	0.4170	0.4020
9214	0.0416	1.7206	9.6102	0.0823	0.0083	0.0165	0.2469	0.2346
9215	0.0520	1.6989	7.8154	0.0945	0.0078	0.0189	0.2746	0.2679
9216	0.0454	1.4653	6.5140	0.0869	0.0056	0.0157	0.4006	0.3774
9217	0.0437	1.5161	7.6977	0.0682	0.0074	0.0164	0.3106	0.2907
9218	0.0457	1.8404	8.0804	0.1108	0.0094	0.0159	0.8463	0.5727
9219	0.0557	1.7422	8.6762	0.0969	0.0076	0.0130	0.3604	0.3430
9220	0.0512	1.5082	7.3512	0.0838	0.0080	0.0170	0.3782	0.3630

Appendix 13 (continued)
Individual Organ Weights (Grams) – F_1 Females
Pubertal Toxicity Study of Vinclozolin, Flutamide and Phenobarbital in Male Sprague Dawley Rats and Methoxychlor, Ethinyl Estradiol and Phenobarbital in Female Sprague Dawley Rats when Administered in Corn Oil by Oral Gavage

Group 5 Dose Level: 25 mg/kg/day Methoxychlor

Animal Number	Adrenal Glands (paired)	Kidneys (paired)	Liver	Ovaries (paired)	Pituitary	Thyroid /Parathyroid (post-fixation)	Uterus and Cervix (with fluid)	Uterus and Cervix (without fluid)
9221	0.0395	1.8072	9.3528	0.0590	0.0063	0.0202	0.2829	0.2696
9222	0.0425	1.8825	10.5109	0.1005	0.0068	0.0208	0.4403	0.3989
9223	0.0471	1.7875	9.3478	0.0889	0.0088	0.0158	0.4187	0.3801
9224	0.0467	1.4685	6.8680	0.0838	0.0102	0.0187	0.3618	0.3508
9225	0.0343	1.8506	8.2014	0.0781	0.0061	0.0240	0.2248	0.2069
9226	0.0404	1.6732	8.9603	0.0710	0.0080	0.0256	0.2585	0.2550
9227	0.0418	1.6252	9.0954	0.0566	0.0067	0.0146	0.2553	0.2354
9228	0.0537	1.6027	8.1973	0.0928	0.0092	0.0143	0.3350	0.3173
9229	0.0572	1.5953	8.1027	0.1103	0.0074	0.0146	0.3234	0.3083
9230	0.0578	1.5668	7.8217	0.0918	0.0073	0.0180	0.3095	0.2928
9231	0.0486	1.7280	9.0216	0.0946	0.0068	0.0190	0.4351	0.3728
9232	0.0332	1.4981	7.7669	0.0903	0.0063	0.0203	0.3460	0.3341
9233	0.0610	1.5530	7.2323	0.0743	0.0088	0.0186	0.3831	0.3679
9234	0.0502	1.6123	7.7781	0.0805	0.0089	0.0153	0.4391	0.4220
9235	0.0401	1.7451	7.6845	0.1031	0.0081	0.0179	0.5566	0.4536

Appendix 13 (continued)
Individual Organ Weights (Grams) – F_1 Females
Pubertal Toxicity Study of Vinclozolin, Flutamide and Phenobarbital in Male Sprague Dawley Rats and Methoxychlor, Ethinyl Estradiol and Phenobarbital in Female Sprague Dawley Rats when Administered in Corn Oil by Oral Gavage

Group 6 Dose Level: 50 mg/kg/day Methoxychlor

Animal Number	Adrenal Glands (paired)	Kidneys (paired)	Liver	Ovaries (paired)	Pituitary	Thyroid /Parathyroid (post-fixation)	Uterus and Cervix (with fluid)	Uterus and Cervix (without fluid)
9236	0.0429	1.5552	8.5374	0.0744	0.0068	0.0254	0.6507	0.4196
9237	0.0520	1.6251	7.5002	0.0733	0.0054	0.0138	0.6949	0.4344
9238	0.0496	1.6839	8.4551	0.0827	0.0012	0.0152	0.4464	0.4203
9239	0.0352	1.7484	8.0210	0.0641	0.0065	0.0180	0.2062	0.1849
9240	0.0495	1.5940	7.3503	0.0670	0.0037	0.0212	0.2519	0.2408
9241	0.0553	1.6729	8.0079	0.0693	0.0086	0.0183	0.8246	0.4613
9242	0.0339	1.7260	6.8607	0.0900	0.0080	0.0167	0.7813	0.4159
9243	0.0222	1.7866	8.2602	0.0728	0.0083	0.0179	0.3226	0.2936
9244	0.0426	1.5807	8.2425	0.0788	0.0069	0.0111	0.2849	0.2746
9245	0.0464	1.8700	9.3116	0.1082	0.0085	0.0164	0.3069	0.2959
9246	0.0446	1.8765	7.6540	0.0618	0.0078	0.0189	0.2714	0.2571
9247	0.0530	1.5751	7.9952	0.0787	0.0083	0.0114	0.3021	0.2828
9248	0.0493	1.7141	7.9945	0.1023	0.0079	0.0148	0.6390	0.4529
9249	0.0440	1.5706	7.4336	0.0788	0.0072	0.0163	0.2355	0.2229
9250	0.0509	1.4136	6.8465	0.0810	0.0087	0.0186	0.3414	0.3166

Appendix 13 (continued)
Individual Organ Weights (Grams) – F_1 Females
Pubertal Toxicity Study of Vinclozolin, Flutamide and Phenobarbital in Male Sprague Dawley Rats and Methoxychlor, Ethinyl Estradiol and Phenobarbital in Female Sprague Dawley Rats when Administered in Corn Oil by Oral Gavage

Group 7 Dose Level: 25 mg/kg/day Phenobarbital

Animal Number	Adrenal Glands (paired)	Kidneys (paired)	Liver	Ovaries (paired)	Pituitary	Thyroid /Parathyroid (post-fixation)	Uterus and Cervix (with fluid)	Uterus and Cervix (without fluid)
9251	0.0479	2.3003	12.4677	0.0906	0.0080	0.0170	0.3193	0.3032
9252	0.0377	1.7771	9.4948	0.1081	0.0093	0.0119	0.2799	0.2728
9253	0.0535	1.7124	8.8944	0.0961	0.0099	0.0157	0.6384	0.4153
9254	0.0538	1.6693	9.5732	0.1002	0.0055	0.0148	0.4092	0.3900
9255	0.0503	1.5962	10.6647	0.0664	0.0049	0.0151	0.2766	0.2678
9256	0.0378	1.7313	9.9974	0.0849	0.0086	0.0168	0.4771	0.3718
9257	0.0548	1.6162	10.9524	0.1010	0.0086	0.0167	0.4091	0.3972
9258	0.0478	1.5813	9.6759	0.0932	0.0111	0.0278	0.3855	0.3764
9259	0.0625	2.0346	10.2958	0.1107	0.0127	0.0172	0.8227	0.5051
9260	0.0460	1.8142	10.2807	0.0854	0.0082	0.0187	0.2476	0.2375
9261	0.0500	1.7429	9.6533	0.0803	0.0057	0.0155	0.3211	0.3009
9262	0.0703	4.7836	9.3955	0.1239	0.0089	0.0194	0.3950	0.3740
9263	0.0455	1.3781	7.2564	0.0925	0.0069	0.0172	0.3213	0.3209
9264	0.0708	1.7738	10.1332	0.1118	0.0196	0.0240	0.3352	0.3256
9265	0.0518	1.8004	8.0428	0.1202	0.0055	0.0170	0.4671	0.4136

Appendix 13 (continued)
Individual Organ Weights (Grams) – F_1 Females
Pubertal Toxicity Study of Vinclozolin, Flutamide and Phenobarbital in Male Sprague Dawley Rats and Methoxychlor, Ethinyl Estradiol and Phenobarbital in Female Sprague Dawley Rats when Administered in Corn Oil by Oral Gavage

Group 8 Dose Level: 50 mg/kg/day Phenobarbital

Animal Number	Adrenal Glands (paired)	Kidneys (paired)	Liver	Ovaries (paired)	Pituitary	Thyroid /Parathyroid (post-fixation)	Uterus and Cervix (with fluid)	Uterus and Cervix (without fluid)
9266	0.0656	1.8158	14.3276	0.1215	0.0072	0.0196	0.3816	0.3657
9267	0.0477	1.6992	10.9662	0.1253	0.0066	0.0194	0.3144	0.3028
9268	0.0559	1.9367	10.7854	0.1056	0.0083	0.0211	0.4768	0.4610
9269	0.0520	1.7590	11.0983	0.0971	0.0101	0.0155	0.2475	0.2404
9270	0.0425	1.5199	11.6160	0.0945	0.0089	0.0138	0.3080	0.2998
9271	0.0484	1.7518	10.8592	0.1047	0.0104	0.0116	0.3232	0.3186
9272	0.0533	1.6163	9.7903	0.1039	0.0088	0.0126	0.2901	0.2825
9273	0.0514	1.6489	10.1216	0.0974	0.0032	0.0187	0.4333	0.4064
9274	0.0618	1.5219	9.4624	0.1122	0.0080	0.0147	0.3734	0.3535
9275	0.0489	1.8220	11.1277	0.0868	0.0053	0.0141	0.7906	0.4560
9276	0.0604	2.6677	10.4040	0.1094	0.0058	0.0222	0.5701	0.4989
9277	0.0510	1.6411	9.8803	0.0931	0.0104	0.0208	0.3181	0.2993
9278	0.0518	1.5419	8.6770	0.0759	0.0078	0.0167	0.5090	0.3980
9279	0.0498	1.6981	12.9797	0.0939	0.0091	0.0265	0.2777	0.2648
9280	0.0487	1.4884	9.2688	0.0890	0.0086	0.0246	0.2846	0.2640

Appendix 13 (continued)
Individual Organ Weights (Grams) – F_1 Females
Pubertal Toxicity Study of Vinclozolin, Flutamide and Phenobarbital in Male Sprague Dawley Rats and Methoxychlor, Ethinyl Estradiol and Phenobarbital in Female Sprague Dawley Rats when Administered in Corn Oil by Oral Gavage

Group 9 Dose Level: 100 mg/kg/day Phenobarbital

Animal Number	Adrenal Glands (paired)	Kidneys (paired)	Liver	Ovaries (paired)	Pituitary	Thyroid /Parathyroid (post-fixation)	Uterus and Cervix (with fluid)	Uterus and Cervix (without fluid)
9281	0.0524	1.7192	12.0770	0.1214	0.0108	0.0240	0.5224	0.4817
9282	0.0646	1.7001	10.4778	0.0871	0.0100	0.0203	0.3569	0.3492
9283	0.0498	1.8161	11.6144	0.1003	0.0093	0.0157	0.3085	0.3000
9284	0.0426	1.6929	11.5726	0.0968	0.0084	0.0185	0.4666	0.3994
9285	0.0556	1.5789	11.0592	0.0932	0.0091	0.0208	0.6029	0.4452
9286	0.0591	1.6438	12.8044	0.1035	0.0067	0.0173	0.6776	0.4756
9287	0.0416	1.7605	11.9102	0.0605	0.0074	0.0201	0.1880	0.1783
9288	0.0457	1.6659	10.8639	0.1152	0.0082	0.0161	0.3037	0.2911
9289	0.0660	1.4919	8.4516	0.1105	0.0064	0.0197	0.3825	0.3590
9290	0.0568	1.6783	13.5561	0.1076	0.0077	0.0229	0.3773	0.3504
9291	0.0432	2.6105	9.5065	0.0699	.	0.0202	0.4623	0.3614
9292	0.0483	1.5693	10.6783	0.0775	0.0079	0.0229	0.2556	0.2394
9293	0.0510	1.8519	11.2284	0.0929	0.0079	0.0242	0.2569	0.2388
9294	0.0461	1.7508	12.6339	0.0871	0.0100	0.0179	0.5669	0.4209
9295	0.0545	1.5742	11.3877	0.0895	0.0067	0.0193	0.4028	0.3880

. = No Data

Appendix 14
Serology Report
Pubertal Toxicity Study of Vinclozolin, Flutamide and Phenobarbital in Male Sprague Dawley Rats and Methoxychlor, Ethinyl Estradiol and Phenobarbital in Female Sprague Dawley Rats when Administered in Corn Oil by Oral Gavage

Taconic ANMED Animal Health and Biopharmaceutical Safety Testing
7642 Standish Pl. • Rockville, MD 20855 • Tel: 301-762-0366 • Fax: 301-762-7438 • Web: www.taconic.com • E-mail: anmed@taconic.com

EVALUATION

Rat (1)

ACCESSION NUMBER

47502

SPONSOR

Therimmune Corp.

STUDIES CONDUCTED

Test 120: Comprehensive Diagnostic Screen
Test 201-102: Comprehensive Rat Serologic Profile

Steven M. Stiefel DVM
Sr. Scientist Pathology

Cinda L. Bell
Sr. QA Specialist

11/27/2002
Date

Taconic ANMED Animal Health and Biopharmaceutical Safety Testing

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SUMMARY PAGE

Client: Therimmune Corp. Accession No. 47502

Species: Rat (1) Date Received: 11/12/2002

Group Description: Date Completed: 11/27/2002

Services Performed: Test 120: Comprehensive Diagnostic Screen
Test 201-102: Rat Comprehensive Virus Serology Panel

INTRODUCTION

One adult, albino, female rat was submitted for a Comprehensive Diagnostic Screen. A serum sample was drawn from this rat at the time of necropsy for a Comprehensive Virus Serology Panel.

RESULTS AND INTERPRETATION

The results of the Comprehensive Diagnostic Screen are summarized in Table 1. Results of the evaluation of serum for the presence of viral antibodies are presented in Table 2. A complete description of all tests conducted is provided in Table 3.

No murine pathogens in the bacterial, viral, mycoplasmal, helminth, protozoan or arthropod groups were isolated or otherwise detected. It is brought to the reader's attention that this animal was detected as a nasopharyngeal carrier of the opportunistic organism *Staphylococcus aureus*. The colony should be regarded as free of all primary murine pathogens in the test profile and entirely suitable for any research protocol, including chronic studies in barrier facilities.

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TABLE 1
DIAGNOSTIC SCREEN RESULTS

Client: Therimmune Corp. Accession No. 47502

Species: Rat (1) Date Received: 11/12/2002

Group Designation: _____ Date Completed: 11/27/2002

1) Physical examination: One adult, albino, female rat with body weight = 260 g was presented for diagnosis. The animal arrived in a filtered shipping carton and was clinically normal in terms of posture and activity. No discharges from the nares, conjunctiva or anus were noted. Euthanasia was conducted in a sterile polycarbonate plastic chamber prior to blood sample collection.

2) Necropsy dissection:	NGL
3) Fecal culture:	No Salmonella, Klebsiella or Citrobacter
4) Direct cecum:	No helminths
5) Intestinal wet mount:	No enteric protozoan forms
6) Oropharyngeal culture:	No Pseudomonas or Klebsiella
7) Nasopharyngeal culture	
a) Pathogenic Bacteria:	None
b) Group II opportunists:	(+) *Staphylococcus aureus*
c) *Mycoplasma*:	None
8) Middle ear:	No exudates
9) Pelage:	No arthropods
10) Liver (histopathology):	NML
11) Lung (histopathology):	NML
12) Kidney (histopathology):	NML
13) Ileum (histopathology):	NML

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TABLE 2
SEROLOGY REPORT

Client: Therimmune Corp. Accession No. 47502

Species: Rat (1) Date Received: 11/12/2002

Group Designation: Date Completed: 11/15/2002

Anmed Ident: 1

Client Ident:

Method		
ELISA	PVM	-
ELISA	REO3	-
ELISA	GD7	-
ELISA	SEN	-
ELISA	LCM	-
ELISA	KRV/ RPV	-
ELISA	TH1	-
ELISA	SDAV/ RCV	-
ELISA	MYCO	-
ELISA	HAN	-

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TABLE 3
TEST PANEL DESCRIPTIONS

COMPREHENSIVE DIAGNOSTIC SCREEN: The full range of clinical examinations, gross necropsy, microbiologic isolations and histopathology to isolate or otherwise detect the presence of the following microorganisms:

(a) Arthropod ectoparasites. Genera include: Myobia, Myocoptes, Radfordia, Polyplax, Psorergates, Notoedres, Demodex, Liponyssus.

(b) Helminth endoparasites. Genera include: Aspicularis, Syphacia, Hymenolepis, Trichosomoides.

(c) Enteric protozoa. Genera include: Hexamita (Spironucleus), Giardia, Entamoeba, Trichomonads, Eimeria.

(d) Bacteria: Group I: Primary Pathogens. Salmonella sp., Streptobacillus moniliformis, Corynebacterium kutscheri, Pasteurella pneumotropica, Streptococcus pneumoniae.

Group II: Secondary Opportunists. Pseudomonas aeruginosa, Staphylococcus aureus, Citrobacter freundii, Type 4280, Klebsiella oxytoca, Klebsiella pneumoniae, Bordetella bronchiseptica, Beta hemolytic Streptococcus.

(e) Hemoprotozoans (Rickettsia). Hemobartonella, Eperythrozoon.

(f) Mycoplasmas. Mycoplasma pulmonis, Mycoplasma. arthritidis.

Virus Serology, Comprehensive Panel: A battery of viral and mycoplasma agents whose presence is detected by various tests for antibodies in serum. The presence of the virus in the colony may be inferred by positive (+) antibody findings.

Rat Panel (Test 201-102)	Virus Name
PVM	Pneumonia Virus of Mice
REO-3	Respiratory Enteric Orphan III
GD-7	Encephalomyelitis Group
SEN	Sendai
LCM	Lymphocytic choriomeningitis
MYCO	Mycoplasma pulmonis
SDAV	Sialodacryoadenitis Virus (coronavirus)
KRV	Kilham's Rat Virus (parvovirus)
TH1	Toolan's H-1 (parvovirus)
HAN	Hantaan Virus

Appendix 15
Food Analysis Data
Pubertal Toxicity Study of Vinclozolin, Flutamide and Phenobarbital in Male Sprague Dawley Rats and Methoxychlor, Ethinyl Estradiol and Phenobarbital in Female Sprague Dawley Rats when Administered in Corn Oil by Oral Gavage

PMI NUTRITION
INTERNATIONAL

ISOFLAVONE PROFILE

PRODUCT: **CERTIFIED RODENT DIET**
Code: 5002

Lot Number: AUG 28 02 3B

Isoflavone Profile

Total Daidzein (Aglycone Units)	170	ppm
Total Genistein (Aglycone Units)	171	ppm
Total Glycitein (Aglycone Units)	37	ppm
Total All Forms (Aglycone Units)	378	ppm

For additional information concerning the report contact the Quality Department at Richmond, In. (Angela Crutcher) 765-962-9561 ext. 229

For additional information concerning the analytical results contact Dr. Dorrance Haught at 314-768-4362.

1401 S. Hanley Road ◆ St. Louis, MO 63144
Phone: 314-768-4100 ◆ Fax: 314-768-4765

LabDiet PMI NUTRITION

Return to Certified Analysis Administration

Product Code: 5002
Product Desc: CERTIFIED RODENT DIET
Lab Number: L0222045-2
Lot Code: AUG 28 02 3B
Entered: 9/10/2002

Assay	Analysis	Units
PROTEIN	21	%
FAT ACID (HYDRO.)	5.99	%
FIBER (CRUDE)	4.02	%
ARSENIC	LESS THAN 0.2	PPM
CADMIUM	0.068	PPM
CALCIUM	0.821	%
LEAD	0.193	PPM
MERCURY	LESS THAN 0.025	PPM
PHOSPHORUS	0.605	%
SELENIUM	0.320	PPM

ORGANOPHOSPHATES	PPM	ORGANOPHOSPHATES	PPM
Diazinon	LESS THAN 0.02	Disulfoton	LESS THAN 0.02
Ethion	LESS THAN 0.02	Malathion	0.04
Methyl Parathion	LESS THAN 0.02	Parathion	LESS THAN 0.02
Thimet	LESS THAN 0.02	Thiodan	LESS THAN 0.02
Trithion	LESS THAN 0.02		

PESTICIDES AND PCB	PPM	PESTICIDES AND PCB	PPM
Aldrin	LESS THAN 0.02	Alpha-BHC	LESS THAN 0.02
Beta-BHC	LESS THAN 0.02	Chlordane	LESS THAN 0.02
DDE	LESS THAN 0.02	DDT	LESS THAN 0.02
Delta-BHC	LESS THAN 0.02	Dieldrin	LESS THAN 0.02
Endrin	LESS THAN 0.02	HCB	LESS THAN 0.02
Heptachlor	LESS THAN 0.02	Heptachlor Epoxide	LESS THAN 0.02
Lindane	LESS THAN 0.02	Methoxychlor	LESS THAN 0.02
Mirex	LESS THAN 0.02	PCB	LESS THAN 0.15

Aflatoxin	Aflatoxins	LESS THAN 5 PPB

No notes.
For additional information, please contact:
1) Customer Service at (314) 982-1310 -- for assay methodology
2) Dr. Dorrance Haught at (314) 768-4362 -- for nutritional interpretation
3) Richmond, IN Manufacturing Plant at (765) 962-9561 -- all other questions

The term "Less Than" is used to signify the lower limit of quantitation of the procedure under the conditions employed. The use of the term "Less Than" does not imply that traces of analyte were present.

Certified Rodent Diet 5002*

DESCRIPTION

Certified Rodent Diet is a constant-nutrient formulation that has yielded highly favorable results for the maintenance, growth and reproduction of rats and mice. It has been developed as a complete life-cycle diet that can also be used by breeders to assure animals do not develop undesirable tissue residues of contaminants. A sample of this product will have been assayed prior to shipment.

Features and Benefits

- Each package is assayed for environmental contaminants prior to shipment
- Preanalysis monitoring assures maximum diet control
- Fulfills GLP requirements

Product Forms Available

- Oval pellet, 10 mm x 16 mm x 25 mm length (3/8"x5/8"x1")
- Meal (ground pellets)

GUARANTEED ANALYSIS

Crude protein not less than	20.0%
Crude fat not less than	4.5%
Crude fiber not more than	5.5%
Ash not more than	7.0%
Added minerals not more than	2.5%

INGREDIENTS

Ground corn, dehulled soybean meal, ground wheat, fish meal, wheat middlings, brewers dried yeast, cane molasses, wheat germ, dried beet pulp, dehydrated alfalfa meal, ground oats, dried whey, ground soybean hulls, soybean oil, calcium carbonate, casein, salt, dicalcium phosphate, choline chloride, DL-methionine, cholecalciferol, vitamin A acetate, pyridoxine hydrochloride, dl-alpha tocopheryl acetate, thiamin mononitrate, nicotinic acid, calcium pantothenate, riboflavin, cyanocobalamin, folic acid, manganous oxide, zinc oxide, ferrous carbonate, copper sulfate, zinc sulfate, calcium iodate, cobalt carbonate.

FEEDING DIRECTIONS

Feed ad libitum to rodents. Plenty of fresh, clean water should be available to the animals at all times. Refer to the "Animal Care and Biological Values" section of this manual for detailed feeding directions.

Rats- All rats will eat varying amounts of feed depending on their genetic origin. Larger strains will eat between 15-30 grams per day. Smaller strains will eat between 12-15 grams per day. Feeders in rat cages should be designed to hold two to three days supply of feed at one time.

Mice-Adult mice will eat 4 to 5 grams of pelleted ration daily. Some of the larger strains may eat as much as 8 grams per day per animal. Feed should be available on a free choice basis in wire feeders above the floor of the cage.

Hamsters-Adults will eat 10 to 14 grams per day.

CHEMICAL COMPOSITION[1]

***Nutrients*[2]**	
Protein, %	**20.1**
Arginine, %	1.13
Cystine, %	0.27
Glycine, %	0.86
Histidine, %	0.49
Isoleucine, %	1.03
Leucine, %	1.58
Lysine, %	1.18
Methionine, %	0.43
Phenylalanine, %	0.88
Tyrosine, %	0.59
Threonine, %	0.78
Tryptophan, %	0.24
Valine, %	1.05
Serine, %	1.01
Aspartic Acid, %	2.19
Glutamic Acid, %	4.20
Alanine, %	1.24
Proline, %	1.47
Taurine, %	0.01
Fat (ether extract), %	**4.5**
Fat (acid hydrolysis), %	**5.1**
Cholesterol, ppm	150
Linoleic Acid, %	2.15
Linolenic Acid, %	0.16
Arachidonic Acid, %	<0.01
Omega-3 Fatty Acids, %	0.34
Total Saturated Fatty Acids, %	0.86
Total Monounsaturated Fatty Acids, %	1.14
Fiber (Crude), %	**4.6**
Neutral Detergent Fiber[3], %	13.8
Acid Detergent Fiber[4], %	5.9
Nitrogen-Free Extract (by difference), %	**55.0**
Starch, %	36.3
Glucose, %	0.25
Fructose, %	0.30
Sucrose, %	3.13
Lactose, %	1.11
Total Digestible Nutrients, %	**77.0**
Gross Energy, kcal/gm	**4.04**
Physiological Fuel Value[5], kcal/gm	**3.41**
Metabolizable Energy, kcal/gm	**3.10**

Minerals	
Ash, %	**5.8**
Calcium, %	0.80
Phosphorus, %	0.60
Phosphorus (non-phytate), %	0.34
Potassium, %	0.86
Magnesium, %	0.21
Sulfur, %	0.25
Sodium, %	0.30
Chlorine, %	0.47
Fluorine, ppm	13
Iron, ppm	210
Zinc, ppm	76
Manganese, ppm	75
Copper, ppm	11
Cobalt, ppm	0.6
Iodine, ppm	0.77
Chromium, ppm	2.0
Selenium, ppm	0.25

Vitamins	
Carotene, ppm	5.6
Vitamin K (as menadione), ppm	0.4
Thiamin Hydrochloride, ppm	16
Riboflavin, ppm	8.0
Niacin, ppm	95
Pantothenic Acid, ppm	17
Choline Chloride, ppm	1800
Folic Acid, ppm	4.0
Pyridoxine, ppm	6.0
Biotin, ppm	0.13
B_{12}, mcg/kg	20
Vitamin A, IU/gm	18
Vitamin D_3 (added), IU/gm	2.2
Vitamin E, IU/kg	66

Calories provided by:	
Protein, %	23.585
Fat (ether extract), %	11.880
Carbohydrates, %	64.535

*Product Code

1. Based on the latest ingredient analysis information. Since nutrient composition of natural ingredients varies, analysis will differ accordingly.
2. Nutrients expressed as percent of ration except where otherwise indicated. Moisture content is assumed to be 10.0% for the purpose of calculations.
3. NDF = approximately cellulose, hemi-cellulose and lignin.
4. ADF = approximately cellulose and lignin.
5. Physiological Fuel Value (kcal/gm) = Sum of decimal fractions of protein, fat and carbo- hydrate (use Nitrogen Free Extract) x 4,9,4 kcal/gm respectively.

8/14/98

LabDiet
www.labdiet.com

Appendix 16
Determination of Peroxide in Corn Oil
Pubertal Toxicity Study of Vinclozolin, Flutamide and Phenobarbital in Male Sprague Dawley Rats and Methoxychlor, Ethinyl Estradiol and Phenobarbital in Female Sprague Dawley Rats when Administered in Corn Oil by Oral Gavage

Appendix 16
Determination of Peroxide in Corn Oil
Pubertal Toxicity Study of Vinclozolin, Flutamide and Phenobarbital in Male Sprague Dawley Rats and Methoxychlor, Ethinyl Estradiol and Phenobarbital in Female Sprague Dawley Rats when Administered in Corn Oil by Oral Gavage

Sample	Peroxide Value (Meq)
Sample 1	2.62
Sample 2	2.62
Sample 3	2.12
Averaged Peroxide Value	2.45

Appendix 17
Histopathology Report
Pubertal Toxicity Study of Vinclozolin, Flutamide and Phenobarbital in Male Sprague Dawley Rats and Methoxychlor, Ethinyl Estradiol and Phenobarbital in Female Sprague Dawley Rats when Administered in Corn Oil by Oral Gavage

CHARLES RIVER
LABORATORIES
Discovery and Development Services
Pathology Associates Division

FINAL PATHOLOGY REPORT

PUBERTAL TOXICITY STUDY OF VINCLOZOLIN, FLUTAMIDE AND PHENOBARBITAL IN MALE SPRAGUE DAWLEY RATS AND METHOXYCHLOR, ETHINYL ESTRADIOL AND PHENOBARBITAL IN FEMALE SPRAGUE DAWLEY RATS WHEN ADMINISTERED IN CORN OIL BY ORAL GAVAGE

THERIMMUNE RESEARCH CORPORATION STUDY NUMBER 7244-600

NTP/NIEHS CONTRACT N0.: N01-ES-75409

PREPARED FOR
THERIMMUNE RESEARCH CORPORATION

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I. Pathology Narrative

FINAL PATHOLOGY REPORT

PUBERTAL TOXICITY STUDY OF VINCLOZOLIN, FLUTAMIDE AND PHENOBARBITAL IN MALE SPRAGUE DAWLEY RATS AND METHOXYCHLOR, ETHINYL ESTRADIOL AND PHENOBARBITAL IN FEMALE SPRAGUE DAWLEY RATS WHEN ADMINISTERED IN CORN OIL BY ORAL GAVAGE

THERIMMUNE STUDY NO.: 7244-600
NTP/NIEHS CONTRACT NO.: NO1-ES-75409

INTRODUCTION

This report by Pathology Associates Division of Charles River Laboratories, Inc. (PAI), for TherImmune Research Corporation, 15 Firstfield Road, Gaithersburg, MD 20878, presents the results of pathology support for TherImmune Study No. 7244-600. The purpose of this study was to provide data on proposed procedures to evaluate the effects of Vinclozolin, Methoxychlor, Flutamide, Estradiol, and Phenobarbital on pubertal development in the intact juvenile/peripubertal male and female rat. The portions of this study performed by PAI were conducted in compliance with the Food and Drug Administration Good Laboratory Practice Regulations for Nonclinical Laboratory Studies (1987).

EXPERIMENTAL DESIGN AND METHODS

This was a blinded study. Tissues received by PAI had been blinded by group and were processed and evaluated without knowledge of the test article or dose. The test articles and dosage levels corresponding to the treatment groups (as presented below in text Tables 1 and 2) were disclosed through communication with the study director after the histological evaluation of the tissues was completed.

According to the study protocol, fifty time-mated Sprague Dawley Crl:CD®(SD) IGS BR rats were allowed to litter and produce juvenile animals. There was no treatment for the time-mated females. On post natal day (PND) 21, all pups were separated from the dams.

One hundred thirty-five males were assigned to one of nine treatment groups, fifteen animals per group, in a randomized block-fashion based upon weight. The study design for the male juvenile study is shown below in text Table 1. Starting on PND 23, animals were administered the corn oil vehicle or test article in corn oil by oral gavage at 2.5 mL/kg/day. The test articles administered to the males were either vinclozolin, flutamide, or phenobarbital. Animals were dosed once a day up to and including the day of necropsy, PND 53-54.

Table 1. Juvenile Male Cohort Study

Group	Test Material	Dose Level (mg/kg/day)	Number of Males
1	Corn Oil Vehicle	(control)	15
2	Phenobarbital	25	15
3	Phenobarbital	50	15
4	Phenobarbital	100	15
5	Vinclozolin	10	15
6	Vinclozolin	30	15
7	Vinclozolin	100	15
8	Flutamide	25	15
9	Flutamide	50	15

One hundred thirty-five females were assigned to one of nine treatment groups, fifteen animals per group, in a randomized block-fashion based upon weight. The female juvenile study is shown below in text Table 2. Starting on PND 22, animals were administered the corn oil vehicle or test article in corn oil by oral gavage at 2.5 mL/kg/day. The test articles administered to the females were either methoxychlor, ethinyl estradiol, or phenobarbital. Animals were dosed once a day, up to and including the day of necropsy, PND 42-43.

Table 2. Juvenile Female Cohort Study

Group	Test Material	Dose Level (mg/kg/day)	Number of Females
1	Corn Oil	(control)	15
2	Ethinyl Estradiol	0.0025	15
3	Ethinyl Estradiol	0.005	15
4	Methoxychlor	12.5	15
5	Methoxychlor	25	15
6	Methoxychlor	50	15
7	Phenobarbital	25	15
8	Phenobarbital	50	15
9	Phenobarbital	100	15

Animals were euthanized by carbon dioxide inhalation and exsanguination. All animals, including animals sacrificed moribund or found dead, were subjected to a gross necropsy, which included the examination of the external surface of the body and brain, all orifices, the cranial, thoracic, and abdominal cavities and their contents. Protocol required tissues (with the exception of the thyroid and parathyroid) were fixed in Bouin's and then transferred into 70% ethanol within 24 – 48 hours. The thyroid and parathyroid were fixed in 10% neutral buffered formalin.

Tissues were sent to PAI. The protocol required tissues, thyroid, testes, epididymides, ovaries, uterus, cervix, and gross lesions, were processed through paraffin, sectioned at approximately 5 microns, and stained with hematoxylin and eosin (H&E). Histopathological evaluation was performed by the undersigned, board certified veterinary pathologist.

All microscopic pathology data were directly entered into the histopathology module of the LABCAT® pathology data system. The Reports Codes and Abbreviations List (Appendix 1 and 2, respectively) contain explanations for codes and abbreviations used in the data tables. The Project Summary Tables (Section II) summarizes the data by sex and group. The results of the histopathologic evaluation of individual animals are presented in the Tabulated Animal Data Table in Section III. Gross lesions and their microscopic correlates are listed for all groups in the Correlation of Gross & Micro Table in Section IV. All comments are listed in the Comment Report in Section V. The Quality Assurance Statement is found in Section VI.

RESULTS AND DISCUSSION

Early Deaths

Early Death animals were not evaluated in this study.

Gross Lesions

The majority of the gross lesions concerned the accessory sex glands of the males (excised, weighed with fluid, blotted with paper towels, weighed without fluid, and discarded) and dilation of the kidneys of both males and females, which corresponded to hydronephrosis of variable degree. Hydronephrosis was seen in both males and females throughout all groups (except Group 8 males and Group 5 females) and is considered an incidental finding.

Microscopic Findings

Microscopic findings in these groups of animals were variable, but, except for the Group 9 males (50 mg/kg Flutamide), are considered to be incidental and similar to findings expected in any similar groups of animals.

Group 9 males exhibited a variable testicular response to 50 mg/kg Flutamide administered in corn oil via oral gavage. Of 15 animals examined, 5 were normal. Nine of the 15 had luminal dilation of the testicular tubules, one of which was a unilateral dilation, and one animal had multifocal (as opposed to diffuse) luminal dilation of the testicular tubules. However, of those 9 animals exhibiting testicular tubular dilation, only 4 had a corresponding degeneration of the germinal epithelium, minimal in intensity. In addition, 3 of those 4 animals also

had sperm granulomas in the epididymis; a fourth animal exhibiting testicular tubular dilation had a corresponding epididymal sperm granuloma with no degeneration of the germinal epithelium of the testicle.

SUMMARY AND CONCLUSION

This was a blinded study. Tissues received by PAI were blinded by group and were processed and evaluated without knowledge of the test article and dose. The test articles and dosage levels corresponding to the treatment groups were disclosed after the histological evaluation of the tissues was completed.

One hundred thirty-five males were assigned to one of nine treatment groups, fifteen animals per group, in a randomized block-fashion based upon weight. Starting on PND 23, animals were administered the corn oil vehicle or test article in corn oil by oral gavage at 2.5 mL/kg/day. The test articles administered to the males were either vinclozolin, flutamide, or phenobarbital. Animals were dosed once a day up to and including the day of necropsy, PND 53-54.

One hundred thirty-five females were assigned to one of nine treatment groups, fifteen animals per group, in a randomized block-fashion based upon weight. Starting on PND 22, animals were administered the corn oil vehicle or test article in corn oil by oral gavage at 2.5 mL/kg/day. The test articles administered to the females were either methoxychlor, ethinyl estradiol, or phenobarbital. Animals were dosed once a day, up to and including the day of necropsy, PND 42-43.

The protocol required tissues evaluated were thyroid, testes, epididymides, ovaries, uterus, cervix and gross lesions.

Microscopic findings for all groups of animals examined, both males and females, were incidental and would be expected in any like grouping of animals of similar age and environment, except for the Group 9 males.

Group 9 males exhibited a variable testicular response after receiving 50 mg/kg of Flutamide in corn oil via oral gavage. Although 5 animals were microscopically normal, changes in the remaining 10 included dilation of the testicular tubules, degeneration of the germinal epithelium and edema. Four of these animals also had sperm granulomas in the epididymis.

Jerry L. Quance, DVM, DACVP
Study Pathologist

5-27-04
Date

Appendix 1:
Report Codes Table

PATHOLOGY ASSOCIATES

PUBERTAL TOXICITY STUDY OF VINCLOZOLIN, FLUTAMIDE AND PHENOBARBITAL IN MALE SPRAGUE DAWLEY RATS AND METHOXYCHLOR, ETHINYL ESTRADIOL AND PHENOBARBITAL IN FEMALE SPRAGUE DAWLEY RATS WHEN ADMINISTERED IN CORN OIL BY ORAL GAVAGE

THERIMMUNE RESEARCH CORPORATION STUDY NUMBER 7244-600

Report Codes Table

N	Tissues within normal histological limits
A	Autolysis precluding adequate evaluation
U	Tissues unavailable/unsuitable for evaluation
S	Tissues not applicable to animal
R	Recut/Retrim requested
*	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

PUBERTAL TOXICITY STUDY OF VINCLOZOLIN, FLUTAMIDE AND PHENOBARBITAL IN MALE SPRAGUE DAWLEY RATS AND METHOXYCHLOR, ETHINYL ESTRADIOL AND PHENOBARBITAL IN FEMALE SPRAGUE DAWLEY RATS WHEN ADMINISTERED IN CORN OIL BY ORAL GAVAGE

THERIMMUNE RESEARCH CORPORATION STUDY NUMBER 7244-600

Abbreviations List

# EX	Number Examined
7244600	7244-600
BULBCAV	Bulbocavernosus
DORSAL LATERAL	Dorsal-lateral
ID	Identification

II. Project Summary Tables

PATHOLOGY ASSOCIATES

PUBERTAL TOXICITY STUDY OF VINCLOZOLIN, FLUTAMIDE AND PHENOBARBITAL IN MALE SPRAGUE DAWLEY RATS AND METHOXYCHLOR, ETHINYL ESTRADIOL AND PHENOBARBITAL IN FEMALE SPRAGUE DAWLEY RATS WHEN ADMINISTERED IN CORN OIL BY ORAL GAVAGE

PROJECT SUMMARY

STUDY ID : 7244-600 STUDY NUMBER: 7244600
FATE: Terminal Kill
SEX: MALE

INCIDENCE OF NEOPLASTIC and NON-NEOPLASTIC MICROSCOPIC FINDINGS

GROUP:		1 (1)	2 (2)	3 (3)	4 (4)	5 (5)
NUMBER OF ANIMALS:		15	15	15	14	15
		#	#	#	#	#
THYROID	# EX	15	15	15	14	15
Ultimobranchial cyst		3	0	4	4	8
Ectopic thymus		0	0	1	0	0
TESTES	# EX	15	15	15	14	15
Degeneration, germinal epithelium		0	0	0	2	0
Edema		0	0	0	0	0
Dilation, tubule lumen		0	0	0	0	0
EPIDIDYMIDES	# EX	15	15	15	14	15
Infiltrate, lymphocytic		1	0	0	2	2
Sperm granuloma		0	0	0	0	0
Edema		0	0	0	0	0
KIDNEYS	# EX	1	3	4	3	3
Hydronephrosis		1	2	3	3	3
Cyst, cortical		0	1	1	0	0
Chronic progressive nephropathy		0	0	0	1	0
LIVER	# EX	0	1	0	0	0
Infiltrate, mixed cell		0	1	0	0	0
Hemorrhage, capsular		0	1	0	0	0
SPLEEN	# EX	2	0	0	0	1
Lymphocytic hyperplasia		2	0	0	0	1

(1) - Corn Oil Vehicle
(2) - 25 mg/kg Phenobarbital
(3) - 50 mg/kg Phenobarbital
(4) - 100 mg/kg Phenobarbital
(5) - 10 mg/kg Vinclozolin

THERIMUNE RESEARCH CORPORATION STUDY NUMBER 7244-600

LABCAT HP4.33 14-FEB-2003

PATHOLOGY ASSOCIATES

PUBERTAL TOXICITY STUDY OF VINCLOZOLIN, FLUTAMIDE AND PHENOBARBITAL IN MALE SPRAGUE DAWLEY RATS AND METHOXYCHLOR, ETHINYL ESTRADIOL AND PHENOBARBITAL IN FEMALE SPRAGUE DAWLEY RATS WHEN ADMINISTERED IN CORN OIL BY ORAL GAVAGE

PROJECT SUMMARY

STUDY ID : 7244-600 STUDY NUMBER: 7244600
FATE: Terminal Kill
SEX: MALE

INCIDENCE OF NEOPLASTIC and NON-NEOPLASTIC MICROSCOPIC FINDINGS

GROUP:		1	2	3	4	5
		(1)	(2)	(3)	(4)	(5)
NUMBER OF ANIMALS:		15	15	15	14	15
		#	#	#	#	#
THYMUS	# EX	2	1	1	1	0
Hemorrhage		0	0	1	1	0
SKIN	# EX	0	0	0	0	0
Ulceration with scab formation		0	0	0	0	0
Infiltrate, mixed cell		0	0	0	0	0

(1) - Corn Oil Vehicle
(2) - 25 mg/kg Phenobarbital
(3) - 50 mg/kg Phenobarbital
(4) - 100 mg/kg Phenobarbital
(5) - 10 mg/kg Vinclozolin

THERIMUNE RESEARCH CORPORATION STUDY NUMBER 7244-600

LABCAT HP4.33 14-FEB-2003

PATHOLOGY ASSOCIATES

PUBERTAL TOXICITY STUDY OF VINCLOZOLIN, FLUTAMIDE AND PHENOBARBITAL IN MALE SPRAGUE DAWLEY RATS AND METHOXYCHLOR, ETHINYL ESTRADIOL AND PHENOBARBITAL IN FEMALE SPRAGUE DAWLEY RATS WHEN ADMINISTERED IN CORN OIL BY ORAL GAVAGE

PROJECT SUMMARY

STUDY ID : 7244-600 STUDY NUMBER: 7244600
FATE: Terminal Kill
SEX: MALE

INCIDENCE OF NEOPLASTIC and NON-NEOPLASTIC MICROSCOPIC FINDINGS

GROUP:		6 (1)	7 (2)	8 (3)	9 (4)
NUMBER OF ANIMALS:		15	15	15	15
		#	#	#	#
THYROID	# EX	15	15	15	15
Ultimobranchial cyst		5	4	3	4
Ectopic thymus		2	1	1	1
TESTES	# EX	15	15	15	15
Degeneration, germinal epithelium		0	1	1	4
Edema		0	0	0	5
Dilation, tubule lumen		0	0	0	9
EPIDIDYMIDES	# EX	15	15	15	15
Infiltrate, lymphocytic		4	0	1	1
Sperm granuloma		0	0	0	4
Edema		0	0	0	2
KIDNEYS	# EX	4	6	0	2
Hydronephrosis		4	6	0	2
Cyst, cortical		0	0	0	0
Chronic progressive nephropathy		1	0	0	0
LIVER	# EX	0	1	0	0
Infiltrate, mixed cell		0	1	0	0
Hemorrhage, capsular		0	0	0	0
SPLEEN	# EX	1	0	0	0
Lymphocytic hyperplasia		1	0	0	0
THYMUS	# EX	0	0	0	0
Hemorrhage		0	0	0	0

(1) - 30 mg/kg Vinclozolin
(2) - 100 mg/kg Vinclozolin
(3) - 25 mg/kg Flutamide
(4) - 50 mg/kg Flutamide
THERIMUNE RESEARCH CORPORATION STUDY NUMBER 7244-600

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PATHOLOGY ASSOCIATES

PUBERTAL TOXICITY STUDY OF VINCLOZOLIN, FLUTAMIDE AND PHENOBARBITAL IN MALE SPRAGUE DAWLEY RATS AND METHOXYCHLOR, ETHINYL ESTRADIOL AND PHENOBARBITAL IN FEMALE SPRAGUE DAWLEY RATS WHEN ADMINISTERED IN CORN OIL BY ORAL GAVAGE

PROJECT SUMMARY

STUDY ID : 7244-600 STUDY NUMBER: 7244600

FATE: Terminal Kill

SEX: MALE

INCIDENCE OF NEOPLASTIC and NON-NEOPLASTIC MICROSCOPIC FINDINGS

GROUP:		6 (1)	7 (2)	8 (3)	9 (4)
NUMBER OF ANIMALS:		15	15	15	15
		#	#	#	#
SKIN	# EX	1	0	0	0
Ulceration with scab formation		1	0	0	0
Infiltrate, mixed cell		1	0	0	0

(1) - 30 mg/kg Vinclozolin
(2) - 100 mg/kg Vinclozolin
(3) - 25 mg/kg Flutamide
(4) - 50 mg/kg Flutamide

THERIMUNE RESEARCH CORPORATION STUDY NUMBER 7244-600

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PATHOLOGY ASSOCIATES

PUBERTAL TOXICITY STUDY OF VINCLOZOLIN, FLUTAMIDE AND PHENOBARBITAL IN MALE SPRAGUE DAWLEY RATS AND METHOXYCHLOR, ETHINYL ESTRADIOL AND PHENOBARBITAL IN FEMALE SPRAGUE DAWLEY RATS WHEN ADMINISTERED IN CORN OIL BY ORAL GAVAGE

PROJECT SUMMARY

STUDY ID : 7244-600 STUDY NUMBER: 7244600
FATE: Terminal Kill
SEX: FEMALE

INCIDENCE OF NEOPLASTIC and NON-NEOPLASTIC MICROSCOPIC FINDINGS

GROUP:		1	2	3	4	5
		(1)	(2)	(3)	(4)	(5)
NUMBER OF ANIMALS:		15	15	15	15	15
		#	#	#	#	#
THYROID	# EX	15	15	15	14	15
Ultimobranchial cyst		4	5	7	8	4
OVARIES	# EX	15	15	15	15	15
Cyst, oviduct		1	0	0	0	0
Hemorrhage		1	1	0	0	0
Inflammation, mixed cell, oviduct serosa		0	1	0	0	0
Interstitial cell hyperplasia		0	0	0	1	0
Hemorrhage/fluid, bursa		0	0	0	0	0
UTERUS	# EX	15	15	15	15	15
CERVIX	# EX	15	15	15	15	15
KIDNEYS	# EX	2	1	2	4	1
Hydronephrosis		1	1	1	2	0
Fibrosis, interstitial		0	0	0	0	0
Cyst, cortical		0	0	0	0	0
Infiltrate, mixed cell		0	0	0	0	0
Infiltrate, mixed cell, renal pelvis		0	0	0	0	0
Calculi		0	0	0	0	0
LIVER	# EX	1	0	0	0	0
Infiltrate, mixed cell		1	0	0	0	0
TAIL	# EX	0	0	1	0	0
Vertebral dysplasia		0	0	1	0	0

(1) - Corn Oil Vehicle
(2) - 0.0025 mg/kg Ethinyl Estradiol
(3) - 0.005 mg/kg Ethinyl Estradiol
(4) - 12.5 mg/kg Methoxychlor
(5) - 25 mg/kg Methoxychlor

THERIMUNE RESEARCH CORPORATION STUDY NUMBER 7244-600

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PATHOLOGY ASSOCIATES

PUBERTAL TOXICITY STUDY OF VINCLOZOLIN, FLUTAMIDE AND PHENOBARBITAL IN MALE SPRAGUE DAWLEY RATS AND METHOXYCHLOR, ETHINYL ESTRADIOL AND PHENOBARBITAL IN FEMALE SPRAGUE DAWLEY RATS WHEN ADMINISTERED IN CORN OIL BY ORAL GAVAGE

PROJECT SUMMARY

STUDY ID : 7244-600 STUDY NUMBER: 7244600
FATE: Terminal Kill
SEX: FEMALE

INCIDENCE OF NEOPLASTIC and NON-NEOPLASTIC MICROSCOPIC FINDINGS

GROUP:		1 (1)	2 (2)	3 (3)	4 (4)	5 (5)
NUMBER OF ANIMALS:		15	15	15	15	15
		#	#	#	#	#
TAIL	# EX	0	0	1	0	0
Necrosis, tail tip		0	0	0	0	0
SPLEEN	# EX	0	0	0	0	2
Lymphocytic hyperplasia		0	0	0	0	2
Congestion		0	0	0	0	0
ADRENAL GLANDS	# EX	0	0	0	0	1
URETER	# EX	0	0	0	0	0
Dilation		0	0	0	0	0
Inflammation, mixed cell		0	0	0	0	0
URINARY BLADDER	# EX	0	0	0	0	0
Infiltrate, mixed cell		0	0	0	0	0
Hyperplasia, epithelium		0	0	0	0	0
Fibrosis, sub-epithelial		0	0	0	0	0
Ulceration, epithelium		0	0	0	0	0
Calculi		0	0	0	0	0

(1) - Corn Oil Vehicle
(2) - 0.0025 mg/kg Ethinyl Estradiol
(3) - 0.005 mg/kg Ethinyl Estradiol
(4) - 12.5 mg/kg Methoxychlor
(5) - 25 mg/kg Methoxychlor
THERIMUNE RESEARCH CORPORATION STUDY NUMBER 7244-600

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PATHOLOGY ASSOCIATES

PUBERTAL TOXICITY STUDY OF VINCLOZOLIN, FLUTAMIDE AND PHENOBARBITAL IN MALE SPRAGUE DAWLEY RATS AND METHOXYCHLOR, ETHINYL ESTRADIOL AND PHENOBARBITAL IN FEMALE SPRAGUE DAWLEY RATS WHEN ADMINISTERED IN CORN OIL BY ORAL GAVAGE

PROJECT SUMMARY

STUDY ID : 7244-600 STUDY NUMBER: 7244600
FATE: Terminal Kill
SEX: FEMALE

INCIDENCE OF NEOPLASTIC and NON-NEOPLASTIC MICROSCOPIC FINDINGS

GROUP:		6 (1)	7 (2)	8 (3)	9 (4)
NUMBER OF ANIMALS:		15	15	15	15
		#	#	#	#
THYROID	# EX	15	15	15	15
Ultimobranchial cyst		3	8	5	7
OVARIES	# EX	15	15	15	15
Cyst, oviduct		0	0	1	0
Hemorrhage		0	0	0	0
Inflammation, mixed cell, oviduct serosa		0	0	0	0
Interstitial cell hyperplasia		0	0	0	0
Hemorrhage/fluid, bursa		0	1	0	0
UTERUS	# EX	15	15	15	15
CERVIX	# EX	15	15	15	15
KIDNEYS	# EX	1	4	4	3
Hydronephrosis		1	2	3	3
Fibrosis, interstitial		0	1	0	0
Cyst, cortical		0	1	0	0
Infiltrate, mixed cell		0	0	1	0
Infiltrate, mixed cell, renal pelvis		0	0	1	1
Calculi		0	0	0	1
LIVER	# EX	0	0	0	0
Infiltrate, mixed cell		0	0	0	0
TAIL	# EX	0	1	0	0
Vertebral dysplasia		0	0	0	0
Necrosis, tail tip		0	1	0	0

(1) - 50 mg/kg Methoxychlor
(2) - 25 mg/kg Phenobarbital
(3) - 50 mg/kg Phenobarbital
(4) - 100 mg/kg Phenobarbital
THERIMUNE RESEARCH CORPORATION STUDY NUMBER 7244-600

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PATHOLOGY ASSOCIATES

PUBERTAL TOXICITY STUDY OF VINCLOZOLIN, FLUTAMIDE AND PHENOBARBITAL IN MALE SPRAGUE DAWLEY RATS AND METHOXYCHLOR, ETHINYL ESTRADIOL AND PHENOBARBITAL IN FEMALE SPRAGUE DAWLEY RATS WHEN ADMINISTERED IN CORN OIL BY ORAL GAVAGE

PROJECT SUMMARY

STUDY ID : 7244-600 STUDY NUMBER: 7244600
FATE: Terminal Kill
SEX: FEMALE

INCIDENCE OF NEOPLASTIC and NON-NEOPLASTIC MICROSCOPIC FINDINGS

GROUP:		6 (1)	7 (2)	8 (3)	9 (4)
NUMBER OF ANIMALS:		15	15	15	15
		#	#	#	#
SPLEEN	# EX	0	0	1	0
Lymphocytic hyperplasia		0	0	0	0
Congestion		0	0	1	0
ADRENAL GLANDS	# EX	0	1	0	0
URETER	# EX	0	1	0	0
Dilation		0	1	0	0
Inflammation, mixed cell		0	1	0	0
URINARY BLADDER	# EX	0	0	1	1
Infiltrate, mixed cell		0	0	1	1
Hyperplasia, epithelium		0	0	1	1
Fibrosis, sub-epithelial		0	0	0	1
Ulceration, epithelium		0	0	0	1
Calculi		0	0	0	1

(1) - 50 mg/kg Methoxychlor
(2) - 25 mg/kg Phenobarbital
(3) - 50 mg/kg Phenobarbital
(4) - 100 mg/kg Phenobarbital
THERIMUNE RESEARCH CORPORATION STUDY NUMBER 7244-600

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III. Tabulated Animal Data

PATHOLOGY ASSOCIATES

PUBERTAL TOXICITY STUDY OF VINCLOZOLIN, FLUTAMIDE AND PHENOBARBITAL IN MALE SPRAGUE DAWLEY RATS AND METHOXYCHLOR, ETHINYL ESTRADIOL AND PHENOBARBITAL IN FEMALE SPRAGUE DAWLEY RATS WHEN ADMINISTERED IN CORN OIL BY ORAL GAVAGE

TABULATED ANIMAL DATA

STUDY ID : 7244-600 STUDY NUMBER: 7244600
FATE: Terminal Kill GROUP: 1: Corn Oil Vehicle
SEX: MALE

ANIMAL ID:	9026	9027	9028	9029	9030	9031
THYROID	N	N	N	N	N	N
TESTES	N	N	N	N	N	N
EPIDIDYMIDES	N	N	N	N	N	N
Non-Protocol Tissues:						
KIDNEYS	-	-	-	-	-	-
Hydronephrosis	-	-	-	-	-	2L

See Reports Code Table for Symbol Definitions
THERIMUNE RESEARCH CORPORATION STUDY NUMBER 7244-600

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PATHOLOGY ASSOCIATES

PUBERTAL TOXICITY STUDY OF VINCLOZOLIN, FLUTAMIDE AND PHENOBARBITAL IN MALE SPRAGUE DAWLEY RATS AND METHOXYCHLOR, ETHINYL ESTRADIOL AND PHENOBARBITAL IN FEMALE SPRAGUE DAWLEY RATS WHEN ADMINISTERED IN CORN OIL BY ORAL GAVAGE

TABULATED ANIMAL DATA

STUDY ID : 7244-600 STUDY NUMBER: 7244600
FATE: Terminal Kill GROUP: 1: Corn Oil Vehicle
SEX: MALE

ANIMAL ID:	9032	9033	9034	9035	9036	9037
THYROID	N	N	N	N	N	-
Ultimobranchial cyst	-	-	-	-	-	(1)
TESTES	N	N	N	N	N	N
EPIDIDYMIDES	N	N	N	-	N	N
Infiltrate, lymphocytic	-	-	-	(1)	-	-
Non-Protocol Tissues:						
THYMUS	-	-	-	-	-	N

See Reports Code Table for Symbol Definitions
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PATHOLOGY ASSOCIATES

PUBERTAL TOXICITY STUDY OF VINCLOZOLIN, FLUTAMIDE AND PHENOBARBITAL IN MALE SPRAGUE DAWLEY RATS AND METHOXYCHLOR, ETHINYL ESTRADIOL AND PHENOBARBITAL IN FEMALE SPRAGUE DAWLEY RATS WHEN ADMINISTERED IN CORN OIL BY ORAL GAVAGE

TABULATED ANIMAL DATA

STUDY ID : 7244-600 STUDY NUMBER: 7244600
FATE: Terminal Kill GROUP: 1: Corn Oil Vehicle
SEX: MALE

ANIMAL ID:	9038	9039	9040
THYROID	N	-	-
Ultimobranchial cyst	-	<1>	(1)
TESTES	N	N	N
EPIDIDYMIDES	N	N	N
Non-Protocol Tissues:			
SPLEEN	-	-	-
Lymphocytic hyperplasia	-	[2]	[2]
Non-Protocol Tissues:			
THYMUS	-	-	N

See Reports Code Table for Symbol Definitions
THERIMUNE RESEARCH CORPORATION STUDY NUMBER 7244-600

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PATHOLOGY ASSOCIATES

PUBERTAL TOXICITY STUDY OF VINCLOZOLIN, FLUTAMIDE AND PHENOBARBITAL IN MALE SPRAGUE DAWLEY RATS AND METHOXYCHLOR, ETHINYL ESTRADIOL AND PHENOBARBITAL IN FEMALE SPRAGUE DAWLEY RATS WHEN ADMINISTERED IN CORN OIL BY ORAL GAVAGE

TABULATED ANIMAL DATA

STUDY ID : 7244-600 STUDY NUMBER: 7244600
FATE: Terminal Kill GROUP: 2: 25 mg/kg Phenobarbital
SEX: MALE

ANIMAL ID:	9041	9042	9043	9044	9045	9046
THYROID	N	N	N	N	N	N
TESTES	N	N	N	N	N	N
EPIDIDYMIDES	N	N	N	N	N	N
Non-Protocol Tissues:						
KIDNEYS	-	-	-	-	-	-
Cyst, cortical	(2)	-	-	-	-	-
Non-Protocol Tissues:						
LIVER	-	-	-	-	-	-
Infiltrate, mixed cell	-	<1>	-	-	-	-
Hemorrhage, capsular	-	(3)	-	-	-	-

See Reports Code Table for Symbol Definitions
THERIMUNE RESEARCH CORPORATION STUDY NUMBER 7244-600

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PATHOLOGY ASSOCIATES

PUBERTAL TOXICITY STUDY OF VINCLOZOLIN, FLUTAMIDE AND PHENOBARBITAL IN MALE SPRAGUE DAWLEY RATS AND METHOXYCHLOR, ETHINYL ESTRADIOL AND PHENOBARBITAL IN FEMALE SPRAGUE DAWLEY RATS WHEN ADMINISTERED IN CORN OIL BY ORAL GAVAGE

TABULATED ANIMAL DATA

STUDY ID : 7244-600 STUDY NUMBER: 7244600
FATE: Terminal Kill GROUP: 2: 25 mg/kg Phenobarbital
SEX: MALE

ANIMAL ID:	9047	9048	9049	9050	9051	9052
THYROID	N	N	N	N	N	N
TESTES	N	N	N	N	N	N
EPIDIDYMIDES	N	N	N	N	N	N
Non-Protocol Tissues:						
KIDNEYS	-	-	-	-	-	-
Hydronephrosis	2L	-	-	-	-	-

See Reports Code Table for Symbol Definitions
THERIMUNE RESEARCH CORPORATION STUDY NUMBER 7244-600

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PATHOLOGY ASSOCIATES

PUBERTAL TOXICITY STUDY OF VINCLOZOLIN, FLUTAMIDE AND PHENOBARBITAL IN MALE SPRAGUE DAWLEY RATS AND METHOXYCHLOR, ETHINYL ESTRADIOL AND PHENOBARBITAL IN FEMALE SPRAGUE DAWLEY RATS WHEN ADMINISTERED IN CORN OIL BY ORAL GAVAGE

TABULATED ANIMAL DATA

STUDY ID : 7244-600 STUDY NUMBER: 7244600
FATE: Terminal Kill GROUP: 2: 25 mg/kg Phenobarbital
SEX: MALE

ANIMAL ID:	9053	9054	9055
THYROID	N	N	N
TESTES	N	N	N
EPIDIDYMIDES	N	N	N
Non-Protocol Tissues:			
KIDNEYS	-	-	-
Hydronephrosis	2L	-	-
Non-Protocol Tissues:			
THYMUS	-	N	-

See Reports Code Table for Symbol Definitions
THERIMUNE RESEARCH CORPORATION STUDY NUMBER 7244-600

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PATHOLOGY ASSOCIATES

PUBERTAL TOXICITY STUDY OF VINCLOZOLIN, FLUTAMIDE AND PHENOBARBITAL IN MALE SPRAGUE DAWLEY RATS AND METHOXYCHLOR, ETHINYL ESTRADIOL AND PHENOBARBITAL IN FEMALE SPRAGUE DAWLEY RATS WHEN ADMINISTERED IN CORN OIL BY ORAL GAVAGE

TABULATED ANIMAL DATA

STUDY ID : 7244-600 STUDY NUMBER: 7244600
FATE: Terminal Kill GROUP: 3: 50 mg/kg Phenobarbital
SEX: MALE

ANIMAL ID:	9056	9057	9058	9059	9060	9061
THYROID	-	-	N	-	N	N
Ultimobranchial cyst	-	(1)	-	<1>	-	-
Ectopic thymus	P	-	-	-	-	-
TESTES	N	N	N	N	N	N
EPIDIDYMIDES	N	N	N	N	N	N
Non-Protocol Tissues: KIDNEYS	-	-	-	-	-	-
Cyst, cortical	-	-	-	(2)	-	-
Non-Protocol Tissues: SEMINAL VESICLES	-	-	-	-	U	-
Non-Protocol Tissues: COAGULATING GLANDS	-	-	-	-	U	-

See Reports Code Table for Symbol Definitions
THERIMUNE RESEARCH CORPORATION STUDY NUMBER 7244-600

LABCAT HP4.33 14-FEB-2003

PATHOLOGY ASSOCIATES

PUBERTAL TOXICITY STUDY OF VINCLOZOLIN, FLUTAMIDE AND PHENOBARBITAL IN MALE SPRAGUE DAWLEY RATS AND METHOXYCHLOR, ETHINYL ESTRADIOL AND PHENOBARBITAL IN FEMALE SPRAGUE DAWLEY RATS WHEN ADMINISTERED IN CORN OIL BY ORAL GAVAGE

TABULATED ANIMAL DATA

STUDY ID : 7244-600 STUDY NUMBER: 7244600
FATE: Terminal Kill GROUP: 3: 50 mg/kg Phenobarbital
SEX: MALE

ANIMAL ID:	9062	9063	9064	9065	9066	9067
THYROID	N	N	N	N	-	N
Ultimobranchial cyst	-	-	-	-	<1>	-
TESTES	N	N	N	N	N	N
EPIDIDYMIDES	N	N	N	N	N	N
Non-Protocol Tissues:						
KIDNEYS	-	-	-	-	-	-
Hydronephrosis	2L	3L	-	-	3L	-
Non-Protocol Tissues:						
THYMUS	-	-	-	-	-	-
Hemorrhage	-	-	-	<2>	-	-

See Reports Code Table for Symbol Definitions
THERIMUNE RESEARCH CORPORATION STUDY NUMBER 7244-600

LABCAT HP4.33 14-FEB-2003

PATHOLOGY ASSOCIATES

PUBERTAL TOXICITY STUDY OF VINCLOZOLIN, FLUTAMIDE AND PHENOBARBITAL IN MALE SPRAGUE DAWLEY RATS AND METHOXYCHLOR, ETHINYL ESTRADIOL AND PHENOBARBITAL IN FEMALE SPRAGUE DAWLEY RATS WHEN ADMINISTERED IN CORN OIL BY ORAL GAVAGE

TABULATED ANIMAL DATA

STUDY ID : 7244-600 STUDY NUMBER: 7244600
FATE: Terminal Kill GROUP: 3: 50 mg/kg Phenobarbital
SEX: MALE

ANIMAL ID:	9068	9069	9070
THYROID	N	-	N
Ultimobranchial cyst	-	<1>	-
TESTES	N	N	N
EPIDIDYMIDES	N	N	N

See Reports Code Table for Symbol Definitions
THERIMUNE RESEARCH CORPORATION STUDY NUMBER 7244-600

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PATHOLOGY ASSOCIATES

PUBERTAL TOXICITY STUDY OF VINCLOZOLIN, FLUTAMIDE AND PHENOBARBITAL IN MALE SPRAGUE DAWLEY RATS AND METHOXYCHLOR, ETHINYL ESTRADIOL AND PHENOBARBITAL IN FEMALE SPRAGUE DAWLEY RATS WHEN ADMINISTERED IN CORN OIL BY ORAL GAVAGE

TABULATED ANIMAL DATA

STUDY ID : 7244-600 STUDY NUMBER: 7244600
FATE: Terminal Kill GROUP: 4: 100 mg/kg Phenobarbital
SEX: MALE

ANIMAL ID:	9071	9072	9073	9074	9075	9076
THYROID	N	N	N	N	-	-
Ultimobranchial cyst	-	-	-	-	(1)	(1)
TESTES	N	N	-	N	N	N
Degeneration, germinal epithelium	-	-	<3>	-	-	-
EPIDIDYMIDES	-	N	N	-	N	N
Infiltrate, lymphocytic	<1>	-	-	(1)	-	-
Non-Protocol Tissues:						
KIDNEYS	-	-	-	-	-	-
Hydronephrosis	-	-	-	-	3	-
Non-Protocol Tissues:						
SEMINAL VESICLES	-	-	U	-	-	-
Non-Protocol Tissues:						
COAGULATING GLANDS	-	-	U	-	-	-

See Reports Code Table for Symbol Definitions
THERIMUNE RESEARCH CORPORATION STUDY NUMBER 7244-600

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PATHOLOGY ASSOCIATES

PUBERTAL TOXICITY STUDY OF VINCLOZOLIN, FLUTAMIDE AND PHENOBARBITAL IN MALE SPRAGUE DAWLEY RATS AND METHOXYCHLOR, ETHINYL ESTRADIOL AND PHENOBARBITAL IN FEMALE SPRAGUE DAWLEY RATS WHEN ADMINISTERED IN CORN OIL BY ORAL GAVAGE

TABULATED ANIMAL DATA

STUDY ID : 7244-600 STUDY NUMBER: 7244600
FATE: Terminal Kill GROUP: 4: 100 mg/kg Phenobarbital
SEX: MALE

ANIMAL ID:	9078	9079	9080	9081	9082	9083
THYROID	N	N	-	N	N	N
Ultimobranchial cyst	-	-	(1)	-	-	-
TESTES	N	-	N	N	N	N
Degeneration, germinal epithelium	-	<1>	-	-	-	-
EPIDIDYMIDES	N	N	N	N	N	N
Non-Protocol Tissues:						
KIDNEYS	-	-	-	-	-	-
Hydronephrosis	2L	-	-	-	-	2L
Chronic progressive nephropathy	-	-	-	-	-	(1)

See Reports Code Table for Symbol Definitions
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PATHOLOGY ASSOCIATES

PUBERTAL TOXICITY STUDY OF VINCLOZOLIN, FLUTAMIDE AND PHENOBARBITAL IN MALE SPRAGUE DAWLEY RATS AND METHOXYCHLOR, ETHINYL ESTRADIOL AND PHENOBARBITAL IN FEMALE SPRAGUE DAWLEY RATS WHEN ADMINISTERED IN CORN OIL BY ORAL GAVAGE

TABULATED ANIMAL DATA

STUDY ID : 7244-600 STUDY NUMBER: 7244600
FATE: Terminal Kill GROUP: 4: 100 mg/kg Phenobarbital
SEX: MALE

ANIMAL ID:	9084	9085
THYROID	-	N
Ultimobranchial cyst	(1)	-
TESTES	N	N
EPIDIDYMIDES	N	N
Non-Protocol Tissues:		
THYMUS	-	-
Hemorrhage	<1>	-

See Reports Code Table for Symbol Definitions
THERIMUNE RESEARCH CORPORATION STUDY NUMBER 7244-600

LABCAT HP4.33 14-FEB-2003

PATHOLOGY ASSOCIATES

PUBERTAL TOXICITY STUDY OF VINCLOZOLIN, FLUTAMIDE AND PHENOBARBITAL IN MALE SPRAGUE DAWLEY RATS AND METHOXYCHLOR, ETHINYL ESTRADIOL AND PHENOBARBITAL IN FEMALE SPRAGUE DAWLEY RATS WHEN ADMINISTERED IN CORN OIL BY ORAL GAVAGE

TABULATED ANIMAL DATA

STUDY ID : 7244-600 STUDY NUMBER: 7244600
FATE: Terminal Kill GROUP: 5: 10 mg/kg Vinclozolin
SEX: MALE

ANIMAL ID:	9086	9087	9088	9089	9090	9091
THYROID	N	N	N	-	-	N
Ultimobranchial cyst	-	-	-	(1)	<1>	-
TESTES	N	N	N	N	N	N
EPIDIDYMIDES	N	N	N	-	N	N
Infiltrate, lymphocytic	-	-	-	(1)	-	-
Non-Protocol Tissues:						
KIDNEYS	-	-	-	-	-	-
Hydronephrosis	-	-	3L	-	-	-

See Reports Code Table for Symbol Definitions
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PATHOLOGY ASSOCIATES

PUBERTAL TOXICITY STUDY OF VINCLOZOLIN, FLUTAMIDE AND PHENOBARBITAL IN MALE SPRAGUE DAWLEY RATS AND METHOXYCHLOR, ETHINYL ESTRADIOL AND PHENOBARBITAL IN FEMALE SPRAGUE DAWLEY RATS WHEN ADMINISTERED IN CORN OIL BY ORAL GAVAGE

TABULATED ANIMAL DATA

STUDY ID : 7244-600 STUDY NUMBER: 7244600
FATE: Terminal Kill GROUP: 5: 10 mg/kg Vinclozolin
SEX: MALE

ANIMAL ID:	9092	9093	9094	9095	9096	9097
THYROID	-	-	N	-	N	-
Ultimobranchial cyst	(1)	(1)	-	(1)	-	<1>
TESTES	N	N	N	N	N	N
EPIDIDYMIDES	N	N	N	N	N	-
Infiltrate, lymphocytic	-	-	-	-	-	(1)
Non-Protocol Tissues:						
SPLEEN	-	-	-	-	-	-
Lymphocytic hyperplasia	-	-	-	-	-	[2]
Non-Protocol Tissues:						
DORSAL LATERAL PROSTATE	-	-	-	-	-	U
Non-Protocol Tissues:						
VENTRAL PROSTATE	-	-	-	-	-	U

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PATHOLOGY ASSOCIATES

PUBERTAL TOXICITY STUDY OF VINCLOZOLIN, FLUTAMIDE AND PHENOBARBITAL IN MALE SPRAGUE DAWLEY RATS AND METHOXYCHLOR, ETHINYL ESTRADIOL AND PHENOBARBITAL IN FEMALE SPRAGUE DAWLEY RATS WHEN ADMINISTERED IN CORN OIL BY ORAL GAVAGE

TABULATED ANIMAL DATA

STUDY ID : 7244-600 STUDY NUMBER: 7244600
FATE: Terminal Kill GROUP: 5: 10 mg/kg Vinclozolin
SEX: MALE

ANIMAL ID:	9098	9099	9100
THYROID	-	N	-
Ultimobranchial cyst	<1>	-	<1>
TESTES	N	N	N
EPIDIDYMIDES	N	N	N
Non-Protocol Tissues:			
KIDNEYS	-	-	-
Hydronephrosis	-	1L	3L
Non-Protocol Tissues:			
SEMINAL VESICLES	-	U	-
Non-Protocol Tissues:			
COAGULATING GLANDS	-	U	-

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PUBERTAL TOXICITY STUDY OF VINCLOZOLIN, FLUTAMIDE AND PHENOBARBITAL IN MALE SPRAGUE DAWLEY RATS AND METHOXYCHLOR, ETHINYL ESTRADIOL AND PHENOBARBITAL IN FEMALE SPRAGUE DAWLEY RATS WHEN ADMINISTERED IN CORN OIL BY ORAL GAVAGE

TABULATED ANIMAL DATA

STUDY ID : 7244-600 STUDY NUMBER: 7244600
FATE: Terminal Kill GROUP: 6: 30 mg/kg Vinclozolin
SEX: MALE

ANIMAL ID:	9101	9102	9103	9104	9105	9106
THYROID	N	-	N	-	N	N
Ultimobranchial cyst	-	<1>	-	-	-	-
Ectopic thymus	-	-	-	P	-	-
TESTES	N	N	N	N	N	N
EPIDIDYMIDES	-	-	N	N	N	N
Infiltrate, lymphocytic	<1>	(1)	-	-	-	-
Non-Protocol Tissues:						
KIDNEYS	-	-	-	-	-	-
Hydronephrosis	-	-	-	3L	-	-
Non-Protocol Tissues:						
SPLEEN	-	-	-	-	-	-
Lymphocytic hyperplasia	-	-	-	-	-	[2]

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PATHOLOGY ASSOCIATES

PUBERTAL TOXICITY STUDY OF VINCLOZOLIN, FLUTAMIDE AND PHENOBARBITAL IN MALE SPRAGUE DAWLEY RATS AND METHOXYCHLOR, ETHINYL ESTRADIOL AND PHENOBARBITAL IN FEMALE SPRAGUE DAWLEY RATS WHEN ADMINISTERED IN CORN OIL BY ORAL GAVAGE

TABULATED ANIMAL DATA

STUDY ID : 7244-600 STUDY NUMBER: 7244600
FATE: Terminal Kill GROUP: 6: 30 mg/kg Vinclozolin
SEX: MALE

ANIMAL ID:	9107	9108	9109	9110	9111	9112
THYROID	-	N	-	N	-	N
Ultimobranchial cyst	(1)	-	<1>	-	<1>	-
Ectopic thymus	P	-	-	-	-	-
TESTES	N	N	N	N	N	N
EPIDIDYMIDES	N	N	N	N	N	N
Non-Protocol Tissues:						
KIDNEYS	-	-	-	-	-	-
Hydronephrosis	-	-	2L	2L	-	3L
Chronic progressive nephropathy	-	-	-	-	-	(1)
Non-Protocol Tissues:						
SKIN	-	-	-	-	-	-
Ulceration with scab formation	-	-	-	-	[3]	-
Infiltrate, mixed cell	-	-	-	-	[3]	-

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PATHOLOGY ASSOCIATES

PUBERTAL TOXICITY STUDY OF VINCLOZOLIN, FLUTAMIDE AND PHENOBARBITAL IN MALE SPRAGUE DAWLEY RATS AND METHOXYCHLOR, ETHINYL ESTRADIOL AND PHENOBARBITAL IN FEMALE SPRAGUE DAWLEY RATS WHEN ADMINISTERED IN CORN OIL BY ORAL GAVAGE

TABULATED ANIMAL DATA

STUDY ID : 7244-600 STUDY NUMBER: 7244600
FATE: Terminal Kill GROUP: 6: 30 mg/kg Vinclozolin
SEX: MALE

ANIMAL ID:	9113	9114	9115
THYROID	-	N	N
Ultimobranchial cyst	(1)	-	-
TESTES	N	N	N
EPIDIDYMIDES	N	-	-
Infiltrate, lymphocytic	-	<1>	<1>

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PATHOLOGY ASSOCIATES

PUBERTAL TOXICITY STUDY OF VINCLOZOLIN, FLUTAMIDE AND PHENOBARBITAL IN MALE SPRAGUE DAWLEY RATS AND METHOXYCHLOR, ETHINYL ESTRADIOL AND PHENOBARBITAL IN FEMALE SPRAGUE DAWLEY RATS WHEN ADMINISTERED IN CORN OIL BY ORAL GAVAGE

TABULATED ANIMAL DATA

STUDY ID : 7244-600 STUDY NUMBER: 7244600
FATE: Terminal Kill GROUP: 7: 100 mg/kg Vinclozolin
SEX: MALE

ANIMAL ID:	9116	9117	9118	9119	9120	9121
THYROID	N	-	N	N	N	-
Ultimobranchial cyst	-	<1>	-	-	-	<1>
TESTES	N	N	N	N	N	N
EPIDIDYMIDES	N	N	N	N	N	N
Non-Protocol Tissues: KIDNEYS	-	-	-	-	-	-
Hydronephrosis	-	-	-	3L	2L	2L
Non-Protocol Tissues: LIVER	-	-	-	-	-	-
Infiltrate, mixed cell	-	-	-	<1>	-	-
Non-Protocol Tissues: SEMINAL VESICLES	-	-	U	-	-	U
Non-Protocol Tissues: COAGULATING GLANDS	-	-	U	-	-	U
Non-Protocol Tissues: DORSAL LATERAL PROSTATE	-	-	U	-	-	-
Non-Protocol Tissues: VENTRAL PROSTATE	-	-	U	-	-	-

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PATHOLOGY ASSOCIATES

PUBERTAL TOXICITY STUDY OF VINCLOZOLIN, FLUTAMIDE AND PHENOBARBITAL IN MALE SPRAGUE DAWLEY RATS AND METHOXYCHLOR, ETHINYL ESTRADIOL AND PHENOBARBITAL IN FEMALE SPRAGUE DAWLEY RATS WHEN ADMINISTERED IN CORN OIL BY ORAL GAVAGE

TABULATED ANIMAL DATA

STUDY ID : 7244-600 STUDY NUMBER: 7244600
FATE: Terminal Kill GROUP: 7: 100 mg/kg Vinclozolin
SEX: MALE

ANIMAL ID:	9122	9123	9124	9125	9126	9127
THYROID	N	N	N	N	N	-
Ultimobranchial cyst	-	-	-	-	-	<1>
Ectopic thymus	-	-	-	-	-	P
TESTES	N	N	N	N	N	N
EPIDIDYMIDES	N	N	N	N	N	N
Non-Protocol Tissues:						
KIDNEYS	-	-	-	-	-	-
Hydronephrosis	2L	-	-	-	-	2L

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PUBERTAL TOXICITY STUDY OF VINCLOZOLIN, FLUTAMIDE AND PHENOBARBITAL IN MALE SPRAGUE DAWLEY RATS AND METHOXYCHLOR, ETHINYL ESTRADIOL AND PHENOBARBITAL IN FEMALE SPRAGUE DAWLEY RATS WHEN ADMINISTERED IN CORN OIL BY ORAL GAVAGE

TABULATED ANIMAL DATA

STUDY ID : 7244-600 STUDY NUMBER: 7244600
FATE: Terminal Kill GROUP: 7: 100 mg/kg Vinclozolin
SEX: MALE

ANIMAL ID:	9128	9129	9130
THYROID	N	N	-
Ultimobranchial cyst	-	-	<1>
TESTES	N	-	N
Degeneration, germinal epithelium	-	<1>	-
EPIDIDYMIDES	N	N	N
Non-Protocol Tissues:			
KIDNEYS	-	-	-
Hydronephrosis	3L	-	-

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PATHOLOGY ASSOCIATES

PUBERTAL TOXICITY STUDY OF VINCLOZOLIN, FLUTAMIDE AND PHENOBARBITAL IN MALE SPRAGUE DAWLEY RATS AND METHOXYCHLOR, ETHINYL ESTRADIOL AND PHENOBARBITAL IN FEMALE SPRAGUE DAWLEY RATS WHEN ADMINISTERED IN CORN OIL BY ORAL GAVAGE

TABULATED ANIMAL DATA

STUDY ID : 7244-600
FATE: Terminal Kill

STUDY NUMBER: 7244600
GROUP: 8: 25 mg/kg Flutamide
SEX: MALE

ANIMAL ID:	9131	9132	9133	9134	9135	9136
THYROID	N	N	N	N	N	-
Ultimobranchial cyst	-	-	-	-	-	(1)
TESTES	N	N	N	N	N	N
EPIDIDYMIDES	N	N	N	N	N	N
Non-Protocol Tissues: SEMINAL VESICLES	U	U	U	U	U	U
Non-Protocol Tissues: COAGULATING GLANDS	U	U	U	U	U	U
Non-Protocol Tissues: DORSAL LATERAL PROSTATE	U	-	-	U	-	U
Non-Protocol Tissues: VENTRAL PROSTATE	U	-	-	U	-	U

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PUBERTAL TOXICITY STUDY OF VINCLOZOLIN, FLUTAMIDE AND PHENOBARBITAL IN MALE SPRAGUE DAWLEY RATS AND METHOXYCHLOR, ETHINYL ESTRADIOL AND PHENOBARBITAL IN FEMALE SPRAGUE DAWLEY RATS WHEN ADMINISTERED IN CORN OIL BY ORAL GAVAGE

TABULATED ANIMAL DATA

STUDY ID : 7244-600 STUDY NUMBER: 7244600
FATE: Terminal Kill GROUP: 8: 25 mg/kg Flutamide
SEX: MALE

ANIMAL ID:	9137	9138	9139	9140	9141	9142
THYROID	N	-	-	-	N	N
Ultimobranchial cyst	-	(1)	-	(1)	-	-
Ectopic thymus	-	-	P	-	-	-
TESTES	-	N	N	N	N	N
Degeneration, germinal epithelium	<2>	-	-	-	-	-
EPIDIDYMIDES	N	N	N	-	N	N
Infiltrate, lymphocytic	-	-	-	<1>	-	-
Non-Protocol Tissues: SEMINAL VESICLES	U	U	U	U	U	-
Non-Protocol Tissues: COAGULATING GLANDS	U	U	U	U	U	-
Non-Protocol Tissues: DORSAL LATERAL PROSTATE	U	U	-	U	U	-
Non-Protocol Tissues: VENTRAL PROSTATE	U	U	-	U	U	-

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TABULATED ANIMAL DATA

STUDY ID : 7244-600 STUDY NUMBER: 7244600
FATE: Terminal Kill GROUP: 8: 25 mg/kg Flutamide
SEX: MALE

ANIMAL ID:	9143	9144	9145
THYROID	N	N	N
TESTES	N	N	N
EPIDIDYMIDES	N	N	N
Non-Protocol Tissues: SEMINAL VESICLES	U	U	U
Non-Protocol Tissues: COAGULATING GLANDS	U	U	U
Non-Protocol Tissues: DORSAL LATERAL PROSTATE	U	U	U
Non-Protocol Tissues: VENTRAL PROSTATE	U	U	U

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TABULATED ANIMAL DATA

STUDY ID : 7244-600 STUDY NUMBER: 7244600
FATE: Terminal Kill GROUP: 9: 50 mg/kg Flutamide
SEX: MALE

ANIMAL ID:	9146	9147	9148	9149	9150	9151
THYROID	N	N	N	-	N	-
Ultimobranchial cyst	-	-	-	(1)	-	(1)
Ectopic thymus	-	-	-	P	-	-
TESTES	N	-	-	N	-	-
Degeneration, germinal epithelium	-	<1>	<1>	-	-	-
Dilation, tubule lumen	-	[3]	[3]	-	[3]	<1>
EPIDIDYMIDES	N	-	-	N	N	N
Sperm granuloma	-	<2>	(2)	-	-	-
Non-Protocol Tissues: KIDNEYS	-	-	-	-	-	-
Hydronephrosis	-	-	-	2L	-	-
Non-Protocol Tissues: SEMINAL VESICLES	U	U	U	U	U	U
Non-Protocol Tissues: COAGULATING GLANDS	U	U	U	U	U	U
Non-Protocol Tissues: DORSAL LATERAL PROSTATE	-	U	-	U	U	U
Non-Protocol Tissues: VENTRAL PROSTATE	-	U	-	U	U	U
Non-Protocol Tissues: LEVATOR ANI PLUS BULBCAV	-	U	-	-	-	-

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PUBERTAL TOXICITY STUDY OF VINCLOZOLIN, FLUTAMIDE AND PHENOBARBITAL IN MALE SPRAGUE DAWLEY RATS AND METHOXYCHLOR, ETHINYL ESTRADIOL AND PHENOBARBITAL IN FEMALE SPRAGUE DAWLEY RATS WHEN ADMINISTERED IN CORN OIL BY ORAL GAVAGE

TABULATED ANIMAL DATA

STUDY ID : 7244-600 STUDY NUMBER: 7244600
FATE: Terminal Kill GROUP: 9: 50 mg/kg Flutamide
SEX: MALE

ANIMAL ID:	9152	9153	9154	9155	9156	9157
THYROID	N	N	N	N	N	N
TESTES	-	N	-	-	-	-
Degeneration, germinal epithelium	-	-	<1>	-	-	-
Edema	2	-	2	1	1	1
Dilation, tubule lumen	[3]	-	[3]	[2]	[2]	-
EPIDIDYMIDES	-	N	-	-	N	N
Sperm granuloma	(2)	-	(2)	-	-	-
Edema	-	-	-	2	-	-
Non-Protocol Tissues: SEMINAL VESICLES	U	U	U	U	U	U
Non-Protocol Tissues: COAGULATING GLANDS	U	U	U	U	U	U
Non-Protocol Tissues: DORSAL LATERAL PROSTATE	-	U	U	U	U	U
Non-Protocol Tissues: VENTRAL PROSTATE	-	U	U	U	U	U
Non-Protocol Tissues: LEVATOR ANI PLUS BULBCAV	-	-	U	-	U	-

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TABULATED ANIMAL DATA

STUDY ID : 7244-600 STUDY NUMBER: 7244600
FATE: Terminal Kill GROUP: 9: 50 mg/kg Flutamide
SEX: MALE

ANIMAL ID:	9158	9159	9160
THYROID	N	-	-
Ultimobranchial cyst	-	(1)	(1)
TESTES	N	-	N
Degeneration, germinal epithelium	-	<1>L	-
Dilation, tubule lumen	-	[3]L	-
EPIDIDYMIDES	N	-	-
Infiltrate, lymphocytic	-	(1)	-
Edema	-	-	<2>
Non-Protocol Tissues: KIDNEYS	-	-	-
Hydronephrosis	-	2L	-
Non-Protocol Tissues: SEMINAL VESICLES	U	U	U
Non-Protocol Tissues: COAGULATING GLANDS	U	U	U
Non-Protocol Tissues: DORSAL LATERAL PROSTATE	U	U	U
Non-Protocol Tissues: VENTRAL PROSTATE	U	U	U
Non-Protocol Tissues: LEVATOR ANI PLUS BULBCAV	-	-	U

See Reports Code Table for Symbol Definitions
THERIMUNE RESEARCH CORPORATION STUDY NUMBER 7244-600

LABCAT HP4.33 14-FEB-2003

PATHOLOGY ASSOCIATES

PUBERTAL TOXICITY STUDY OF VINCLOZOLIN, FLUTAMIDE AND PHENOBARBITAL IN MALE SPRAGUE DAWLEY RATS AND METHOXYCHLOR, ETHINYL ESTRADIOL AND PHENOBARBITAL IN FEMALE SPRAGUE DAWLEY RATS WHEN ADMINISTERED IN CORN OIL BY ORAL GAVAGE

TABULATED ANIMAL DATA

STUDY ID : 7244-600
FATE: Terminal Kill

STUDY NUMBER: 7244600
GROUP: 1: Corn Oil Vehicle
SEX: FEMALE

ANIMAL ID:	9161	9162	9163	9164	9165	9166
THYROID	-	-	N	N	N	N
Ultimobranchial cyst	(1)	(1)	-	-	-	-
OVARIES	N	N	-	N	N	N
Cyst, oviduct	-	-	(1)	-	-	-
UTERUS	N	N	N	N	N	N
CERVIX	N	N	N	N	N	N

See Reports Code Table for Symbol Definitions
THERIMUNE RESEARCH CORPORATION STUDY NUMBER 7244-600

LABCAT HP4.33 14-FEB-2003

PATHOLOGY ASSOCIATES

PUBERTAL TOXICITY STUDY OF VINCLOZOLIN, FLUTAMIDE AND PHENOBARBITAL IN MALE SPRAGUE DAWLEY RATS AND METHOXYCHLOR, ETHINYL ESTRADIOL AND PHENOBARBITAL IN FEMALE SPRAGUE DAWLEY RATS WHEN ADMINISTERED IN CORN OIL BY ORAL GAVAGE

TABULATED ANIMAL DATA

STUDY ID : 7244-600
FATE: Terminal Kill

STUDY NUMBER: 7244600
GROUP: 1: Corn Oil Vehicle
SEX: FEMALE

ANIMAL ID:	9167	9168	9169	9170	9171	9172
THYROID	N	N	N	N	N	N
OVARIES	N	N	-	N	N	N
Hemorrhage	-	-	[2]L	-	-	-
UTERUS	N	N	N	N	N	N
CERVIX	N	N	N	N	N	N
Non-Protocol Tissues:						
KIDNEYS	-	-	N	-	-	-
Hydronephrosis	2L	-	-	-	-	-
Non-Protocol Tissues:						
LIVER	-	-	-	-	-	-
Infiltrate, mixed cell	-	-	-	-	<1>	-

See Reports Code Table for Symbol Definitions
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PATHOLOGY ASSOCIATES

PUBERTAL TOXICITY STUDY OF VINCLOZOLIN, FLUTAMIDE AND PHENOBARBITAL IN MALE SPRAGUE DAWLEY RATS AND METHOXYCHLOR, ETHINYL ESTRADIOL AND PHENOBARBITAL IN FEMALE SPRAGUE DAWLEY RATS WHEN ADMINISTERED IN CORN OIL BY ORAL GAVAGE

TABULATED ANIMAL DATA

STUDY ID : 7244-600
FATE: Terminal Kill

STUDY NUMBER: 7244600
GROUP: 1: Corn Oil Vehicle
SEX: FEMALE

ANIMAL ID:	9173	9174	9175
THYROID	-	-	N
Ultimobranchial cyst	<1>	(1)	-
OVARIES	N	N	N
UTERUS	N	N	N
CERVIX	N	N	N

See Reports Code Table for Symbol Definitions
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PATHOLOGY ASSOCIATES

PUBERTAL TOXICITY STUDY OF VINCLOZOLIN, FLUTAMIDE AND PHENOBARBITAL IN MALE SPRAGUE DAWLEY RATS AND METHOXYCHLOR, ETHINYL ESTRADIOL AND PHENOBARBITAL IN FEMALE SPRAGUE DAWLEY RATS WHEN ADMINISTERED IN CORN OIL BY ORAL GAVAGE

TABULATED ANIMAL DATA

STUDY ID : 7244-600 STUDY NUMBER: 7244600
FATE: Terminal Kill GROUP: 2: 0.0025 mg/kg Ethinyl Estradiol
SEX: FEMALE

ANIMAL ID:	9176	9177	9178	9179	9180	9181
THYROID	N	-	N	-	-	N
Ultimobranchial cyst	-	<1>	-	(1)	(1)	-
OVARIES	N	N	N	N	N	N
UTERUS	N	N	N	N	N	N
CERVIX	N	N	N	N	N	N
Non-Protocol Tissues:						
KIDNEYS	-	-	-	-	-	-
Hydronephrosis	-	-	-	-	-	2L

See Reports Code Table for Symbol Definitions
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PATHOLOGY ASSOCIATES

PUBERTAL TOXICITY STUDY OF VINCLOZOLIN, FLUTAMIDE AND PHENOBARBITAL IN MALE SPRAGUE DAWLEY RATS AND METHOXYCHLOR, ETHINYL ESTRADIOL AND PHENOBARBITAL IN FEMALE SPRAGUE DAWLEY RATS WHEN ADMINISTERED IN CORN OIL BY ORAL GAVAGE

TABULATED ANIMAL DATA

STUDY ID : 7244-600 STUDY NUMBER: 7244600
FATE: Terminal Kill GROUP: 2: 0.0025 mg/kg Ethinyl Estradiol
SEX: FEMALE

ANIMAL ID:	9182	9183	9184	9185	9186	9187
THYROID	N	N	N	N	N	-
Ultimobranchial cyst	-	-	-	-	-	(1)
OVARIES	-	N	N	N	-	N
Hemorrhage	-	-	-	-	[1]	-
Inflammation, mixed cell, oviduct serosa	(1)	-	-	-	-	-
UTERUS	N	N	N	N	N	N
CERVIX	N	N	N	N	N	N

See Reports Code Table for Symbol Definitions
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PATHOLOGY ASSOCIATES

PUBERTAL TOXICITY STUDY OF VINCLOZOLIN, FLUTAMIDE AND PHENOBARBITAL IN MALE SPRAGUE DAWLEY RATS AND METHOXYCHLOR, ETHINYL ESTRADIOL AND PHENOBARBITAL IN FEMALE SPRAGUE DAWLEY RATS WHEN ADMINISTERED IN CORN OIL BY ORAL GAVAGE

TABULATED ANIMAL DATA

STUDY ID : 7244-600 STUDY NUMBER: 7244600
FATE: Terminal Kill GROUP: 2: 0.0025 mg/kg Ethinyl Estradiol
SEX: FEMALE

ANIMAL ID:	9188	9189	9190
THYROID	N	N	-
Ultimobranchial cyst	-	-	(1)
OVARIES	N	N	N
UTERUS	N	N	N
CERVIX	N	N	N

See Reports Code Table for Symbol Definitions
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PATHOLOGY ASSOCIATES

PUBERTAL TOXICITY STUDY OF VINCLOZOLIN, FLUTAMIDE AND PHENOBARBITAL IN MALE SPRAGUE DAWLEY RATS AND METHOXYCHLOR, ETHINYL ESTRADIOL AND PHENOBARBITAL IN FEMALE SPRAGUE DAWLEY RATS WHEN ADMINISTERED IN CORN OIL BY ORAL GAVAGE

TABULATED ANIMAL DATA

STUDY ID : 7244-600 STUDY NUMBER: 7244600
FATE: Terminal Kill GROUP: 3: 0.005 mg/kg Ethinyl Estradiol
SEX: FEMALE

ANIMAL ID:	9191	9192	9193	9194	9195	9196
THYROID	N	-	N	N	-	-
Ultimobranchial cyst	-	<1>	-	-	<1>	<1>
OVARIES	N	N	N	N	N	N
UTERUS	N	N	N	N	N	N
CERVIX	N	N	N	N	N	N

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PATHOLOGY ASSOCIATES

PUBERTAL TOXICITY STUDY OF VINCLOZOLIN, FLUTAMIDE AND PHENOBARBITAL IN MALE SPRAGUE DAWLEY RATS AND METHOXYCHLOR, ETHINYL ESTRADIOL AND PHENOBARBITAL IN FEMALE SPRAGUE DAWLEY RATS WHEN ADMINISTERED IN CORN OIL BY ORAL GAVAGE

TABULATED ANIMAL DATA

STUDY ID : 7244-600 STUDY NUMBER: 7244600
FATE: Terminal Kill GROUP: 3: 0.005 mg/kg Ethinyl Estradiol
SEX: FEMALE

ANIMAL ID:	9197	9198	9199	9200	9201	9202
THYROID	-	N	-	N	N	N
Ultimobranchial cyst	<1>	-	(1)	-	-	-
OVARIES	N	N	N	N	N	N
UTERUS	N	N	N	N	N	N
CERVIX	N	N	N	N	N	N
Non-Protocol Tissues:						
KIDNEYS	-	-	N	-	-	-
Hydronephrosis	1L	-	-	-	-	-
Non-Protocol Tissues:						
TAIL	-	-	-	-	-	-
Vertebral dysplasia	<1>	-	-	-	-	-

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PATHOLOGY ASSOCIATES

PUBERTAL TOXICITY STUDY OF VINCLOZOLIN, FLUTAMIDE AND PHENOBARBITAL IN MALE SPRAGUE DAWLEY RATS AND METHOXYCHLOR, ETHINYL ESTRADIOL AND PHENOBARBITAL IN FEMALE SPRAGUE DAWLEY RATS WHEN ADMINISTERED IN CORN OIL BY ORAL GAVAGE

TABULATED ANIMAL DATA

STUDY ID : 7244-600 STUDY NUMBER: 7244600
FATE: Terminal Kill GROUP: 3: 0.005 mg/kg Ethinyl Estradiol
SEX: FEMALE

ANIMAL ID:	9203	9204	9205
THYROID	-	-	N
Ultimobranchial cyst	(1)	<1>	-
OVARIES	N	N	N
UTERUS	N	N	N
CERVIX	N	N	N

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PATHOLOGY ASSOCIATES

PUBERTAL TOXICITY STUDY OF VINCLOZOLIN, FLUTAMIDE AND PHENOBARBITAL IN MALE SPRAGUE DAWLEY RATS AND METHOXYCHLOR, ETHINYL ESTRADIOL AND PHENOBARBITAL IN FEMALE SPRAGUE DAWLEY RATS WHEN ADMINISTERED IN CORN OIL BY ORAL GAVAGE

TABULATED ANIMAL DATA

STUDY ID : 7244-600 STUDY NUMBER: 7244600
FATE: Terminal Kill GROUP: 4: 12.5 mg/kg Methoxychlor
SEX: FEMALE

ANIMAL ID:	9206	9207	9208	9209	9210	9211
THYROID	N	-	N	N	-	-
Ultimobranchial cyst	-	<1>	-	-	(1)	(1)
OVARIES	N	N	N	N	N	N
UTERUS	N	N	N	N	N	N
CERVIX	N	N	N	N	N	N
Non-Protocol Tissues:						
KIDNEYS	-	-	-	N	-	-
Hydronephrosis	-	-	-	-	-	2L

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PATHOLOGY ASSOCIATES

PUBERTAL TOXICITY STUDY OF VINCLOZOLIN, FLUTAMIDE AND PHENOBARBITAL IN MALE SPRAGUE DAWLEY RATS AND METHOXYCHLOR, ETHINYL ESTRADIOL AND PHENOBARBITAL IN FEMALE SPRAGUE DAWLEY RATS WHEN ADMINISTERED IN CORN OIL BY ORAL GAVAGE

TABULATED ANIMAL DATA

STUDY ID : 7244-600 STUDY NUMBER: 7244600
FATE: Terminal Kill GROUP: 4: 12.5 mg/kg Methoxychlor
SEX: FEMALE

ANIMAL ID:	9212	9213	9214	9215	9216	9217
THYROID	N	U	N	-	-	-
Ultimobranchial cyst	-	-	-	<1>	<1>	(1)
OVARIES	N	-	N	N	N	N
Interstitial cell hyperplasia	-	(1)	-	-	-	-
UTERUS	N	N	N	N	N	N
CERVIX	N	N	N	N	N	N
Non-Protocol Tissues:						
KIDNEYS	-	-	N	-	-	-

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PATHOLOGY ASSOCIATES

PUBERTAL TOXICITY STUDY OF VINCLOZOLIN, FLUTAMIDE AND PHENOBARBITAL IN MALE SPRAGUE DAWLEY RATS AND METHOXYCHLOR, ETHINYL ESTRADIOL AND PHENOBARBITAL IN FEMALE SPRAGUE DAWLEY RATS WHEN ADMINISTERED IN CORN OIL BY ORAL GAVAGE

TABULATED ANIMAL DATA

STUDY ID : 7244-600 STUDY NUMBER: 7244600
FATE: Terminal Kill GROUP: 4: 12.5 mg/kg Methoxychlor
SEX: FEMALE

ANIMAL ID:	9218	9219	9220
THYROID	N	-	-
Ultimobranchial cyst	-	<1>	<1>
OVARIES	N	N	N
UTERUS	N	N	N
CERVIX	N	N	N
Non-Protocol Tissues:			
KIDNEYS	-	-	-
Hydronephrosis	-	-	2L

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PATHOLOGY ASSOCIATES

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TABULATED ANIMAL DATA

STUDY ID : 7244-600 STUDY NUMBER: 7244600
FATE: Terminal Kill GROUP: 5: 25 mg/kg Methoxychlor
SEX: FEMALE

ANIMAL ID:	9221	9222	9223	9224	9225	9226
THYROID	N	N	-	N	N	-
Ultimobranchial cyst	-	-	(1)	-	-	<1>
OVARIES	N	N	N	N	N	N
UTERUS	N	N	N	N	N	N
CERVIX	N	N	N	N	N	N

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PATHOLOGY ASSOCIATES

PUBERTAL TOXICITY STUDY OF VINCLOZOLIN, FLUTAMIDE AND PHENOBARBITAL IN MALE SPRAGUE DAWLEY RATS AND METHOXYCHLOR, ETHINYL ESTRADIOL AND PHENOBARBITAL IN FEMALE SPRAGUE DAWLEY RATS WHEN ADMINISTERED IN CORN OIL BY ORAL GAVAGE

TABULATED ANIMAL DATA

STUDY ID : 7244-600 STUDY NUMBER: 7244600
FATE: Terminal Kill GROUP: 5: 25 mg/kg Methoxychlor
SEX: FEMALE

ANIMAL ID:	9227	9228	9229	9230	9231	9232
THYROID	N	N	N	N	-	N
Ultimobranchial cyst	-	-	-	-	<1>	-
OVARIES	N	N	N	N	N	N
UTERUS	N	N	N	N	N	N
CERVIX	N	N	N	N	N	N
Non-Protocol Tissues:						
KIDNEYS	-	-	N	-	-	-
Non-Protocol Tissues:						
SPLEEN	-	-	-	-	-	-
Lymphocytic hyperplasia	[2]	-	-	-	[2]	-
Non-Protocol Tissues:						
ADRENAL GLANDS	-	-	-	-	-	N

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PATHOLOGY ASSOCIATES

PUBERTAL TOXICITY STUDY OF VINCLOZOLIN, FLUTAMIDE AND PHENOBARBITAL IN MALE SPRAGUE DAWLEY RATS AND METHOXYCHLOR, ETHINYL ESTRADIOL AND PHENOBARBITAL IN FEMALE SPRAGUE DAWLEY RATS WHEN ADMINISTERED IN CORN OIL BY ORAL GAVAGE

TABULATED ANIMAL DATA

STUDY ID : 7244-600 STUDY NUMBER: 7244600
FATE: Terminal Kill GROUP: 5: 25 mg/kg Methoxychlor
SEX: FEMALE

ANIMAL ID:	9233	9234	9235
THYROID	N	-	N
Ultimobranchial cyst	-	<1>	-
OVARIES	N	N	N
UTERUS	N	N	N
CERVIX	N	N	N

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PATHOLOGY ASSOCIATES

PUBERTAL TOXICITY STUDY OF VINCLOZOLIN, FLUTAMIDE AND PHENOBARBITAL IN MALE SPRAGUE DAWLEY RATS AND METHOXYCHLOR, ETHINYL ESTRADIOL AND PHENOBARBITAL IN FEMALE SPRAGUE DAWLEY RATS WHEN ADMINISTERED IN CORN OIL BY ORAL GAVAGE

TABULATED ANIMAL DATA

STUDY ID : 7244-600 STUDY NUMBER: 7244600
FATE: Terminal Kill GROUP: 6: 50 mg/kg Methoxychlor
SEX: FEMALE

ANIMAL ID:	9236	9237	9238	9239	9240	9241
THYROID	N	N	N	N	N	N
OVARIES	N	N	N	N	N	N
UTERUS	N	N	N	N	N	N
CERVIX	N	N	N	N	N	N

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PATHOLOGY ASSOCIATES

PUBERTAL TOXICITY STUDY OF VINCLOZOLIN, FLUTAMIDE AND PHENOBARBITAL IN MALE SPRAGUE DAWLEY RATS AND METHOXYCHLOR, ETHINYL ESTRADIOL AND PHENOBARBITAL IN FEMALE SPRAGUE DAWLEY RATS WHEN ADMINISTERED IN CORN OIL BY ORAL GAVAGE

TABULATED ANIMAL DATA

STUDY ID : 7244-600 STUDY NUMBER: 7244600
FATE: Terminal Kill GROUP: 6: 50 mg/kg Methoxychlor
SEX: FEMALE

ANIMAL ID:	9242	9243	9244	9245	9246	9247
THYROID	-	N	-	N	N	N
Ultimobranchial cyst	<1>	-	<1>	-	-	-
OVARIES	N	N	N	N	N	N
UTERUS	N	N	N	N	N	N
CERVIX	N	N	N	N	N	N
Non-Protocol Tissues:						
KIDNEYS	-	-	-	-	-	-
Hydronephrosis	-	3L	-	-	-	-

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PATHOLOGY ASSOCIATES

PUBERTAL TOXICITY STUDY OF VINCLOZOLIN, FLUTAMIDE AND PHENOBARBITAL IN MALE SPRAGUE DAWLEY RATS AND METHOXYCHLOR, ETHINYL ESTRADIOL AND PHENOBARBITAL IN FEMALE SPRAGUE DAWLEY RATS WHEN ADMINISTERED IN CORN OIL BY ORAL GAVAGE

TABULATED ANIMAL DATA

STUDY ID : 7244-600 STUDY NUMBER: 7244600
FATE: Terminal Kill GROUP: 6: 50 mg/kg Methoxychlor
SEX: FEMALE

ANIMAL ID:	9248	9249	9250
THYROID	N	-	N
Ultimobranchial cyst	-	<2>	-
OVARIES	N	N	N
UTERUS	N	N	N
CERVIX	N	N	N

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TABULATED ANIMAL DATA

STUDY ID : 7244-600 STUDY NUMBER: 7244600
FATE: Terminal Kill GROUP: 7: 25 mg/kg Phenobarbital
SEX: FEMALE

ANIMAL ID:	9251	9252	9253	9254	9255	9256
THYROID	-	N	N	-	N	N
Ultimobranchial cyst	(1)	-	-	<1>	-	-
OVARIES	N	N	N	N	N	N
UTERUS	N	N	N	N	N	N
CERVIX	N	N	N	N	N	N
Non-Protocol Tissues:						
KIDNEYS	N	-	-	-	-	-
Hydronephrosis	-	2L	-	-	-	-
Non-Protocol Tissues:						
TAIL	-	-	-	-	-	-
Necrosis, tail tip	-	-	[4]	-	-	-

See Reports Code Table for Symbol Definitions
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PATHOLOGY ASSOCIATES

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TABULATED ANIMAL DATA

STUDY ID : 7244-600 STUDY NUMBER: 7244600
FATE: Terminal Kill GROUP: 7: 25 mg/kg Phenobarbital
SEX: FEMALE

ANIMAL ID:	9257	9258	9259	9260	9261	9262
THYROID	-	-	-	-	-	N
Ultimobranchial cyst	(1)	(1)	<1>	(1)	<1>	-
OVARIES	N	N	N	N	N	-
Hemorrhage/fluid, bursa	-	-	-	-	-	<2>
UTERUS	N	N	N	N	N	N
CERVIX	N	N	N	N	N	N
Non-Protocol Tissues:						
KIDNEYS	-	-	-	-	-	-
Hydronephrosis	-	-	-	-	-	1L
Fibrosis, interstitial	-	-	-	<1>	-	-
Cyst, cortical	-	-	-	-	-	(4)L
Non-Protocol Tissues:						
ADRENAL GLANDS	-	-	-	-	-	N
Non-Protocol Tissues:						
URETER	-	-	-	-	-	-
Dilation	-	-	-	-	-	[4]
Inflammation, mixed cell	-	-	-	-	-	[1]

See Reports Code Table for Symbol Definitions
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PUBERTAL TOXICITY STUDY OF VINCLOZOLIN, FLUTAMIDE AND PHENOBARBITAL IN MALE SPRAGUE DAWLEY RATS AND METHOXYCHLOR, ETHINYL ESTRADIOL AND PHENOBARBITAL IN FEMALE SPRAGUE DAWLEY RATS WHEN ADMINISTERED IN CORN OIL BY ORAL GAVAGE

TABULATED ANIMAL DATA

STUDY ID : 7244-600 STUDY NUMBER: 7244600
FATE: Terminal Kill GROUP: 7: 25 mg/kg Phenobarbital
SEX: FEMALE

ANIMAL ID:	9263	9264	9265
THYROID	N	N	-
Ultimobranchial cyst	-	-	<1>
OVARIES	N	N	N
UTERUS	N	N	N
CERVIX	N	N	N

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PATHOLOGY ASSOCIATES

PUBERTAL TOXICITY STUDY OF VINCLOZOLIN, FLUTAMIDE AND PHENOBARBITAL IN MALE SPRAGUE DAWLEY RATS AND METHOXYCHLOR, ETHINYL ESTRADIOL AND PHENOBARBITAL IN FEMALE SPRAGUE DAWLEY RATS WHEN ADMINISTERED IN CORN OIL BY ORAL GAVAGE

TABULATED ANIMAL DATA

STUDY ID : 7244-600 STUDY NUMBER: 7244600
FATE: Terminal Kill GROUP: 8: 50 mg/kg Phenobarbital
SEX: FEMALE

ANIMAL ID:	9266	9267	9268	9269	9270	9271
THYROID	-	N	N	N	-	N
Ultimobranchial cyst	<1>	-	-	-	(1)	-
OVARIES	N	N	N	-	N	N
Cyst, oviduct	-	-	-	(2)	-	-
UTERUS	N	N	N	N	N	N
CERVIX	N	N	N	N	N	N

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TABULATED ANIMAL DATA

STUDY ID : 7244-600 STUDY NUMBER: 7244600
FATE: Terminal Kill GROUP: 8: 50 mg/kg Phenobarbital
SEX: FEMALE

ANIMAL ID:	9272	9273	9274	9275	9276	9277
THYROID	-	N	N	N	-	-
Ultimobranchial cyst	(1)	-	-	-	<1>	<1>
OVARIES	N	N	N	N	N	N
UTERUS	N	N	N	N	N	N
CERVIX	N	N	N	N	N	N
Non-Protocol Tissues:						
KIDNEYS	-	-	N	-	-	-
Hydronephrosis	2L	-	-	2L	2L	-
Infiltrate, mixed cell	-	-	-	-	[4]L	-
Infiltrate, mixed cell, renal pelvis	-	-	-	-	<2>	-
Non-Protocol Tissues:						
SPLEEN	-	-	-	-	-	-
Congestion	-	-	-	-	[2]	-
Non-Protocol Tissues:						
URINARY BLADDER	-	-	-	-	-	-
Infiltrate, mixed cell	-	-	-	-	[3]	-
Hyperplasia, epithelium	-	-	-	-	[3]	-

See Reports Code Table for Symbol Definitions
THERIMUNE RESEARCH CORPORATION STUDY NUMBER 7244-600

LABCAT HP4.33 14-FEB-2003

PATHOLOGY ASSOCIATES

PUBERTAL TOXICITY STUDY OF VINCLOZOLIN, FLUTAMIDE AND PHENOBARBITAL IN MALE SPRAGUE DAWLEY RATS AND METHOXYCHLOR, ETHINYL ESTRADIOL AND PHENOBARBITAL IN FEMALE SPRAGUE DAWLEY RATS WHEN ADMINISTERED IN CORN OIL BY ORAL GAVAGE

TABULATED ANIMAL DATA

STUDY ID : 7244-600 STUDY NUMBER: 7244600
FATE: Terminal Kill GROUP: 8: 50 mg/kg Phenobarbital
SEX: FEMALE

ANIMAL ID:	9278	9279	9280
THYROID	N	N	N
OVARIES	N	N	N
UTERUS	N	N	N
CERVIX	N	N	N

See Reports Code Table for Symbol Definitions
THERIMUNE RESEARCH CORPORATION STUDY NUMBER 7244-600

LABCAT HP4.33 14-FEB-2003

PATHOLOGY ASSOCIATES

PUBERTAL TOXICITY STUDY OF VINCLOZOLIN, FLUTAMIDE AND PHENOBARBITAL IN MALE SPRAGUE DAWLEY RATS AND METHOXYCHLOR, ETHINYL ESTRADIOL AND PHENOBARBITAL IN FEMALE SPRAGUE DAWLEY RATS WHEN ADMINISTERED IN CORN OIL BY ORAL GAVAGE

TABULATED ANIMAL DATA

STUDY ID : 7244-600 STUDY NUMBER: 7244600
FATE: Terminal Kill GROUP: 9: 100 mg/kg Phenobarbital
SEX: FEMALE

ANIMAL ID:	9281	9282	9283	9284	9285	9286
THYROID	-	N	N	N	-	N
Ultimobranchial cyst	(1)	-	-	-	<1>	-
OVARIES	N	N	N	N	N	N
UTERUS	N	N	N	N	N	N
CERVIX	N	N	N	N	N	N
Non-Protocol Tissues:						
KIDNEYS	-	-	-	-	-	-
Hydronephrosis	-	-	2L	-	-	-

See Reports Code Table for Symbol Definitions
THERIMUNE RESEARCH CORPORATION STUDY NUMBER 7244-600

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PATHOLOGY ASSOCIATES

PUBERTAL TOXICITY STUDY OF VINCLOZOLIN, FLUTAMIDE AND PHENOBARBITAL IN MALE SPRAGUE DAWLEY RATS AND METHOXYCHLOR, ETHINYL ESTRADIOL AND PHENOBARBITAL IN FEMALE SPRAGUE DAWLEY RATS WHEN ADMINISTERED IN CORN OIL BY ORAL GAVAGE

TABULATED ANIMAL DATA

STUDY ID : 7244-600 STUDY NUMBER: 7244600
FATE: Terminal Kill GROUP: 9: 100 mg/kg Phenobarbital
SEX: FEMALE

ANIMAL ID:	9287	9288	9289	9290	9291	9292
THYROID	N	-	N	-	N	N
Ultimobranchial cyst	-	(1)	-	(1)	-	-
OVARIES	N	N	N	N	N	N
UTERUS	N	N	N	N	N	N
CERVIX	N	N	N	N	N	N
Non-Protocol Tissues:						
KIDNEYS	-	-	-	-	-	-
Hydronephrosis	-	-	-	-	3	-
Infiltrate, mixed cell, renal pelvis	-	-	-	-	<3>	-
Calculi	-	-	-	-	P	-
Non-Protocol Tissues:						
URINARY BLADDER	-	-	-	-	-	-
Infiltrate, mixed cell	-	-	-	-	<2>	-
Hyperplasia, epithelium	-	-	-	-	[3]	-
Fibrosis, sub-epithelial	-	-	-	-	<3>	-
Ulceration, epithelium	-	-	-	-	(3)	-
Calculi	-	-	-	-	P	-

See Reports Code Table for Symbol Definitions
THERIMUNE RESEARCH CORPORATION STUDY NUMBER 7244-600

LABCAT HP4.33 14-FEB-2003

PATHOLOGY ASSOCIATES

PUBERTAL TOXICITY STUDY OF VINCLOZOLIN, FLUTAMIDE AND PHENOBARBITAL IN MALE SPRAGUE DAWLEY RATS AND METHOXYCHLOR, ETHINYL ESTRADIOL AND PHENOBARBITAL IN FEMALE SPRAGUE DAWLEY RATS WHEN ADMINISTERED IN CORN OIL BY ORAL GAVAGE

TABULATED ANIMAL DATA

STUDY ID : 7244-600 STUDY NUMBER: 7244600
FATE: Terminal Kill GROUP: 9: 100 mg/kg Phenobarbital
SEX: FEMALE

ANIMAL ID:	9293	9294	9295
THYROID	-	-	-
Ultimobranchial cyst	<1>	(1)	<1>
OVARIES	N	N	N
UTERUS	N	N	N
CERVIX	N	N	N
Non-Protocol Tissues:			
KIDNEYS	-	-	-
Hydronephrosis	3L	-	-

See Reports Code Table for Symbol Definitions
THERIMUNE RESEARCH CORPORATION STUDY NUMBER 7244-600

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IV. Correlation of Gross and Microscopic Findings

PATHOLOGY ASSOCIATES

PUBERTAL TOXICITY STUDY OF VINCLOZOLIN, FLUTAMIDE AND PHENOBARBITAL IN MALE SPRAGUE DAWLEY RATS AND METHOXYCHLOR, ETHINYL ESTRADIOL AND PHENOBARBITAL IN FEMALE SPRAGUE DAWLEY RATS WHEN ADMINISTERED IN CORN OIL BY ORAL GAVAGE

CORRELATION OF GROSS & MICRO

STUDY ID: 7244-600 STUDY NUMBER: 7244600
SEX: MALE GROUP: 1: Corn Oil Vehicle

Animal ID: 9031 Pathologist: JLQ
Animal Fate: Terminal Kill Days on Test: 53

Reference to Necropsy Record:	Related Histopathology:
KIDNEYS - DILATION; RIGHT; MODERATE (TGL): RENAL PELVIS	KIDNEYS - Hydronephrosis

---

Animal ID: 9037 Pathologist: JLQ
Animal Fate: Terminal Kill Days on Test: 54

Reference to Necropsy Record:	Related Histopathology:
THYMUS - DISCOLORATION; RED; BILATERAL; DIFFUSE (TGL)	THYMUS - No Corollary change detected

---

Animal ID: 9039 Pathologist: JLQ
Animal Fate: Terminal Kill Days on Test: 54

Reference to Necropsy Record:	Related Histopathology:
EPIDIDYMIDES - REDUCTION IN SIZE; BILATERAL; MODERATE (TGL)	EPIDIDYMIDES - No Corollary change detected
SPLEEN - ENLARGEMENT (TGL): 55X15X10MM	SPLEEN - Lymphocytic hyperplasia
SPLEEN - ROUGH	SPLEEN - Lymphocytic hyperplasia
TESTES - REDUCTION IN SIZE; BILATERAL; MINIMAL (TGL)	TESTES - No Corollary change detected

---

THERIMUNE RESEARCH CORPORATION STUDY NUMBER 7244-600

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PATHOLOGY ASSOCIATES

PUBERTAL TOXICITY STUDY OF VINCLOZOLIN, FLUTAMIDE AND PHENOBARBITAL IN MALE SPRAGUE DAWLEY RATS AND METHOXYCHLOR, ETHINYL ESTRADIOL AND PHENOBARBITAL IN FEMALE SPRAGUE DAWLEY RATS WHEN ADMINISTERED IN CORN OIL BY ORAL GAVAGE

CORRELATION OF GROSS & MICRO

STUDY ID: 7244-600 STUDY NUMBER: 7244600
SEX: MALE GROUP: 1: Corn Oil Vehicle

Animal ID: 9040 Pathologist: JLQ
Animal Fate: Terminal Kill Days on Test: 54

Reference to Necropsy Record:	Related Histopathology:
SPLEEN - ENLARGEMENT (TGL): 55X20X10MM	SPLEEN - Lymphocytic hyperplasia
SPLEEN - NODULE(S); WHITE (TGL): SINGLE; 4X4X4MM	SPLEEN - Lymphocytic hyperplasia
THYMUS - DISCOLORATION; RED; BILATERAL (TGL)	THYMUS - No Corollary change detected

---

THERIMUNE RESEARCH CORPORATION STUDY NUMBER 7244-600

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PATHOLOGY ASSOCIATES

PUBERTAL TOXICITY STUDY OF VINCLOZOLIN, FLUTAMIDE AND PHENOBARBITAL IN MALE SPRAGUE DAWLEY RATS AND METHOXYCHLOR, ETHINYL ESTRADIOL AND PHENOBARBITAL IN FEMALE SPRAGUE DAWLEY RATS WHEN ADMINISTERED IN CORN OIL BY ORAL GAVAGE

CORRELATION OF GROSS & MICRO

STUDY ID: 7244-600 STUDY NUMBER: 7244600
SEX: MALE GROUP: 2: 25 mg/kg Phenobarbital

Animal ID: 9041 Pathologist: JLQ
Animal Fate: Terminal Kill Days on Test: 53

Reference to Necropsy Record:	Related Histopathology:
KIDNEYS - DISCOLORATION; DARK; LEFT; FOCAL (TGL)	KIDNEYS - Cyst, cortical

---

Animal ID: 9042 Pathologist: JLQ
Animal Fate: Terminal Kill Days on Test: 53

Reference to Necropsy Record:	Related Histopathology:
LIVER - NODULE(S); WHITE (TGL): FOCAL; CAUDATE LOBE	LIVER - Hemorrhage, capsular

---

Animal ID: 9047 Pathologist: JLQ
Animal Fate: Terminal Kill Days on Test: 53

Reference to Necropsy Record:	Related Histopathology:
KIDNEYS - DILATION; RIGHT; MINIMAL (TGL): RENAL PELVIS	KIDNEYS - Hydronephrosis

---

Animal ID: 9053 Pathologist: JLQ
Animal Fate: Terminal Kill Days on Test: 54

Reference to Necropsy Record:	Related Histopathology:
KIDNEYS - DILATION; RIGHT; MINIMAL (TGL): RENAL PELVIS	KIDNEYS - Hydronephrosis

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THERIMUNE RESEARCH CORPORATION STUDY NUMBER 7244-600

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PATHOLOGY ASSOCIATES

PUBERTAL TOXICITY STUDY OF VINCLOZOLIN, FLUTAMIDE AND PHENOBARBITAL IN MALE SPRAGUE DAWLEY RATS AND METHOXYCHLOR, ETHINYL ESTRADIOL AND PHENOBARBITAL IN FEMALE SPRAGUE DAWLEY RATS WHEN ADMINISTERED IN CORN OIL BY ORAL GAVAGE

CORRELATION OF GROSS & MICRO

STUDY ID: 7244-600 STUDY NUMBER: 7244600
SEX: MALE GROUP: 2: 25 mg/kg Phenobarbital

Animal ID: 9054 Pathologist: JLQ
Animal Fate: Terminal Kill Days on Test: 54

Reference to Necropsy Record:
THYMUS - DISCOLORATION; DARK; BILATERAL (TGL)

Related Histopathology:
THYMUS - No Corollary change detected

THERIMUNE RESEARCH CORPORATION STUDY NUMBER 7244-600

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PATHOLOGY ASSOCIATES

PUBERTAL TOXICITY STUDY OF VINCLOZOLIN, FLUTAMIDE AND PHENOBARBITAL IN MALE SPRAGUE DAWLEY RATS AND METHOXYCHLOR, ETHINYL ESTRADIOL AND PHENOBARBITAL IN FEMALE SPRAGUE DAWLEY RATS WHEN ADMINISTERED IN CORN OIL BY ORAL GAVAGE

CORRELATION OF GROSS & MICRO

STUDY ID: 7244-600 STUDY NUMBER: 7244600
SEX: MALE GROUP: 3: 50 mg/kg Phenobarbital

Animal ID: 9059 Pathologist: JLQ
Animal Fate: Terminal Kill Days on Test: 53

Reference to Necropsy Record:	Related Histopathology:
KIDNEYS - DISCOLORATION; DARK; LEFT; SINGLE (TGL): FOCAL	KIDNEYS - Cyst, cortical

---

Animal ID: 9060 Pathologist: JLQ
Animal Fate: Terminal Kill Days on Test: 53

Reference to Necropsy Record:	Related Histopathology:
SEMINAL VESICLES - WITH FLUID; REDUCTION IN SIZE; BILATERAL; MINIMAL (TGL)	SEMINAL VESICLES - (Tissue unavailable)
COAGULATING GLANDS - WITH FLUID; REDUCTION IN SIZE; BILATERAL; MINIMAL (TGL)	COAGULATING GLANDS - (Tissue unavailable)

Animal ID: 9062 Pathologist: JLQ
Animal Fate: Terminal Kill Days on Test: 53

Reference to Necropsy Record:	Related Histopathology:
KIDNEYS - DILATION; RIGHT; MODERATE (TGL): RENAL PELVIS	KIDNEYS - Hydronephrosis

---

Animal ID: 9063 Pathologist: JLQ
Animal Fate: Terminal Kill Days on Test: 53

Reference to Necropsy Record:	Related Histopathology:
KIDNEYS - DILATION; RIGHT; MODERATE (TGL): RENAL PELVIS	KIDNEYS - Hydronephrosis

---

THERIMUNE RESEARCH CORPORATION STUDY NUMBER 7244-600

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PATHOLOGY ASSOCIATES

PUBERTAL TOXICITY STUDY OF VINCLOZOLIN, FLUTAMIDE AND PHENOBARBITAL IN MALE SPRAGUE DAWLEY RATS AND METHOXYCHLOR, ETHINYL ESTRADIOL AND PHENOBARBITAL IN FEMALE SPRAGUE DAWLEY RATS WHEN ADMINISTERED IN CORN OIL BY ORAL GAVAGE

CORRELATION OF GROSS & MICRO

STUDY ID: 7244-600 STUDY NUMBER: 7244600
SEX: MALE GROUP: 3: 50 mg/kg Phenobarbital

Animal ID: 9065 Pathologist: JLQ
Animal Fate: Terminal Kill Days on Test: 54

Reference to Necropsy Record:	Related Histopathology:
THYMUS - DISCOLORATION; DARK; LEFT (TGL)	THYMUS - Hemorrhage

Animal ID: 9066 Pathologist: JLQ
Animal Fate: Terminal Kill Days on Test: 54

Reference to Necropsy Record:	Related Histopathology:
KIDNEYS - DILATION; BILATERAL; MODERATE (TGL): RENAL PELVIS	KIDNEYS - Hydronephrosis

---

Animal ID: 9067 Pathologist: JLQ
Animal Fate: Terminal Kill Days on Test: 54

Reference to Necropsy Record:	Related Histopathology:
THYROID - DISCOLORATION; BILATERAL (TGL): DARK RED	THYROID - No Corollary change detected

---

THERIMUNE RESEARCH CORPORATION STUDY NUMBER 7244-600

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PATHOLOGY ASSOCIATES

PUBERTAL TOXICITY STUDY OF VINCLOZOLIN, FLUTAMIDE AND PHENOBARBITAL IN MALE SPRAGUE DAWLEY RATS AND METHOXYCHLOR, ETHINYL ESTRADIOL AND PHENOBARBITAL IN FEMALE SPRAGUE DAWLEY RATS WHEN ADMINISTERED IN CORN OIL BY ORAL GAVAGE

CORRELATION OF GROSS & MICRO

STUDY ID: 7244-600 STUDY NUMBER: 7244600
SEX: MALE GROUP: 4: 100 mg/kg Phenobarbital

Animal ID: 9073 Pathologist: JLQ
Animal Fate: Terminal Kill Days on Test: 53

Reference to Necropsy Record:	Related Histopathology:
TESTES - REDUCTION IN SIZE; BILATERAL; MODERATE (TGL)	TESTES - Degeneration, germinal epithelium
SEMINAL VESICLES - WITH FLUID; REDUCTION IN SIZE; BILATERAL; MODERATE (TGL)	SEMINAL VESICLES - (Tissue unavailable)
COAGULATING GLANDS - WITH FLUID: REDUCTION IN SIZE; BILATERAL; MODERATE (TGL)	COAGULATING GLANDS - (Tissue unavailable)

Animal ID: 9075 Pathologist: JLQ
Animal Fate: Terminal Kill Days on Test: 53

Reference to Necropsy Record:	Related Histopathology:
KIDNEYS - DILATION; BILATERAL; MODERAT (TGL): RENAL PELVIS	KIDNEYS - Hydronephrosis

---

Animal ID: 9078 Pathologist: JLQ
Animal Fate: Terminal Kill Days on Test: 53

Reference to Necropsy Record:	Related Histopathology:
KIDNEYS - DILATION; RIGHT; MODERATE (TGL): RENAL PELVIS	KIDNEYS - Hydronephrosis

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THERIMUNE RESEARCH CORPORATION STUDY NUMBER 7244-600

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PATHOLOGY ASSOCIATES

PUBERTAL TOXICITY STUDY OF VINCLOZOLIN, FLUTAMIDE AND PHENOBARBITAL IN MALE SPRAGUE DAWLEY RATS AND METHOXYCHLOR, ETHINYL ESTRADIOL AND PHENOBARBITAL IN FEMALE SPRAGUE DAWLEY RATS WHEN ADMINISTERED IN CORN OIL BY ORAL GAVAGE

CORRELATION OF GROSS & MICRO

STUDY ID: 7244-600 STUDY NUMBER: 7244600
SEX: MALE GROUP: 4: 100 mg/kg Phenobarbital

Animal ID: 9083 Pathologist: JLQ
Animal Fate: Terminal Kill Days on Test: 54

Reference to Necropsy Record:	Related Histopathology:
KIDNEYS - DILATION; RIGHT; MODERATE (TGL): RENAL PELVIS	KIDNEYS - Hydronephrosis

---

Animal ID: 9084 Pathologist: JLQ
Animal Fate: Terminal Kill Days on Test: 54

Reference to Necropsy Record:	Related Histopathology:
EPIDIDYMIDES - REDUCTION IN SIZE; BILATERAL; MINIMAL (TGL)	EPIDIDYMIDES - No Corollary change detected
TESTES - REDUCTION IN SIZE; BILATERAL; MINIMAL (TGL)	TESTES - No Corollary change detected
THYMUS - DISCOLORATION; LEFT (TGL): DARK RED	THYMUS - Hemorrhage

---

Animal ID: 9085 Pathologist: JLQ
Animal Fate: Terminal Kill Days on Test: 54

Reference to Necropsy Record:	Related Histopathology:
EPIDIDYMIDES - ENLARGEMENT; BILATERAL; MINIMAL (TGL)	EPIDIDYMIDES - No Corollary change detected

---

THERIMUNE RESEARCH CORPORATION STUDY NUMBER 7244-600

LABCAT HP4.33 14-FEB-2003

PATHOLOGY ASSOCIATES

PUBERTAL TOXICITY STUDY OF VINCLOZOLIN, FLUTAMIDE AND PHENOBARBITAL IN MALE SPRAGUE DAWLEY RATS AND METHOXYCHLOR, ETHINYL ESTRADIOL AND PHENOBARBITAL IN FEMALE SPRAGUE DAWLEY RATS WHEN ADMINISTERED IN CORN OIL BY ORAL GAVAGE

CORRELATION OF GROSS & MICRO

STUDY ID: 7244-600 STUDY NUMBER: 7244600
SEX: MALE GROUP: 5: 10 mg/kg Vinclozolin

Animal ID: 9088 Pathologist: JLQ
Animal Fate: Terminal Kill Days on Test: 53

Reference to Necropsy Record:	Related Histopathology:
KIDNEYS - DILATION; RIGHT; MODERATE (TGL): RENAL PELVIS	KIDNEYS - Hydronephrosis

---

Animal ID: 9097 Pathologist: JLQ
Animal Fate: Terminal Kill Days on Test: 54

Reference to Necropsy Record:	Related Histopathology:
DORSAL LATERAL PROSTATE - REDUCTION IN SIZE; MINIMAL (TGL)	DORSAL LATERAL PROSTATE - (Tissue unavailable)
VENTRAL PROSTATE - REDUCTION IN SIZE; MINIMAL (TGL)	VENTRAL PROSTATE - (Tissue unavailable)
SPLEEN - ENLARGEMENT (TGL): 52X10X6MM	SPLEEN - Lymphocytic hyperplasia

---

Animal ID: 9099 Pathologist: JLQ
Animal Fate: Terminal Kill Days on Test: 54

Reference to Necropsy Record:	Related Histopathology:
KIDNEYS - DILATION; RIGHT; MINIMAL (TGL): RENAL PELVIS	KIDNEYS - Hydronephrosis
SEMINAL VESICLES - WITH FLUID; REDUCTION IN SIZE; LEFT; MODERATE (TGL)	SEMINAL VESICLES - (Tissue unavailable)
COAGULATING GLANDS - WITH FLUID; REDUCTION IN SIZE; LEFT MODERATE (TGL)	COAGULATING GLANDS - (Tissue unavailable)

PATHOLOGY ASSOCIATES

PUBERTAL TOXICITY STUDY OF VINCLOZOLIN, FLUTAMIDE AND PHENOBARBITAL IN MALE SPRAGUE DAWLEY RATS AND METHOXYCHLOR, ETHINYL ESTRADIOL AND PHENOBARBITAL IN FEMALE SPRAGUE DAWLEY RATS WHEN ADMINISTERED IN CORN OIL BY ORAL GAVAGE

CORRELATION OF GROSS & MICRO

STUDY ID: 7244-600 STUDY NUMBER: 7244600
SEX: MALE GROUP: 5: 10 mg/kg Vinclozolin

Animal ID: 9100 Pathologist: JLQ
Animal Fate: Terminal Kill Days on Test: 54

Reference to Necropsy Record:
KIDNEYS - DILATION; RIGHT; MODERATE (TGL): RENAL PELVIS

Related Histopathology:
KIDNEYS - Hydronephrosis

THERIMUNE RESEARCH CORPORATION STUDY NUMBER 7244-600

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PATHOLOGY ASSOCIATES

PUBERTAL TOXICITY STUDY OF VINCLOZOLIN, FLUTAMIDE AND PHENOBARBITAL IN MALE SPRAGUE DAWLEY RATS AND METHOXYCHLOR, ETHINYL ESTRADIOL AND PHENOBARBITAL IN FEMALE SPRAGUE DAWLEY RATS WHEN ADMINISTERED IN CORN OIL BY ORAL GAVAGE

CORRELATION OF GROSS & MICRO

STUDY ID: 7244-600 STUDY NUMBER: 7244600
SEX: MALE GROUP: 6: 30 mg/kg Vinclozolin

Animal ID: 9104 Pathologist: JLQ
Animal Fate: Terminal Kill Days on Test: 53

Reference to Necropsy Record:	Related Histopathology:
KIDNEYS - DILATION; RIGHT; MINIMAL (TGL): RENAL PELVIS	KIDNEYS - Hydronephrosis

---

Animal ID: 9106 Pathologist: JLQ
Animal Fate: Terminal Kill Days on Test: 53

Reference to Necropsy Record:	Related Histopathology:
SPLEEN - NODULE(S); RED (TGL): SINGLE; 7X6X3MM	SPLEEN - Lymphocytic hyperplasia

---

Animal ID: 9109 Pathologist: JLQ
Animal Fate: Terminal Kill Days on Test: 54

Reference to Necropsy Record:	Related Histopathology:
KIDNEYS - DILATION; RIGHT; MINIMAL (TGL): RENAL PELVIS	KIDNEYS - Hydronephrosis

---

Animal ID: 9110 Pathologist: JLQ
Animal Fate: Terminal Kill Days on Test: 54

Reference to Necropsy Record:	Related Histopathology:
KIDNEYS - DILATION; RIGHT; MODERATE (TGL): RENAL PELVIS	KIDNEYS - Hydronephrosis

---

THERIMUNE RESEARCH CORPORATION STUDY NUMBER 7244-600

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PATHOLOGY ASSOCIATES

PUBERTAL TOXICITY STUDY OF VINCLOZOLIN, FLUTAMIDE AND PHENOBARBITAL IN MALE SPRAGUE DAWLEY RATS AND METHOXYCHLOR, ETHINYL ESTRADIOL AND PHENOBARBITAL IN FEMALE SPRAGUE DAWLEY RATS WHEN ADMINISTERED IN CORN OIL BY ORAL GAVAGE

CORRELATION OF GROSS & MICRO

STUDY ID: 7244-600 STUDY NUMBER: 7244600
SEX: MALE GROUP: 6: 30 mg/kg Vinclozolin

Animal ID: 9111 Pathologist: JLQ
Animal Fate: Terminal Kill Days on Test: 54

Reference to Necropsy Record:
SKIN - ABRASION(S); RED; SINGLE; PRESENT, NO GRADE ASSIGNED (TGL): 10X5MM; ADJACENT TO RIGHT EAR

Related Histopathology:
SKIN - Ulceration with scab formation; Infiltrate, mixed cell

---

Animal ID: 9112 Pathologist: JLQ
Animal Fate: Terminal Kill Days on Test: 54

Reference to Necropsy Record:
KIDNEYS - DILATION; RIGHT; MINIMAL (TGL): RENAL PELVIS

Related Histopathology:
KIDNEYS - Hydronephrosis

PATHOLOGY ASSOCIATES

PUBERTAL TOXICITY STUDY OF VINCLOZOLIN, FLUTAMIDE AND PHENOBARBITAL IN MALE SPRAGUE DAWLEY RATS AND METHOXYCHLOR, ETHINYL ESTRADIOL AND PHENOBARBITAL IN FEMALE SPRAGUE DAWLEY RATS WHEN ADMINISTERED IN CORN OIL BY ORAL GAVAGE

CORRELATION OF GROSS & MICRO

STUDY ID: 7244-600 STUDY NUMBER: 7244600
SEX: MALE GROUP: 7: 100 mg/kg Vinclozolin

Animal ID: 9118 Pathologist: JLQ
Animal Fate: Terminal Kill Days on Test: 53

Reference to Necropsy Record:	Related Histopathology:
DORSAL LATERAL PROSTATE - REDUCTION IN SIZE; MODERATE (TGL)	DORSAL LATERAL PROSTATE - (Tissue unavailable)
EPIDIDYMIDES - REDUCTION IN SIZE; BILATERAL; MODERATE (TGL)	EPIDIDYMIDES - No Corollary change detected
VENTRAL PROSTATE - REDUCTION IN SIZE; MODERATE (TGL)	VENTRAL PROSTATE - (Tissue unavailable)
SEMINAL VESICLES - WITH FLUID; REDUCTION IN SIZE; BILATERAL; MODERATE (TGL)	SEMINAL VESICLES - (Tissue unavailable)
COAGULATING GLANDS - WITH FLUID; REDUCTION IN SIZE; BILATERAL; MODERATE (TGL)	COAGULATING GLANDS - (Tissue unavailable)

Animal ID: 9119 Pathologist: JLQ
Animal Fate: Terminal Kill Days on Test: 53

Reference to Necropsy Record:	Related Histopathology:
KIDNEYS - DILATION; RIGHT; MODERATE (TGL): RENAL PELVIS	KIDNEYS - Hydronephrosis
LIVER - DISCOLORATION; PALE (TGL): ALL LOBES	LIVER - No Corollary change detected

---

Animal ID: 9120 Pathologist: JLQ
Animal Fate: Terminal Kill Days on Test: 53

Reference to Necropsy Record:	Related Histopathology:
KIDNEYS - DILATION; LEFT; MODERATE (TGL): RENAL PELVIS	KIDNEYS - Hydronephrosis

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THERIMUNE RESEARCH CORPORATION STUDY NUMBER 7244-600

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PATHOLOGY ASSOCIATES

PUBERTAL TOXICITY STUDY OF VINCLOZOLIN, FLUTAMIDE AND PHENOBARBITAL IN MALE SPRAGUE DAWLEY RATS AND METHOXYCHLOR, ETHINYL ESTRADIOL AND PHENOBARBITAL IN FEMALE SPRAGUE DAWLEY RATS WHEN ADMINISTERED IN CORN OIL BY ORAL GAVAGE

CORRELATION OF GROSS & MICRO

STUDY ID: 7244-600 STUDY NUMBER: 7244600
SEX: MALE GROUP: 7: 100 mg/kg Vinclozolin

Animal ID: 9121 Pathologist: JLQ
Animal Fate: Terminal Kill Days on Test: 53

Reference to Necropsy Record:	Related Histopathology:
KIDNEYS - DILATION; RIGHT; MODERATE (TGL): RENAL PELVIS	KIDNEYS - Hydronephrosis
SEMINAL VESICLES - WITH FLUID; REDUCTION IN SIZE; RIGHT; MODERATE (TGL)	SEMINAL VESICLES - (Tissue unavailable)
COAGULATING GLANDS - WITH FLUID; REDUCTION IN SIZE; RIGHT; MODERATE (TGL)	COAGULATING GLANDS - (Tissue unavailable)

Animal ID: 9122 Pathologist: JLQ
Animal Fate: Terminal Kill Days on Test: 53

Reference to Necropsy Record:	Related Histopathology:
KIDNEYS - DILATION; RIGHT; MODERATE (TGL): RENAL PELVIS	KIDNEYS - Hydronephrosis

---

Animal ID: 9127 Pathologist: JLQ
Animal Fate: Terminal Kill Days on Test: 54

Reference to Necropsy Record:	Related Histopathology:
KIDNEYS - DILATION; RIGHT; MODERATE (TGL): RENAL PELVIS	KIDNEYS - Hydronephrosis

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THERIMUNE RESEARCH CORPORATION STUDY NUMBER 7244-600

LABCAT HP4.33 14-FEB-2003

PATHOLOGY ASSOCIATES

PUBERTAL TOXICITY STUDY OF VINCLOZOLIN, FLUTAMIDE AND PHENOBARBITAL IN MALE SPRAGUE DAWLEY RATS AND METHOXYCHLOR, ETHINYL ESTRADIOL AND PHENOBARBITAL IN FEMALE SPRAGUE DAWLEY RATS WHEN ADMINISTERED IN CORN OIL BY ORAL GAVAGE

CORRELATION OF GROSS & MICRO

STUDY ID: 7244-600 STUDY NUMBER: 7244600
SEX: MALE GROUP: 7: 100 mg/kg Vinclozolin

Animal ID: 9128 Pathologist: JLQ
Animal Fate: Terminal Kill Days on Test: 54

Reference to Necropsy Record:	Related Histopathology:
KIDNEYS - DISCOLORATION; DARK; RIGHT; SINGLE (TGL): FOCAL	KIDNEYS - No Corollary change detected
KIDNEYS - DILATION; RIGHT; MODERATE (TGL): RENAL PELVIS	KIDNEYS - Hydronephrosis

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PATHOLOGY ASSOCIATES

PUBERTAL TOXICITY STUDY OF VINCLOZOLIN, FLUTAMIDE AND PHENOBARBITAL IN MALE SPRAGUE DAWLEY RATS AND METHOXYCHLOR, ETHINYL ESTRADIOL AND PHENOBARBITAL IN FEMALE SPRAGUE DAWLEY RATS WHEN ADMINISTERED IN CORN OIL BY ORAL GAVAGE

CORRELATION OF GROSS & MICRO

STUDY ID: 7244-600 STUDY NUMBER: 7244600
SEX: MALE GROUP: 8: 25 mg/kg Flutamide

Animal ID: 9131 Pathologist: JLQ
Animal Fate: Terminal Kill Days on Test: 53

Reference to Necropsy Record:	Related Histopathology:
DORSAL LATERAL PROSTATE - REDUCTION IN SIZE; MODERATE (TGL)	DORSAL LATERAL PROSTATE - (Tissue unavailable)
VENTRAL PROSTATE - REDUCTION IN SIZE; MODERATE (TGL)	VENTRAL PROSTATE - (Tissue unavailable)
SEMINAL VESICLES - WITH FLUID; REDUCTION IN SIZE; BILATERAL; MODERATE (TGL)	SEMINAL VESICLES - (Tissue unavailable)
COAGULATING GLANDS - WITH FLUID; REDUCTION IN SIZE; BILATERAL; MODERATE	COAGULATING GLANDS - (Tissue unavailable)

Animal ID: 9132 Pathologist: JLQ
Animal Fate: Terminal Kill Days on Test: 53

Reference to Necropsy Record:	Related Histopathology:
SEMINAL VESICLES - WITH FLUID; REDUCTION IN SIZE; BILATERAL; SEVERE (TGL)	SEMINAL VESICLES - (Tissue unavailable)
COAGULATING GLANDS - WITH FLUID; REDUCTION IN SIZE; BILATERAL; SEVERE (TGL)	COAGULATING GLANDS - (Tissue unavailable)

Animal ID: 9133 Pathologist: JLQ
Animal Fate: Terminal Kill Days on Test: 53

Reference to Necropsy Record:	Related Histopathology:
SEMINAL VESICLES - WITH FLUID; REDUCTION IN SIZE; BILATERAL; MODERATE (TGL)	SEMINAL VESICLES - (Tissue unavailable)
COAGULATING GLANDS - WITH FLUID; REDUCTION IN SIZE; BILATERAL; MODERATE (TGL)	COAGULATING GLANDS - (Tissue unavailable)

THERIMUNE RESEARCH CORPORATION STUDY NUMBER 7244-600

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PATHOLOGY ASSOCIATES

PUBERTAL TOXICITY STUDY OF VINCLOZOLIN, FLUTAMIDE AND PHENOBARBITAL IN MALE SPRAGUE DAWLEY RATS AND METHOXYCHLOR, ETHINYL ESTRADIOL AND PHENOBARBITAL IN FEMALE SPRAGUE DAWLEY RATS WHEN ADMINISTERED IN CORN OIL BY ORAL GAVAGE

CORRELATION OF GROSS & MICRO

STUDY ID: 7244-600 STUDY NUMBER: 7244600
SEX: MALE GROUP: 8: 25 mg/kg Flutamide

Animal ID: 9134 Pathologist: JLQ
Animal Fate: Terminal Kill Days on Test: 53

Reference to Necropsy Record:	Related Histopathology:
DORSAL LATERAL PROSTATE - REDUCTION IN SIZE; MODERATE (TGL)	DORSAL LATERAL PROSTATE - (Tissue unavailable)
EPIDIDYMIDES - REDUCTION IN SIZE; BILATERAL; MODERATE (TGL)	EPIDIDYMIDES - No Corollary change detected
VENTRAL PROSTATE - REDUCTION IN SIZE; MODERATE (TGL)	VENTRAL PROSTATE - (Tissue unavailable)
SEMINAL VESICLES - WITH FLUID; REDUCTION IN SIZE; BILATERAL; SEVERE (TGL)	SEMINAL VESICLES - (Tissue unavailable)
COAGULATING GLANDS - WITH FLUID; REDUCTION IN SIZE; BILATERAL; SEVERE (TGL)	COAGULATING GLANDS - (Tissue unavailable)

Animal ID: 9135 Pathologist: JLQ
Animal Fate: Terminal Kill Days on Test: 53

Reference to Necropsy Record:	Related Histopathology:
SEMINAL VESICLES - WITH FLUID; REDUCTION IN SIZE; BILATERAL; MODERATE (TGL)	SEMINAL VESICLES - (Tissue unavailable)
COAGULATING GLANDS - WITH FLUID; REDUCTION IN SIZE; BILATERAL; MODERATE (TGL)	COAGULATING GLANDS - (Tissue unavailable)

THERIMUNE RESEARCH CORPORATION STUDY NUMBER 7244-600

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PATHOLOGY ASSOCIATES

PUBERTAL TOXICITY STUDY OF VINCLOZOLIN, FLUTAMIDE AND PHENOBARBITAL IN MALE SPRAGUE DAWLEY RATS AND METHOXYCHLOR, ETHINYL ESTRADIOL AND PHENOBARBITAL IN FEMALE SPRAGUE DAWLEY RATS WHEN ADMINISTERED IN CORN OIL BY ORAL GAVAGE

CORRELATION OF GROSS & MICRO

STUDY ID: 7244-600 STUDY NUMBER: 7244600
SEX: MALE GROUP: 8: 25 mg/kg Flutamide

Animal ID: 9136 Pathologist: JLQ
Animal Fate: Terminal Kill Days on Test: 53

Reference to Necropsy Record:	Related Histopathology:
DORSAL LATERAL PROSTATE - REDUCTION IS SIZE; MODERATE (TGL)	DORSAL LATERAL PROSTATE - (Tissue unavailable)
EPIDIDYMIDES - REDUCTION IN SIZE; BILATERAL; MODERATE (TGL)	EPIDIDYMIDES - No Corollary change detected
VENTRAL PROSTATE - REDUCTION IN SIZE; MODERATE (TGL)	VENTRAL PROSTATE - (Tissue unavailable)
SEMINAL VESICLES - WITH FLUID; REDUCTION IN SIZE; BILATERAL; SEVERE (TGL)	SEMINAL VESICLES - (Tissue unavailable)
COAGULATING GLANDS - WITH FLUID; REDUCTION IN SIZE; BILATERAL; SEVERE (TGL)	COAGULATING GLANDS - (Tissue unavailable)

Animal ID: 9137 Pathologist: JLQ
Animal Fate: Terminal Kill Days on Test: 53

Reference to Necropsy Record:	Related Histopathology:
DORSAL LATERAL PROSTATE - REDUCTION IN SIZE; MODERATE (TGL)	DORSAL LATERAL PROSTATE - (Tissue unavailable)
VENTRAL PROSTATE - REDUCTION IN SIZE; MODERATE (TGL)	VENTRAL PROSTATE - (Tissue unavailable)
SEMINAL VESICLES - WITH FLUID; REDUCTION IN SIZE; BILATERAL; MODERATE (TGL)	SEMINAL VESICLES - (Tissue unavailable)
COAGULATING GLANDS - WITH FLUID; REDUCTION IS SIZE; BILATERAL; MODERATE (TGL)	COAGULATING GLANDS - (Tissue unavailable)

THERIMUNE RESEARCH CORPORATION STUDY NUMBER 7244-600

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PUBERTAL TOXICITY STUDY OF VINCLOZOLIN, FLUTAMIDE AND PHENOBARBITAL IN MALE SPRAGUE DAWLEY RATS AND METHOXYCHLOR, ETHINYL ESTRADIOL AND PHENOBARBITAL IN FEMALE SPRAGUE DAWLEY RATS WHEN ADMINISTERED IN CORN OIL BY ORAL GAVAGE

CORRELATION OF GROSS & MICRO

STUDY ID: 7244-600 STUDY NUMBER: 7244600
SEX: MALE GROUP: 8: 25 mg/kg Flutamide

Animal ID: 9138 Pathologist: JLQ
Animal Fate: Terminal Kill Days on Test: 53

Reference to Necropsy Record:	Related Histopathology:
DORSAL LATERAL PROSTATE - REDUCTION IN SIZE; MODERATE (TGL)	DORSAL LATERAL PROSTATE - (Tissue unavailable)
VENTRAL PROSTATE - REDUCTION IN SIZE; MODERATE (TGL)	VENTRAL PROSTATE - (Tissue unavailable)
SEMINAL VESICLES - WITH FLUID; REDUCTION IN SIZE; BILATERAL; MODERATE (TGL)	SEMINAL VESICLES - (Tissue unavailable)
COAGULATING GLANDS - WITH FLUID; REDUCTION IN SIZE; BILATERAL; MODERATE (TGL)	COAGULATING GLANDS - (Tissue unavailable)

Animal ID: 9139 Pathologist: JLQ
Animal Fate: Terminal Kill Days on Test: 54

Reference to Necropsy Record:	Related Histopathology:
EPIDIDYMIDES - REDUCTION IN SIZE; BILATERAL; MODERATE (TGL)	EPIDIDYMIDES - No Corollary change detected
SEMINAL VESICLES - WITH FLUID; REDUCTION IN SIZE; BILATERAL; MODERATE (TGL)	SEMINAL VESICLES - (Tissue unavailable)
COAGULATING GLANDS - WITH FLUID; REDUCTION IN SIZE; BILATERAL; MODERATE (TGL)	COAGULATING GLANDS - (Tissue unavailable)

THERIMUNE RESEARCH CORPORATION STUDY NUMBER 7244-600

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PUBERTAL TOXICITY STUDY OF VINCLOZOLIN, FLUTAMIDE AND PHENOBARBITAL IN MALE SPRAGUE DAWLEY RATS AND METHOXYCHLOR, ETHINYL ESTRADIOL AND PHENOBARBITAL IN FEMALE SPRAGUE DAWLEY RATS WHEN ADMINISTERED IN CORN OIL BY ORAL GAVAGE

CORRELATION OF GROSS & MICRO

STUDY ID: 7244-600 STUDY NUMBER: 7244600
SEX: MALE GROUP: 8: 25 mg/kg Flutamide

Animal ID: 9140 Pathologist: JLQ
Animal Fate: Terminal Kill Days on Test: 54

Reference to Necropsy Record:	Related Histopathology:
DORSAL LATERAL PROSTATE - REDUCTION IN SIZE; MODERATE (TGL)	DORSAL LATERAL PROSTATE - (Tissue unavailable)
EPIDIDYMIDES - REDUCTION IN SIZE; BILATERAL; MODERATE (TGL)	EPIDIDYMIDES - No Corollary change detected
VENTRAL PROSTATE - REDUCTION IN SIZE; MODERATE (TGL)	VENTRAL PROSTATE - (Tissue unavailable)
SEMINAL VESICLES - WITH FLUID; REDUCTION IN SIZE; BILATERAL; MODERATE (TGL)	SEMINAL VESICLES - (Tissue unavailable)
COAGULATING GLANDS - WITH FLUID; REDUCTION IN SIZE; BILATERAL; MODERATE (TGL)	COAGULATING GLANDS - (Tissue unavailable)

Animal ID: 9141 Pathologist: JLQ
Animal Fate: Terminal Kill Days on Test: 54

Reference to Necropsy Record:	Related Histopathology:
DORSAL LATERAL PROSTATE - REDUCTION IN SIZE; MODERATE (TGL)	DORSAL LATERAL PROSTATE - (Tissue unavailable)
EPIDIDYMIDES - REDUCTION IN SIZE; BILATERAL; MODERATE (TGL)	EPIDIDYMIDES - No Corollary change detected
VENTRAL PROSTATE - REDUCTION IN SIZE; BILATERAL; MODERATE (TGL)	VENTRAL PROSTATE - (Tissue unavailable)
SEMINAL VESICLES - WITH FLUID; REDUCTION IN SIZE; BILATERAL; MODERATE (TGL)	SEMINAL VESICLES - (Tissue unavailable)
COAGULATING GLANDS - WITH FLUID; REDUCTION IN SIZE; BILATERAL; MODERATE (TGL)	COAGULATING GLANDS - (Tissue unavailable)

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PUBERTAL TOXICITY STUDY OF VINCLOZOLIN, FLUTAMIDE AND PHENOBARBITAL IN MALE SPRAGUE DAWLEY RATS AND METHOXYCHLOR, ETHINYL ESTRADIOL AND PHENOBARBITAL IN FEMALE SPRAGUE DAWLEY RATS WHEN ADMINISTERED IN CORN OIL BY ORAL GAVAGE

CORRELATION OF GROSS & MICRO

STUDY ID: 7244-600 STUDY NUMBER: 7244600
SEX: MALE GROUP: 8: 25 mg/kg Flutamide

Animal ID: 9143 Pathologist: JLQ
Animal Fate: Terminal Kill Days on Test: 54

Reference to Necropsy Record:	Related Histopathology:
DORSAL LATERAL PROSTATE - REDUCTION IN SIZE; MODERATE (TGL)	DORSAL LATERAL PROSTATE - (Tissue unavailable)
VENTRAL PROSTATE - REDUCTION IN SIZE; MODERATE (TGL)	VENTRAL PROSTATE - (Tissue unavailable)
SEMINAL VESICLES - WITH FLUID; REDUCTION IN SIZE; BILATERAL; MODERATE (TGL)	SEMINAL VESICLES - (Tissue unavailable)
COAGULATING GLANDS - WITH FLUID; REDUCTION IN SIZE; BILATERAL; MODERATE (TGL)	COAGULATING GLANDS - (Tissue unavailable)

Animal ID: 9144 Pathologist: JLQ
Animal Fate: Terminal Kill Days on Test: 54

Reference to Necropsy Record:	Related Histopathology:
DORSAL LATERAL PROSTATE - REDUCTION IN SIZE; MODERATE (TGL)	DORSAL LATERAL PROSTATE - (Tissue unavailable)
VENTRAL PROSTATE - REDUCTION IN SIZE; MODERATE (TGL)	VENTRAL PROSTATE - (Tissue unavailable)
SEMINAL VESICLES - WITH FLUID; REDUCTION IN SIZE; BILATERAL; MODERATE (TGL)	SEMINAL VESICLES - (Tissue unavailable)
COAGULATING GLANDS - WITH FLUID; REDUCTION IN SIZE; BILATERAL; MODERATE (TGL)	COAGULATING GLANDS - (Tissue unavailable)

PATHOLOGY ASSOCIATES

PUBERTAL TOXICITY STUDY OF VINCLOZOLIN, FLUTAMIDE AND PHENOBARBITAL IN MALE SPRAGUE DAWLEY RATS AND METHOXYCHLOR, ETHINYL ESTRADIOL AND PHENOBARBITAL IN FEMALE SPRAGUE DAWLEY RATS WHEN ADMINISTERED IN CORN OIL BY ORAL GAVAGE

CORRELATION OF GROSS & MICRO

STUDY ID: 7244-600 STUDY NUMBER: 7244600
SEX: MALE GROUP: 8: 25 mg/kg Flutamide

Animal ID: 9145 Pathologist: JLQ
Animal Fate: Terminal Kill Days on Test: 54

Reference to Necropsy Record:	Related Histopathology:
DORSAL LATERAL PROSTATE - REDUCTION IN SIZE; MINIMAL (TGL)	DORSAL LATERAL PROSTATE - (Tissue unavailable)
VENTRAL PROSTATE - REDUCTION IN SIZE; MINIMAL (TGL)	VENTRAL PROSTATE - (Tissue unavailable)
SEMINAL VESICLES - WITH FLUID; REDUCTION IN SIZE; BILATERAL; MINIMAL (TGL)	SEMINAL VESICLES - (Tissue unavailable)
COAGULATING GLANDS - WITH FLUID; REDUCTION IN SIZE; BILATERAL MINIMAL (TGL)	COAGULATING GLANDS - (Tissue unavailable)

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PATHOLOGY ASSOCIATES

PUBERTAL TOXICITY STUDY OF VINCLOZOLIN, FLUTAMIDE AND PHENOBARBITAL IN MALE SPRAGUE DAWLEY RATS AND METHOXYCHLOR, ETHINYL ESTRADIOL AND PHENOBARBITAL IN FEMALE SPRAGUE DAWLEY RATS WHEN ADMINISTERED IN CORN OIL BY ORAL GAVAGE

CORRELATION OF GROSS & MICRO

STUDY ID: 7244-600 STUDY NUMBER: 7244600
SEX: MALE GROUP: 9: 50 mg/kg Flutamide

Animal ID: 9146 Pathologist: JLQ
Animal Fate: Terminal Kill Days on Test: 53

Reference to Necropsy Record:	Related Histopathology:
SEMINAL VESICLES - WITH FLUID; REDUCTION IN SIZE; BILATERAL; SEVERE (TGL)	SEMINAL VESICLES - (Tissue unavailable)
COAGULATING GLANDS - WITH FLUID; REDUCTION IN SIZE; BILATERAL; SEVERE (TGL)	COAGULATING GLANDS - (Tissue unavailable)

Animal ID: 9147 Pathologist: JLQ
Animal Fate: Terminal Kill Days on Test: 53

Reference to Necropsy Record:	Related Histopathology:
DORSAL LATERAL PROSTATE - REDUCTION IN SIZE; MODERATE (TGL)	DORSAL LATERAL PROSTATE - (Tissue unavailable)
EPIDIDYMIDES - REDUCTION IN SIZE; BILATERAL; MODERATE (TGL)	EPIDIDYMIDES - No Corollary change detected
LEVATOR ANI PLUS BULBCAV - REDUCTION IN SIZE; MINIMAL (TGL)	LEVATOR ANI PLUS BULBCAV - (Tissue unavailable)
VENTRAL PROSTATE - REDUCTION IN SIZE; MODERATE (TGL)	VENTRAL PROSTATE - (Tissue unavailable)
SEMINAL VESICLES - WITH FLUID; REDUCTION IN SIZE; BILATERAL; SEVERE (TGL)	SEMINAL VESICLES - (Tissue unavailable)
COAGULATING GLANDS - WITH FLUID; REDUCTION IN SIZE; BILATERAL; SEVERE (TGL)	COAGULATING GLANDS - (Tissue unavailable)

PATHOLOGY ASSOCIATES

PUBERTAL TOXICITY STUDY OF VINCLOZOLIN, FLUTAMIDE AND PHENOBARBITAL IN MALE SPRAGUE DAWLEY RATS AND METHOXYCHLOR, ETHINYL ESTRADIOL AND PHENOBARBITAL IN FEMALE SPRAGUE DAWLEY RATS WHEN ADMINISTERED IN CORN OIL BY ORAL GAVAGE

CORRELATION OF GROSS & MICRO

STUDY ID: 7244-600 STUDY NUMBER: 7244600
SEX: MALE GROUP: 9: 50 mg/kg Flutamide

Animal ID: 9148 Pathologist: JLQ
Animal Fate: Terminal Kill Days on Test: 53

Reference to Necropsy Record:	Related Histopathology:
SEMINAL VESICLES - WITH FLUID; REDUCTION IN SIZE; BILATERAL; SEVERE (TGL)	SEMINAL VESICLES - (Tissue unavailable)
COAGULATING GLANDS - WITH FLUID; REDUCTION IN SIZE; BILATERAL; SEVERE (TGL)	COAGULATING GLANDS - (Tissue unavailable)

Animal ID: 9149 Pathologist: JLQ
Animal Fate: Terminal Kill Days on Test: 53

Reference to Necropsy Record:	Related Histopathology:
DORSAL LATERAL PROSTATE - REDUCTION IN SIZE; SEVERE (TGL)	DORSAL LATERAL PROSTATE - (Tissue unavailable)
EPIDIDYMIDES - REDUCTION IN SIZE; BILATERAL; MODERATE (TGL)	EPIDIDYMIDES - No Corollary change detected
KIDNEYS - DILATION; RIGHT; MODERATE (TGL): RENAL PELVIS	KIDNEYS - Hydronephrosis
VENTRAL PROSTATE - REDUCTION IN SIZE; SEVERE (TGL)	VENTRAL PROSTATE - (Tissue unavailable)
SEMINAL VESICLES - WITH FLUID; REDUCTION IN SIZE; BILATERAL; SEVERE (TGL)	SEMINAL VESICLES - (Tissue unavailable)
COAGULATING GLANDS - WITH FLUID; REDUCTION IN SIZE; BILATERAL; SEVERE (TGL)	COAGULATING GLANDS - (Tissue unavailable)

PATHOLOGY ASSOCIATES

PUBERTAL TOXICITY STUDY OF VINCLOZOLIN, FLUTAMIDE AND PHENOBARBITAL IN MALE SPRAGUE DAWLEY RATS AND METHOXYCHLOR, ETHINYL ESTRADIOL AND PHENOBARBITAL IN FEMALE SPRAGUE DAWLEY RATS WHEN ADMINISTERED IN CORN OIL BY ORAL GAVAGE

CORRELATION OF GROSS & MICRO

STUDY ID: 7244-600 STUDY NUMBER: 7244600
SEX: MALE GROUP: 9: 50 mg/kg Flutamide

Animal ID: 9150 Pathologist: JLQ
Animal Fate: Terminal Kill Days on Test: 53

Reference to Necropsy Record:	Related Histopathology:
DORSAL LATERAL PROSTATE - REDUCTION IN SIZE; MODERATE (TGL)	DORSAL LATERAL PROSTATE - (Tissue unavailable)
EPIDIDYMIDES - REDUCTION IN SIZE; BILATERAL; MINIMAL (TGL)	EPIDIDYMIDES - No Corollary change detected
VENTRAL PROSTATE - REDUCTION IN SIZE; MODERATE (TGL)	VENTRAL PROSTATE - (Tissue unavailable)
SEMINAL VESICLES - WITH FLUID; REDUCTION IN SIZE; BILATERAL; SEVERE (TGL)	SEMINAL VESICLES - (Tissue unavailable)
COAGULATING GLANDS - WITH FLUID; REDUCTION IN SIZE; BILATERAL; SEVERE (TGL)	COAGULATING GLANDS - (Tissue unavailable)

Animal ID: 9151 Pathologist: JLQ
Animal Fate: Terminal Kill Days on Test: 53

Reference to Necropsy Record:	Related Histopathology:
DORSAL LATERAL PROSTATE - REDUCTION IN SIZE; MODERATE (TGL)	DORSAL LATERAL PROSTATE - (Tissue unavailable)
VENTRAL PROSTATE - REDUCTION IN SIZE; MODERATE (TGL)	VENTRAL PROSTATE - (Tissue unavailable)
SEMINAL VESICLES - WITH FLUID; REDUCTION IN SIZE; BILATERAL; MODERATE (TGL)	SEMINAL VESICLES - (Tissue unavailable)
COAGULATING GLANDS - WITH FLUID; REDUCTION IN SIZE; BILATERAL; MODERATE (TGL)	COAGULATING GLANDS - (Tissue unavailable)

THERIMUNE RESEARCH CORPORATION STUDY NUMBER 7244-600

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PATHOLOGY ASSOCIATES

PUBERTAL TOXICITY STUDY OF VINCLOZOLIN, FLUTAMIDE AND PHENOBARBITAL IN MALE SPRAGUE DAWLEY RATS AND METHOXYCHLOR, ETHINYL ESTRADIOL AND PHENOBARBITAL IN FEMALE SPRAGUE DAWLEY RATS WHEN ADMINISTERED IN CORN OIL BY ORAL GAVAGE

CORRELATION OF GROSS & MICRO

STUDY ID: 7244-600 STUDY NUMBER: 7244600
SEX: MALE GROUP: 9: 50 mg/kg Flutamide

Animal ID: 9152 Pathologist: JLQ
Animal Fate: Terminal Kill Days on Test: 53

Reference to Necropsy Record:	Related Histopathology:
EPIDIDYMIDES - REDUCTION IN SIZE; BILATERAL; MODERATE (TGL)	EPIDIDYMIDES - No Corollary change detected
TESTES - ENLARGEMENT; BILATERAL; MINIMAL (TGL)	TESTES - Edema; Dilation, tubule lumen
SEMINAL VESICLES - WITH FLUID; REDUCTION IN SIZE; BILATERAL; MODERATE (TGL)	SEMINAL VESICLES - (Tissue unavailable)
COAGULATING GLANDS - WITH FLUID; REDUCTION IN SIZE; BILATERAL; MODERATE (TGL)	COAGULATING GLANDS - (Tissue unavailable)

Animal ID: 9153 Pathologist: JLQ
Animal Fate: Terminal Kill Days on Test: 53

Reference to Necropsy Record:	Related Histopathology:
DORSAL LATERAL PROSTATE - REDUCTION IN SIZE; MODERATE (TGL)	DORSAL LATERAL PROSTATE - (Tissue unavailable)
VENTRAL PROSTATE - REDUCTION IN SIZE; MODERATE (TGL)	VENTRAL PROSTATE - (Tissue unavailable)
SEMINAL VESICLES - WITH FLUID; REDUCTION IN SIZE; BILATERAL; MODERATE (TGL)	SEMINAL VESICLES - (Tissue unavailable)
COAGULATING GLANDS - WITH FLUID; REDUCTION IN SIZE; BILATERAL; MODERATE (TGL)	COAGULATING GLANDS - (Tissue unavailable)

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PUBERTAL TOXICITY STUDY OF VINCLOZOLIN, FLUTAMIDE AND PHENOBARBITAL IN MALE SPRAGUE DAWLEY RATS AND METHOXYCHLOR, ETHINYL ESTRADIOL AND PHENOBARBITAL IN FEMALE SPRAGUE DAWLEY RATS WHEN ADMINISTERED IN CORN OIL BY ORAL GAVAGE

CORRELATION OF GROSS & MICRO

STUDY ID: 7244-600 STUDY NUMBER: 7244600
SEX: MALE GROUP: 9: 50 mg/kg Flutamide

Animal ID: 9154 Pathologist: JLQ
Animal Fate: Terminal Kill Days on Test: 54

Reference to Necropsy Record:	Related Histopathology:
DORSAL LATERAL PROSTATE - REDUCTION IN SIZE; MODERATE (TGL)	DORSAL LATERAL PROSTATE - (Tissue unavailable)
EPIDIDYMIDES - REDUCTION IN SIZE; RIGHT; MIMIMAL (TGL)	EPIDIDYMIDES - No Corollary change detected
TESTES - ENLARGEMENT; BILATERAL; MINIMAL (TGL)	TESTES - Edema; Dilation, tubule lumen
LEVATOR ANI PLUS BULBCAV - REDUCTION IN SIZE; BILATERAL; MODERATE (TGL)	LEVATOR ANI PLUS BULBCAV - (Tissue unavailable)
VENTRAL PROSTATE - REDUCTION IN SIZE; MODERATE (TGL)	VENTRAL PROSTATE - (Tissue unavailable)
SEMINAL VESICLES - WITH FLUID; REDUCTION IN SIZE; BILATERAL; MODERATE (TGL)	SEMINAL VESICLES - (Tissue unavailable)
COAGULATING GLANDS - WITH FLUID; REDUCTION IN SIZE; BILATERAL; MODERATE (TGL)	COAGULATING GLANDS - (Tissue unavailable)

Animal ID: 9155 Pathologist: JLQ
Animal Fate: Terminal Kill Days on Test: 54

Reference to Necropsy Record:	Related Histopathology:
DORSAL LATERAL PROSTATE - REDUCTION IN SIZE; MODERATE (TGL)	DORSAL LATERAL PROSTATE - (Tissue unavailable)
VENTRAL PROSTATE - REDUCTION IN SIZE; MODERATE (TGL)	VENTRAL PROSTATE - (Tissue unavailable)
SEMINAL VESICLES - WITH FLUID; REDUCTION IN SIZE; BILATERAL; MODERATE (TGL)	SEMINAL VESICLES - (Tissue unavailable)

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PUBERTAL TOXICITY STUDY OF VINCLOZOLIN, FLUTAMIDE AND PHENOBARBITAL IN MALE SPRAGUE DAWLEY RATS AND METHOXYCHLOR, ETHINYL ESTRADIOL AND PHENOBARBITAL IN FEMALE SPRAGUE DAWLEY RATS WHEN ADMINISTERED IN CORN OIL BY ORAL GAVAGE

CORRELATION OF GROSS & MICRO

STUDY ID: 7244-600 STUDY NUMBER: 7244600
SEX: MALE GROUP: 9: 50 mg/kg Flutamide

Animal ID: 9155 Pathologist: JLQ
Animal Fate: Terminal Kill Days on Test: 54

Reference to Necropsy Record:	Related Histopathology:
COAGULATING GLANDS - WITH FLUID; REDUCTION IS SIZE; BILATERAL MODERATE (TGL)	COAGULATING GLANDS - (Tissue unavailable)

Animal ID: 9156 Pathologist: JLQ
Animal Fate: Terminal Kill Days on Test: 54

Reference to Necropsy Record:	Related Histopathology:
DORSAL LATERAL PROSTATE - REDUCTION IN SIZE; MODERATE (TGL)	DORSAL LATERAL PROSTATE - (Tissue unavailable)
EPIDIDYMIDES - REDUCTION IN SIZE; BILATERAL; MODERATE (TGL)	EPIDIDYMIDES - No Corollary change detected
LEVATOR ANI PLUS BULBCAV - REDUCTION IN SIZE; BILATERAL; MODERATE (TGL)	LEVATOR ANI PLUS BULBCAV - (Tissue unavailable)
VENTRAL PROSTATE - REDUCTION IN SIZE; MODERATE (TGL)	VENTRAL PROSTATE - (Tissue unavailable)
SEMINAL VESICLES - REDUCTION IN SIZE; BILATERAL; MODERATE (TGL)	SEMINAL VESICLES - (Tissue unavailable)
COAGULATING GLANDS - REDUCTION IN SIZE; BILATERAL; MODERATE (TGL)	COAGULATING GLANDS - (Tissue unavailable)

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PUBERTAL TOXICITY STUDY OF VINCLOZOLIN, FLUTAMIDE AND PHENOBARBITAL IN MALE SPRAGUE DAWLEY RATS AND METHOXYCHLOR, ETHINYL ESTRADIOL AND PHENOBARBITAL IN FEMALE SPRAGUE DAWLEY RATS WHEN ADMINISTERED IN CORN OIL BY ORAL GAVAGE

CORRELATION OF GROSS & MICRO

STUDY ID: 7244-600 STUDY NUMBER: 7244600
SEX: MALE GROUP: 9: 50 mg/kg Flutamide

Animal ID: 9157 Pathologist: JLQ
Animal Fate: Terminal Kill Days on Test: 54

Reference to Necropsy Record:	Related Histopathology:
DORSAL LATERAL PROSTATE - REDUCTION IN SIZE; MODERATE (TGL)	DORSAL LATERAL PROSTATE - (Tissue unavailable)
VENTRAL PROSTATE - REDUCTION IN SIZE; MODERATE (TGL)	VENTRAL PROSTATE - (Tissue unavailable)
SEMINAL VESICLES - WITH FLUID; REDUCTION IN SIZE; BILATERAL; MODERATE (TGL)	SEMINAL VESICLES - (Tissue unavailable)
COAGULATING GLANDS - WITH FLUID; REDUCTION IN SIZE; BILATERAL; MODERAT (TGL)	COAGULATING GLANDS - (Tissue unavailable)

Animal ID: 9158 Pathologist: JLQ
Animal Fate: Terminal Kill Days on Test: 54

Reference to Necropsy Record:	Related Histopathology:
DORSAL LATERAL PROSTATE - REDUCTION IN SIZE; MODERATE (TGL)	DORSAL LATERAL PROSTATE - (Tissue unavailable)
VENTRAL PROSTATE - REDUCTION IN SIZE; MODERATE (TGL)	VENTRAL PROSTATE - (Tissue unavailable)
SEMINAL VESICLES - WITH FLUID; REDUCTION IN SIZE; BILATERAL; MODERATE (TGL)	SPLEEN - (Tissue unavailable)
COAGULATING GLANDS - WITH FLUID; REDUCTION IN SIZE; BILATERAL; MODERATE (TGL)	COAGULATING GLANDS - (Tissue unavailable)

PATHOLOGY ASSOCIATES

PUBERTAL TOXICITY STUDY OF VINCLOZOLIN, FLUTAMIDE AND PHENOBARBITAL IN MALE SPRAGUE DAWLEY RATS AND METHOXYCHLOR, ETHINYL ESTRADIOL AND PHENOBARBITAL IN FEMALE SPRAGUE DAWLEY RATS WHEN ADMINISTERED IN CORN OIL BY ORAL GAVAGE

CORRELATION OF GROSS & MICRO

STUDY ID: 7244-600 STUDY NUMBER: 7244600
SEX: MALE GROUP: 9: 50 mg/kg Flutamide

Animal ID: 9159 Pathologist: JLQ
Animal Fate: Terminal Kill Days on Test: 54

Reference to Necropsy Record:	Related Histopathology:
DORSAL LATERAL PROSTATE - REDUCTION IN SIZE; MODERATE (TGL)	DORSAL LATERAL PROSTATE - (Tissue unavailable)
KIDNEYS - DILATION; RIGHT; MINIMAL (TGL): RENAL PELVIS	KIDNEYS - Hydronephrosis
TESTES - ENDLARGEMENT; LEFT (TGL): 27X13X12MM	TESTES - Dilation, tubule lumen
VENTRAL PROSTATE - REDUCTION IN SIZE; MODERATE (TGL)	VENTRAL PROSTATE - (Tissue unavailable)
SEMINAL VESICLES - WITH FLUID; REDUCTION IN SIZE; BILATERAL; MODERATE (TGL)	SEMINAL VESICLES - (Tissue unavailable)
COAGULATING GLANDS - WITH FLUID; REDUCTION IN SIZE; BILATERAL; MODERATE (TGL)	COAGULATING GLANDS - (Tissue unavailable)

Animal ID: 9160 Pathologist: JLQ
Animal Fate: Terminal Kill Days on Test: 54

Reference to Necropsy Record:	Related Histopathology:
DORSAL LATERAL PROSTATE - REDUCTION IN SIZE; MODERATE (TGL)	DORSAL LATERAL PROSTATE - (Tissue unavailable)
EPIDIDYMIDES - REDUCTION IN SIZE; BILATERAL; MODERATE (TGL)	EPIDIDYMIDES - No Corollary change detected
LEVATOR ANI PLUS BULBCAV - REDUCTION IN SIZE; BILATERAL; MODERATE (TGL)	LEVATOR ANI PLUS BULBCAV - (Tissue unavailable)
VENTRAL PROSTATE - REDUCTION IN SIZE; MODERATE (TGL)	VENTRAL PROSTATE - (Tissue unavailable)

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CORRELATION OF GROSS & MICRO

STUDY ID: 7244-600 STUDY NUMBER: 7244600
SEX: MALE GROUP: 9: 50 mg/kg Flutamide

Animal ID: 9160 Pathologist: JLQ
Animal Fate: Terminal Kill Days on Test: 54

Reference to Necropsy Record:	Related Histopathology:
SEMINAL VESICLES - WITH FLUID; REDUCTION IN SIZE; BILATERAL; MODERATE (TGL)	SEMINAL VESICLES - (Tissue unavailable)
COAGULATING GLANDS - WITH FLUID; REDUCTION IN SIZE; BILATERAL; MODERATE (TGL)	COAGULATING GLANDS - (Tissue unavailable)

PATHOLOGY ASSOCIATES

PUBERTAL TOXICITY STUDY OF VINCLOZOLIN, FLUTAMIDE AND PHENOBARBITAL IN MALE SPRAGUE DAWLEY RATS AND METHOXYCHLOR, ETHINYL ESTRADIOL AND PHENOBARBITAL IN FEMALE SPRAGUE DAWLEY RATS WHEN ADMINISTERED IN CORN OIL BY ORAL GAVAGE

CORRELATION OF GROSS & MICRO

STUDY ID: 7244-600 STUDY NUMBER: 7244600
SEX: FEMALE GROUP: 1: Corn Oil Vehicle

Animal ID: 9164 Pathologist: JLQ
Animal Fate: Terminal Kill Days on Test: 42

Reference to Necropsy Record:	Related Histopathology:
UTERUS - WITHOUT FLUID; DISTENSION; BILATERAL; MODERATE (TGL): UTERUS	UTERUS - No Corollary change detected
CERVIX - WITHOUT FLUID;	CERVIX - No Corollary change detected

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Animal ID: 9167 Pathologist: JLQ
Animal Fate: Terminal Kill Days on Test: 42

Reference to Necropsy Record:	Related Histopathology:
KIDNEYS - DILATION; RIGHT; MINIMAL (TGL): RENAL PELVIS	KIDNEYS - Hydronephrosis

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Animal ID: 9169 Pathologist: JLQ
Animal Fate: Terminal Kill Days on Test: 43

Reference to Necropsy Record:	Related Histopathology:
KIDNEYS - DISCOLORATION; DARK; BILATERAL; MULTIFOCAL (TGL)	KIDNEYS - No Corollary change detected

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Animal ID: 9170 Pathologist: JLQ
Animal Fate: Terminal Kill Days on Test: 43

Reference to Necropsy Record:	Related Histopathology:
UTERUS - WITHOUT FLUID; DISTENSION; BILATERAL; MODERATE (TGL): UTERUS	UTERUS - No Corollary change detected
CERVIX - WITHOUT FLUID;	CERVIX - No Corollary change detected

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THERIMUNE RESEARCH CORPORATION STUDY NUMBER 7244-600

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PUBERTAL TOXICITY STUDY OF VINCLOZOLIN, FLUTAMIDE AND PHENOBARBITAL IN MALE SPRAGUE DAWLEY RATS AND METHOXYCHLOR, ETHINYL ESTRADIOL AND PHENOBARBITAL IN FEMALE SPRAGUE DAWLEY RATS WHEN ADMINISTERED IN CORN OIL BY ORAL GAVAGE

CORRELATION OF GROSS & MICRO

STUDY ID: 7244-600 STUDY NUMBER: 7244600
SEX: FEMALE GROUP: 1: Corn Oil Vehicle

Animal ID: 9171 Pathologist: JLQ
Animal Fate: Terminal Kill Days on Test: 43

Reference to Necropsy Record:	Related Histopathology:
LIVER - DISCOLORATION; PALE (TGL): ALL LOBES	LIVER - No Corollary change detected
UTERUS - WITHOUT FLUID; DISTENSION; BILATERAL; MODERATE (TGL): UTERUS	UTERUS - No Corollary change detected
CERVIX - WITHOUT FLUID;	CERVIX - No Corollary change detected

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Animal ID: 9174 Pathologist: JLQ
Animal Fate: Terminal Kill Days on Test: 43

Reference to Necropsy Record:	Related Histopathology:
UTERUS - WITHOUT FLUID; DISTENSION; BILATERAL; MINIMAL (TGL): UTERUS	UTERUS - No Corollary change detected
CERVIX - WITHOUT FLUID;	CERVIX - No Corollary change detected

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THERIMUNE RESEARCH CORPORATION STUDY NUMBER 7244-600

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PATHOLOGY ASSOCIATES

PUBERTAL TOXICITY STUDY OF VINCLOZOLIN, FLUTAMIDE AND PHENOBARBITAL IN MALE SPRAGUE DAWLEY RATS AND METHOXYCHLOR, ETHINYL ESTRADIOL AND PHENOBARBITAL IN FEMALE SPRAGUE DAWLEY RATS WHEN ADMINISTERED IN CORN OIL BY ORAL GAVAGE

CORRELATION OF GROSS & MICRO

STUDY ID: 7244-600 STUDY NUMBER: 7244600
SEX: FEMALE GROUP: 2: 0.0025 mg/kg Ethinyl Estradiol

Animal ID: 9181 Pathologist: JLQ
Animal Fate: Terminal Kill Days on Test: 42

Reference to Necropsy Record:	Related Histopathology:
KIDNEYS - DILATION; RIGHT; MINIMAL (TGL): RENAL PELVIS	KIDNEYS - Hydronephrosis
UTERUS - WITHOUT FLUID; DISTENSION; BILATERAL; MODERATE (TGL): UTERUS	UTERUS - No Corollary change detected
CERVIX - WITHOUT FLUID;	CERVIX - No Corollary change detected

---

Animal ID: 9186 Pathologist: JLQ
Animal Fate: Terminal Kill Days on Test: 43

Reference to Necropsy Record:	Related Histopathology:
OVARIES - DISCOLORATION; RED; RIGHT; SINGLE (TGL)	OVARIES - Hemorrhage

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Animal ID: 9188 Pathologist: JLQ
Animal Fate: Terminal Kill Days on Test: 43

Reference to Necropsy Record:	Related Histopathology:
UTERUS - WITHOUT FLUID; DISTENSION; LEFT; MODERATE (TGL): UTERUS	UTERUS - No Corollary change detected
CERVIX - WITHOUT FLUID;	CERVIX - No Corollary change detected

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PUBERTAL TOXICITY STUDY OF VINCLOZOLIN, FLUTAMIDE AND PHENOBARBITAL IN MALE SPRAGUE DAWLEY RATS AND METHOXYCHLOR, ETHINYL ESTRADIOL AND PHENOBARBITAL IN FEMALE SPRAGUE DAWLEY RATS WHEN ADMINISTERED IN CORN OIL BY ORAL GAVAGE

CORRELATION OF GROSS & MICRO

STUDY ID: 7244-600 STUDY NUMBER: 7244600
SEX: FEMALE GROUP: 3: 0.005 mg/kg Ethinyl Estradiol

Animal ID: 9193 Pathologist: JLQ
Animal Fate: Terminal Kill Days on Test: 42

Reference to Necropsy Record:	Related Histopathology:
UTERUS - WITHOUT FLUID; DISTENSION; BILATERAL; MODERATE (TGL): UTERUS	UTERUS - No Corollary change detected
CERVIX - WITHOUT FLUID;	CERVIX - No Corollary change detected

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Animal ID: 9197 Pathologist: JLQ
Animal Fate: Terminal Kill Days on Test: 42

Reference to Necropsy Record:	Related Histopathology:
KIDNEYS - DILATION; RIGHT; MINIMAL (TGL): RENAL PELVIS	KIDNEYS - Hydronephrosis
TAIL - IRREGULARLY SHAPED: MULTIPLE KINKS	TAIL - Vertebral dysplasia

---

Animal ID: 9199 Pathologist: JLQ
Animal Fate: Terminal Kill Days on Test: 43

Reference to Necropsy Record:	Related Histopathology:
KIDNEYS - DILATION; RIGHT; MINIMAL (TGL): RENAL PELVIS	KIDNEYS - No Corollary change detected

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PATHOLOGY ASSOCIATES

PUBERTAL TOXICITY STUDY OF VINCLOZOLIN, FLUTAMIDE AND PHENOBARBITAL IN MALE SPRAGUE DAWLEY RATS AND METHOXYCHLOR, ETHINYL ESTRADIOL AND PHENOBARBITAL IN FEMALE SPRAGUE DAWLEY RATS WHEN ADMINISTERED IN CORN OIL BY ORAL GAVAGE

CORRELATION OF GROSS & MICRO

STUDY ID: 7244-600 STUDY NUMBER: 7244600
SEX: FEMALE GROUP: 4: 12.5 mg/kg Methoxychlor

Animal ID: 9209 Pathologist: JLQ
Animal Fate: Terminal Kill Days on Test: 42

Reference to Necropsy Record:	Related Histopathology:
KIDNEYS - DISCOLORATION; DARK; BILATERAL; MULITFOCAL (TGL)	KIDNEYS - No Corollary change detected

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Animal ID: 9211 Pathologist: JLQ
Animal Fate: Terminal Kill Days on Test: 42

Reference to Necropsy Record:	Related Histopathology:
KIDNEYS - DILATION; RIGHT; MODERATE (TGL): RENAL PELVIS	KIDNEYS - Hydronephrosis

---

Animal ID: 9214 Pathologist: JLQ
Animal Fate: Terminal Kill Days on Test: 43

Reference to Necropsy Record:	Related Histopathology:
KIDNEYS - CYST(S); CLEAR; BILATERAL; FOCAL (TGL): LEFT - ONE; RIGHT - ONE	KIDNEYS - No Corollary change detected

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Animal ID: 9218 Pathologist: JLQ
Animal Fate: Terminal Kill Days on Test: 43

Reference to Necropsy Record:	Related Histopathology:
UTERUS - WITHOUT FLUID; DISTENSION; BILATERAL; MODERATE (TGL): UTERUS	UTERUS - No Corollary change detected
CERVIX - WITHOUT FLUID;	CERVIX - No Corollary change detected

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CORRELATION OF GROSS & MICRO

STUDY ID: 7244-600 STUDY NUMBER: 7244600
SEX: FEMALE GROUP: 4: 12.5 mg/kg Methoxychlor

Animal ID: 9220 Pathologist: JLQ
Animal Fate: Terminal Kill Days on Test: 43

Reference to Necropsy Record:	Related Histopathology:
KIDNEYS - DISCOLORATION; DARK BILATERAL; MULTIFOCAL (TGL)	KIDNEYS - No Corollary change detected
KIDNEYS - DILATION; RIGHT; MINIMAL (TGL): RENAL PELVIS	KIDNEYS - Hydronephrosis
UTERUS - WITHOUT FLUID; DISTENSION; BILATERAL; MINIMAL (TGL): UTERUS	UTERUS - No Corollary change detected
CERVIX - WITHOUT FLUID;	CERVIX - No Corollary change detected

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CORRELATION OF GROSS & MICRO

STUDY ID: 7244-600 STUDY NUMBER: 7244600
SEX: FEMALE GROUP: 5: 25 mg/kg Methoxychlor

Animal ID: 9227 Pathologist: JLQ
Animal Fate: Terminal Kill Days on Test: 42

Reference to Necropsy Record:
SPLEEN - ENLARGEMENT (TGL): 47X12X7MM

Related Histopathology:
SPLEEN - Lymphocytic hyperplasia

Animal ID: 9229 Pathologist: JLQ
Animal Fate: Terminal Kill Days on Test: 43

Reference to Necropsy Record:
KIDNEYS - DILATION; RIGHT; MODERATE (TGL): RENAL PELVIS

Related Histopathology:
KIDNEYS - No Corollary change detected

Animal ID: 9231 Pathologist: JLQ
Animal Fate: Terminal Kill Days on Test: 43

Reference to Necropsy Record:
SPLEEN - ENLARGEMENT (TGL): 46X12X5MM

Related Histopathology:
SPLEEN - Lymphocytic hyperplasia

Animal ID: 9232 Pathologist: JLQ
Animal Fate: Terminal Kill Days on Test: 43

Reference to Necropsy Record:
ADRENAL GLANDS - DISCOLORATION; PALE; BILATERAL (TGL)

Related Histopathology:
ADRENAL GLANDS - No Corollary change detected

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PUBERTAL TOXICITY STUDY OF VINCLOZOLIN, FLUTAMIDE AND PHENOBARBITAL IN MALE SPRAGUE DAWLEY RATS AND METHOXYCHLOR, ETHINYL ESTRADIOL AND PHENOBARBITAL IN FEMALE SPRAGUE DAWLEY RATS WHEN ADMINISTERED IN CORN OIL BY ORAL GAVAGE

CORRELATION OF GROSS & MICRO

STUDY ID: 7244-600 STUDY NUMBER: 7244600
SEX: FEMALE GROUP: 5: 25 mg/kg Methoxychlor

Animal ID: 9235 Pathologist: JLQ
Animal Fate: Terminal Kill Days on Test: 43

Reference to Necropsy Record:	Related Histopathology:
UTERUS - WITHOUT FLUID; DISTENSION; BILATERAL; MODERATE (TGL): UTERUS	UTERUS - No Corollary change detected
CERVIX - WITHOUT FLUID;	CERVIX - No Corollary change detected

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PATHOLOGY ASSOCIATES

PUBERTAL TOXICITY STUDY OF VINCLOZOLIN, FLUTAMIDE AND PHENOBARBITAL IN MALE SPRAGUE DAWLEY RATS AND METHOXYCHLOR, ETHINYL ESTRADIOL AND PHENOBARBITAL IN FEMALE SPRAGUE DAWLEY RATS WHEN ADMINISTERED IN CORN OIL BY ORAL GAVAGE

CORRELATION OF GROSS & MICRO

STUDY ID: 7244-600 STUDY NUMBER: 7244600
SEX: FEMALE GROUP: 6: 50 mg/kg Methoxychlor

Animal ID: 9241 Pathologist: JLQ
Animal Fate: Terminal Kill Days on Test: 42

Reference to Necropsy Record:	Related Histopathology:
UTERUS - WITHOUT FLUID; DISTENSION; BILATERAL; MODERATE (TGL): UTERUS	UTERUS - No Corollary change detected
CERVIX - WITHOUT FLUID;	CERVIX - No Corollary change detected

---

Animal ID: 9242 Pathologist: JLQ
Animal Fate: Terminal Kill Days on Test: 42

Reference to Necropsy Record:	Related Histopathology:
UTERUS - WITHOUT FLUID; DISTENSION; BILATERAL; MODERATE (TGL): UTERUS	UTERUS - No Corollary change detected
CERVIX - WITHOUT FLUID;	CERVIX - No Corollary change detected

---

Animal ID: 9243 Pathologist: JLQ
Animal Fate: Terminal Kill Days on Test: 42

Reference to Necropsy Record:	Related Histopathology:
KIDNEYS - DILATION; RIGHT; SEVERE (TGL): RENAL PELVIS	KIDNEYS - Hydronephrosis

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Animal ID: 9246 Pathologist: JLQ
Animal Fate: Terminal Kill Days on Test: 43

Reference to Necropsy Record:	Related Histopathology:
OVARIES - DISCOLORATION; RED; LEFT; FOCAL (TGL)	OVARIES - No Corollary change detected

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PUBERTAL TOXICITY STUDY OF VINCLOZOLIN, FLUTAMIDE AND PHENOBARBITAL IN MALE SPRAGUE DAWLEY RATS AND METHOXYCHLOR, ETHINYL ESTRADIOL AND PHENOBARBITAL IN FEMALE SPRAGUE DAWLEY RATS WHEN ADMINISTERED IN CORN OIL BY ORAL GAVAGE

CORRELATION OF GROSS & MICRO

STUDY ID: 7244-600 STUDY NUMBER: 7244600
SEX: FEMALE GROUP: 6: 50 mg/kg Methoxychlor

Animal ID: 9248 Pathologist: JLQ
Animal Fate: Terminal Kill Days on Test: 43

Reference to Necropsy Record:	Related Histopathology:
UTERUS - WITHOUT FLUID; DISTENSION; BILATERAL; MODERATE (TGL): UTERUS	UTERUS - No Corollary change detected
CERVIX - WITHOUT FLUID;	CERVIX - No Corollary change detected

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PUBERTAL TOXICITY STUDY OF VINCLOZOLIN, FLUTAMIDE AND PHENOBARBITAL IN MALE SPRAGUE DAWLEY RATS AND METHOXYCHLOR, ETHINYL ESTRADIOL AND PHENOBARBITAL IN FEMALE SPRAGUE DAWLEY RATS WHEN ADMINISTERED IN CORN OIL BY ORAL GAVAGE

CORRELATION OF GROSS & MICRO

STUDY ID: 7244-600 STUDY NUMBER: 7244600
SEX: FEMALE GROUP: 7: 25 mg/kg Phenobarbital

Animal ID: 9251 Pathologist: JLQ
Animal Fate: Terminal Kill Days on Test: 42

Reference to Necropsy Record:	Related Histopathology:
KIDNEYS - DISCOLORATION; DARK; LEFT; SINGLE (TGL): PINPOINT	KIDNEYS - No Corollary change detected

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Animal ID: 9252 Pathologist: JLQ
Animal Fate: Terminal Kill Days on Test: 42

Reference to Necropsy Record:	Related Histopathology:
KIDNEYS - DILATION; RIGHT; PRESENT, NO GRADE ASSIGNED (TGL): RENAL PELVIS	KIDNEYS - Hydronephrosis

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Animal ID: 9253 Pathologist: JLQ
Animal Fate: Terminal Kill Days on Test: 42

Reference to Necropsy Record:	Related Histopathology:
TAIL - ABRASION(S); SINGLE; PRESENT, NO GRADE ASSIGNED (TGL): TIP	TAIL - Necrosis, tail tip
UTERUS - WITHOUT FLUID; DISTENSION; BILATERAL; MODERATE (TGL): UTERUS	UTERUS - No Corollary change detected
CERVIX - WITHOUT FLUID;	CERVIX - No Corollary change detected

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CORRELATION OF GROSS & MICRO

STUDY ID: 7244-600 STUDY NUMBER: 7244600
SEX: FEMALE GROUP: 7: 25 mg/kg Phenobarbital

Animal ID: 9256 Pathologist: JLQ
Animal Fate: Terminal Kill Days on Test: 42

Reference to Necropsy Record:	Related Histopathology:
UTERUS - WITHOUT FLUID; DISTENSION; BILATERAL; MODERATE (TGL): UTERUS	UTERUS - No Corollary change detected
CERVIX - WITHOUT FLUID;	CERVIX - No Corollary change detected

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Animal ID: 9259 Pathologist: JLQ
Animal Fate: Terminal Kill Days on Test: 43

Reference to Necropsy Record:	Related Histopathology:
UTERUS - WITHOUT FLUID; DISTENSION; BILATERAL; MINIMAL (TGL): UTERUS	UTERUS - No Corollary change detected
CERVIX - WITHOUT FLUID;	CERVIX - No Corollary change detected

---

Animal ID: 9260 Pathologist: JLQ
Animal Fate: Terminal Kill Days on Test: 43

Reference to Necropsy Record:	Related Histopathology:
KIDNEYS - DISCOLORATION; DARK; BILATERAL; MULITFOCAL (TGL)	KIDNEYS - Fibrosis, interstitial

---

Animal ID: 9262 Pathologist: JLQ
Animal Fate: Terminal Kill Days on Test: 43

Reference to Necropsy Record:	Related Histopathology:
ADRENAL GLANDS - ENLARGEMENT; RIGHT (TGL): 5X5X5MM	ADRENAL GLANDS - No Corollary change detected

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CORRELATION OF GROSS & MICRO

STUDY ID: 7244-600 STUDY NUMBER: 7244600
SEX: FEMALE GROUP: 7: 25 mg/kg Phenobarbital

Animal ID: 9262 Pathologist: JLQ
Animal Fate: Terminal Kill Days on Test: 43

Reference to Necropsy Record:	Related Histopathology:
KIDNEYS - CYST(S); CLEAR; RIGHT; SINGLE (TGL): 40X25X20MM	KIDNEYS - Cyst, cortical
KIDNEYS - DILATION; LEFT; MINIMAL (TGL)	KIDNEYS - Hydronephrosis
OVARIES - DISCOLORATION; RED; BILATERAL; MULTIFOCAL (TGL)	OVARIES - Hemorrhage/fluid, bursa
URETER - DILATION; RIGHT; SEVERE (TGL)	URETER - Dilation

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CORRELATION OF GROSS & MICRO

STUDY ID: 7244-600 STUDY NUMBER: 7244600
SEX: FEMALE GROUP: 8: 50 mg/kg Phenobarbital

Animal ID: 9269 Pathologist: JLQ
Animal Fate: Terminal Kill Days on Test: 42

Reference to Necropsy Record:	Related Histopathology:
OVARIES - CYST(S); CLEAR; RIGHT; SINGLE; MINIMAL (TGL): 3X3X3MM	OVARIES - Cyst, oviduct

---

Animal ID: 9272 Pathologist: JLQ
Animal Fate: Terminal Kill Days on Test: 42

Reference to Necropsy Record:	Related Histopathology:
KIDNEYS - DILATION; RIGHT; MODERATE (TGL): RENAL PELVIS	KIDNEYS - Hydronephrosis

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Animal ID: 9274 Pathologist: JLQ
Animal Fate: Terminal Kill Days on Test: 43

Reference to Necropsy Record:	Related Histopathology:
KIDNEYS - DISCOLORATION; DARK; BILATERAL; MULTIFOCAL (TGL)	KIDNEYS - No Corollary change detected

---

Animal ID: 9275 Pathologist: JLQ
Animal Fate: Terminal Kill Days on Test: 43

Reference to Necropsy Record:	Related Histopathology:
KIDNEYS - DILATION; RIGHT; MODERATE (TGL): RENAL PELVIS	KIDNEYS - Hydronephrosis
UTERUS - WITHOUT FLUID; DISTENSION; BILATERAL; MINIMAL (TGL): UTERUS	UTERUS - No Corollary change detected
CERVIX - WITHOUT FLUID;	CERVIX - No Corollary change detected

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PUBERTAL TOXICITY STUDY OF VINCLOZOLIN, FLUTAMIDE AND PHENOBARBITAL IN MALE SPRAGUE DAWLEY RATS AND METHOXYCHLOR, ETHINYL ESTRADIOL AND PHENOBARBITAL IN FEMALE SPRAGUE DAWLEY RATS WHEN ADMINISTERED IN CORN OIL BY ORAL GAVAGE

CORRELATION OF GROSS & MICRO

STUDY ID: 7244-600 STUDY NUMBER: 7244600
SEX: FEMALE GROUP: 8: 50 mg/kg Phenobarbital

Animal ID: 9276 Pathologist: JLQ
Animal Fate: Terminal Kill Days on Test: 43

Reference to Necropsy Record:	Related Histopathology:
KIDNEYS - DISCOLORATION; WHITE; BILATERAL; MULTIFOCAL (TGL)	KIDNEYS - Infiltrate, mixed cell; Infiltrate, mixed cell, renal pelvis
SPLEEN - ENLARGEMENT (TGL): 40X14X5MM	SPLEEN - Congestion
URINARY BLADDER - CALCULUS(I); WHITE; MULTIPLE; PRESENT, NO GRADE ASSIGNED (TGL)	URINARY BLADDER - Infiltrate, mixed cell; Hyperplasia, epithelium
UTERUS - WITHOUT FLUID; DISTENSION; BILATERAL; MINIMAL (TGL): UTERUS	UTERUS - No Corollary change detected
CERVIX - WITHOUT FLUID;	CERVIX - No Corollary change detected

---

Animal ID: 9278 Pathologist: JLQ
Animal Fate: Terminal Kill Days on Test: 43

Reference to Necropsy Record:	Related Histopathology:
UTERUS - WITHOUT FLUID; DISTENSION; BILATERAL; MODERATE (TGL): UTERUS	UTERUS - No Corollary change detected
CERVIX - WITHOUT FLUID;	CERVIX - No Corollary change detected

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PATHOLOGY ASSOCIATES

PUBERTAL TOXICITY STUDY OF VINCLOZOLIN, FLUTAMIDE AND PHENOBARBITAL IN MALE SPRAGUE DAWLEY RATS AND METHOXYCHLOR, ETHINYL ESTRADIOL AND PHENOBARBITAL IN FEMALE SPRAGUE DAWLEY RATS WHEN ADMINISTERED IN CORN OIL BY ORAL GAVAGE

CORRELATION OF GROSS & MICRO

STUDY ID: 7244-600 STUDY NUMBER: 7244600
SEX: FEMALE GROUP: 9: 100 mg/kg Phenobarbital

Animal ID: 9283 Pathologist: JLQ
Animal Fate: Terminal Kill Days on Test: 42

Reference to Necropsy Record:	Related Histopathology:
KIDNEYS - DILATION; RIGHT; MINIMAL (TGL): RENAL PELVIS	KIDNEYS - Hydronephrosis

---

Animal ID: 9285 Pathologist: JLQ
Animal Fate: Terminal Kill Days on Test: 42

Reference to Necropsy Record:	Related Histopathology:
UTERUS - WITHOUT FLUID; DISTENSION; BILATERAL; MODERATE (TGL): UTERUS	UTERUS - No Corollary change detected
CERVIX - WITHOUT FLUID;	CERVIX - No Corollary change detected

---

Animal ID: 9286 Pathologist: JLQ
Animal Fate: Terminal Kill Days on Test: 42

Reference to Necropsy Record:	Related Histopathology:
UTERUS - WITHOUT FLUID; DISTENSION; BILATERAL; MODERATE (TGL): UTERUS	UTERUS - No Corollary change detected
CERVIX - WITHOUT FLUID;	CERVIX - No Corollary change detected

---

Animal ID: 9291 Pathologist: JLQ
Animal Fate: Terminal Kill Days on Test: 43

Reference to Necropsy Record:	Related Histopathology:
KIDNEYS - ENLARGEMENT; BILATERAL; MODERATE (TGL)	KIDNEYS - Hydronephrosis
KIDNEYS - DILATION; BILATERAL; SEVERE (TGL)	KIDNEYS - Hydronephrosis

---

THERIMUNE RESEARCH CORPORATION STUDY NUMBER 7244-600

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PATHOLOGY ASSOCIATES

PUBERTAL TOXICITY STUDY OF VINCLOZOLIN, FLUTAMIDE AND PHENOBARBITAL IN MALE SPRAGUE DAWLEY RATS AND METHOXYCHLOR, ETHINYL ESTRADIOL AND PHENOBARBITAL IN FEMALE SPRAGUE DAWLEY RATS WHEN ADMINISTERED IN CORN OIL BY ORAL GAVAGE

CORRELATION OF GROSS & MICRO

STUDY ID: 7244-600 STUDY NUMBER: 7244600
SEX: FEMALE GROUP: 9: 100 mg/kg Phenobarbital

Animal ID: 9291 Pathologist: JLQ
Animal Fate: Terminal Kill Days on Test: 43

Reference to Necropsy Record:	Related Histopathology:
URINARY BLADDER - THICKENING; SEVERE (TGL)	URINARY BLADDER - Hyperplasia, epithelium; Fibrosis, sub-epithelial
URINARY BLADDER - CALCULUS(I); WHITE; MULTIPLE; PRESENT, NO GRADE ASSIGNED (TGL)	URINARY BLADDER - Calculi

---

Animal ID: 9292 Pathologist: JLQ
Animal Fate: Terminal Kill Days on Test: 43

Reference to Necropsy Record:	Related Histopathology:
OVARIES - DISCOLORATION; RED; BILATERAL; MULTIFOCAL (TGL)	OVARIES - No Corollary change detected

---

Animal ID: 9293 Pathologist: JLQ
Animal Fate: Terminal Kill Days on Test: 43

Reference to Necropsy Record:	Related Histopathology:
KIDNEYS - DILATION; RIGHT; MINIMAL (TGL): RENAL PELVIS	KIDNEYS - Hydronephrosis

---

Animal ID: 9294 Pathologist: JLQ
Animal Fate: Terminal Kill Days on Test: 43

Reference to Necropsy Record:	Related Histopathology:
UTERUS - WITHOUT FLUID; DISTENSION; BILATERAL; MODERATE (TGL): UTERUS	UTERUS - No Corollary change detected

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THERIMUNE RESEARCH CORPORATION STUDY NUMBER 7244-600

LABCAT HP4.33 14-FEB-2003

PATHOLOGY ASSOCIATES

PUBERTAL TOXICITY STUDY OF VINCLOZOLIN, FLUTAMIDE AND PHENOBARBITAL IN MALE SPRAGUE DAWLEY RATS AND METHOXYCHLOR, ETHINYL ESTRADIOL AND PHENOBARBITAL IN FEMALE SPRAGUE DAWLEY RATS WHEN ADMINISTERED IN CORN OIL BY ORAL GAVAGE

CORRELATION OF GROSS & MICRO

STUDY ID: 7244-600 STUDY NUMBER: 7244600
SEX: FEMALE GROUP: 9: 100 mg/kg Phenobarbital

Animal ID: 9294 Pathologist: JLQ
Animal Fate: Terminal Kill Days on Test: 43

Reference to Necropsy Record:	Related Histopathology:
CERVIX - WITHOUT FLUID;	CERVIX - No Corollary change detected

---

THERIMUNE RESEARCH CORPORATION STUDY NUMBER 7244-600

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V. Comment Report

PATHOLOGY ASSOCIATES

PUBERTAL TOXICITY STUDY OF VINCLOZOLIN, FLUTAMIDE AND PHENOBARBITAL IN MALE SPRAGUE DAWLEY RATS AND METHOXYCHLOR, ETHINYL ESTRADIOL AND PHENOBARBITAL IN FEMALE SPRAGUE DAWLEY RATS WHEN ADMINISTERED IN CORN OIL BY ORAL GAVAGE

COMMENT REPORT

STUDY ID: 7244-600 STUDY NUMBER: 7244600

SEX: MALE GROUP: 1: Corn Oil Vehicle

No Comments for any animal in this group

PATHOLOGY ASSOCIATES

PUBERTAL TOXICITY STUDY OF VINCLOZOLIN, FLUTAMIDE AND PHENOBARBITAL IN MALE SPRAGUE DAWLEY RATS AND METHOXYCHLOR, ETHINYL ESTRADIOL AND PHENOBARBITAL IN FEMALE SPRAGUE DAWLEY RATS WHEN ADMINISTERED IN CORN OIL BY ORAL GAVAGE

COMMENT REPORT

STUDY ID: 7244-600 STUDY NUMBER: 7244600

SEX: MALE GROUP: 2: 25 mg/kg Phenobarbital

No Comments for any animal in this group

THERIMUNE RESEARCH CORPORATION STUDY NUMBER 7244-600

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PATHOLOGY ASSOCIATES

PUBERTAL TOXICITY STUDY OF VINCLOZOLIN, FLUTAMIDE AND PHENOBARBITAL IN MALE SPRAGUE DAWLEY RATS AND METHOXYCHLOR, ETHINYL ESTRADIOL AND PHENOBARBITAL IN FEMALE SPRAGUE DAWLEY RATS WHEN ADMINISTERED IN CORN OIL BY ORAL GAVAGE

COMMENT REPORT

STUDY ID: 7244-600 STUDY NUMBER: 7244600

SEX: MALE GROUP: 3: 50 mg/kg Phenobarbital

No Comments for any animal in this group

PATHOLOGY ASSOCIATES

PUBERTAL TOXICITY STUDY OF VINCLOZOLIN, FLUTAMIDE AND PHENOBARBITAL IN MALE SPRAGUE DAWLEY RATS AND METHOXYCHLOR, ETHINYL ESTRADIOL AND PHENOBARBITAL IN FEMALE SPRAGUE DAWLEY RATS WHEN ADMINISTERED IN CORN OIL BY ORAL GAVAGE

COMMENT REPORT

STUDY ID: 7244-600 STUDY NUMBER: 7244600

SEX: MALE GROUP: 4: 100 mg/kg Phenobarbital

No Comments for any animal in this group

PATHOLOGY ASSOCIATES

PUBERTAL TOXICITY STUDY OF VINCLOZOLIN, FLUTAMIDE AND PHENOBARBITAL IN MALE SPRAGUE DAWLEY RATS AND METHOXYCHLOR, ETHINYL ESTRADIOL AND PHENOBARBITAL IN FEMALE SPRAGUE DAWLEY RATS WHEN ADMINISTERED IN CORN OIL BY ORAL GAVAGE

COMMENT REPORT

STUDY ID: 7244-600 STUDY NUMBER: 7244600

SEX: MALE GROUP: 5: 10 mg/kg Vinclozolin

No Comments for any animal in this group

PATHOLOGY ASSOCIATES

PUBERTAL TOXICITY STUDY OF VINCLOZOLIN, FLUTAMIDE AND PHENOBARBITAL IN MALE SPRAGUE DAWLEY RATS AND METHOXYCHLOR, ETHINYL ESTRADIOL AND PHENOBARBITAL IN FEMALE SPRAGUE DAWLEY RATS WHEN ADMINISTERED IN CORN OIL BY ORAL GAVAGE

COMMENT REPORT

STUDY ID: 7244-600 STUDY NUMBER: 7244600

SEX: MALE GROUP: 6: 30 mg/kg Vinclozolin

No Comments for any animal in this group

THERIMUNE RESEARCH CORPORATION STUDY NUMBER 7244-600

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PATHOLOGY ASSOCIATES

PUBERTAL TOXICITY STUDY OF VINCLOZOLIN, FLUTAMIDE AND PHENOBARBITAL IN MALE SPRAGUE DAWLEY RATS AND METHOXYCHLOR, ETHINYL ESTRADIOL AND PHENOBARBITAL IN FEMALE SPRAGUE DAWLEY RATS WHEN ADMINISTERED IN CORN OIL BY ORAL GAVAGE

COMMENT REPORT

STUDY ID: 7244-600 STUDY NUMBER: 7244600

SEX: MALE GROUP: 7: 100 mg/kg Vinclozolin

No Comments for any animal in this group

PATHOLOGY ASSOCIATES

PUBERTAL TOXICITY STUDY OF VINCLOZOLIN, FLUTAMIDE AND PHENOBARBITAL IN MALE SPRAGUE DAWLEY RATS AND METHOXYCHLOR, ETHINYL ESTRADIOL AND PHENOBARBITAL IN FEMALE SPRAGUE DAWLEY RATS WHEN ADMINISTERED IN CORN OIL BY ORAL GAVAGE

COMMENT REPORT

STUDY ID: 7244-600 STUDY NUMBER: 7244600

SEX: MALE GROUP: 8: 25 mg/kg Flutamide

No Comments for any animal in this group

THERIMUNE RESEARCH CORPORATION STUDY NUMBER 7244-600

LABCAT HP4.33 14-FEB-2003

PATHOLOGY ASSOCIATES

PUBERTAL TOXICITY STUDY OF VINCLOZOLIN, FLUTAMIDE AND PHENOBARBITAL IN MALE SPRAGUE DAWLEY RATS AND METHOXYCHLOR, ETHINYL ESTRADIOL AND PHENOBARBITAL IN FEMALE SPRAGUE DAWLEY RATS WHEN ADMINISTERED IN CORN OIL BY ORAL GAVAGE

COMMENT REPORT

STUDY ID: 7244-600 STUDY NUMBER: 7244600

SEX: MALE GROUP: 9: 50 mg/kg Flutamide

No Comments for any animal in this group

THERIMUNE RESEARCH CORPORATION STUDY NUMBER 7244-600

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PATHOLOGY ASSOCIATES

PUBERTAL TOXICITY STUDY OF VINCLOZOLIN, FLUTAMIDE AND PHENOBARBITAL IN MALE SPRAGUE DAWLEY RATS AND METHOXYCHLOR, ETHINYL ESTRADIOL AND PHENOBARBITAL IN FEMALE SPRAGUE DAWLEY RATS WHEN ADMINISTERED IN CORN OIL BY ORAL GAVAGE

COMMENT REPORT

STUDY ID: 7244-600 STUDY NUMBER: 7244600

SEX: FEMALE GROUP: 1: Corn Oil Vehicle

No Comments for any animal in this group

PATHOLOGY ASSOCIATES

PUBERTAL TOXICITY STUDY OF VINCLOZOLIN, FLUTAMIDE AND PHENOBARBITAL IN MALE SPRAGUE DAWLEY RATS AND METHOXYCHLOR, ETHINYL ESTRADIOL AND PHENOBARBITAL IN FEMALE SPRAGUE DAWLEY RATS WHEN ADMINISTERED IN CORN OIL BY ORAL GAVAGE

COMMENT REPORT

STUDY ID: 7244-600 STUDY NUMBER: 7244600

SEX: FEMALE GROUP: 2: 0.0025 mg/kg Ethinyl Estradiol

No Comments for any animal in this group

THERIMUNE RESEARCH CORPORATION STUDY NUMBER 7244-600

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PATHOLOGY ASSOCIATES

PUBERTAL TOXICITY STUDY OF VINCLOZOLIN, FLUTAMIDE AND PHENOBARBITAL IN MALE SPRAGUE DAWLEY RATS AND METHOXYCHLOR, ETHINYL ESTRADIOL AND PHENOBARBITAL IN FEMALE SPRAGUE DAWLEY RATS WHEN ADMINISTERED IN CORN OIL BY ORAL GAVAGE

COMMENT REPORT

STUDY ID: 7244-600 STUDY NUMBER: 7244600

SEX: FEMALE GROUP: 3: 0.005 mg/kg Ethinyl Estradiol

No Comments for any animal in this group

PATHOLOGY ASSOCIATES

PUBERTAL TOXICITY STUDY OF VINCLOZOLIN, FLUTAMIDE AND PHENOBARBITAL IN MALE SPRAGUE DAWLEY RATS AND METHOXYCHLOR, ETHINYL ESTRADIOL AND PHENOBARBITAL IN FEMALE SPRAGUE DAWLEY RATS WHEN ADMINISTERED IN CORN OIL BY ORAL GAVAGE

COMMENT REPORT

STUDY ID: 7244-600 STUDY NUMBER: 7244600

SEX: FEMALE GROUP: 4: 12.5 mg/kg Methoxychlor

No Comments for any animal in this group

THERIMUNE RESEARCH CORPORATION STUDY NUMBER 7244-600

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PATHOLOGY ASSOCIATES

PUBERTAL TOXICITY STUDY OF VINCLOZOLIN, FLUTAMIDE AND PHENOBARBITAL IN MALE SPRAGUE DAWLEY RATS AND METHOXYCHLOR, ETHINYL ESTRADIOL AND PHENOBARBITAL IN FEMALE SPRAGUE DAWLEY RATS WHEN ADMINISTERED IN CORN OIL BY ORAL GAVAGE

COMMENT REPORT

STUDY ID: 7244-600 STUDY NUMBER: 7244600

SEX: FEMALE GROUP: 5: 25 mg/kg Methoxychlor

No Comments for any animal in this group

PATHOLOGY ASSOCIATES

PUBERTAL TOXICITY STUDY OF VINCLOZOLIN, FLUTAMIDE AND PHENOBARBITAL IN MALE SPRAGUE DAWLEY RATS AND METHOXYCHLOR, ETHINYL ESTRADIOL AND PHENOBARBITAL IN FEMALE SPRAGUE DAWLEY RATS WHEN ADMINISTERED IN CORN OIL BY ORAL GAVAGE

COMMENT REPORT

STUDY ID: 7244-600 STUDY NUMBER: 7244600

SEX: FEMALE GROUP: 6: 50 mg/kg Methoxychlor

No Comments for any animal in this group

PATHOLOGY ASSOCIATES

PUBERTAL TOXICITY STUDY OF VINCLOZOLIN, FLUTAMIDE AND PHENOBARBITAL IN MALE SPRAGUE DAWLEY RATS AND METHOXYCHLOR, ETHINYL ESTRADIOL AND PHENOBARBITAL IN FEMALE SPRAGUE DAWLEY RATS WHEN ADMINISTERED IN CORN OIL BY ORAL GAVAGE

COMMENT REPORT

STUDY ID: 7244-600 STUDY NUMBER: 7244600
SEX: FEMALE GROUP: 7: 25 mg/kg Phenobarbital

Animal ID: 9262 Pathologist: JLQ
Animal Fate: Terminal Kill Days on Test: 43

TISSUE COMMENTS:
Non-Protocol Tissues:
ADRENAL GLANDS - The adrenal gland is normal. It is surrounded by abundant and closely adherent brown fat, which most likely elicited the gross observation of enlargement.

PATHOLOGY ASSOCIATES

PUBERTAL TOXICITY STUDY OF VINCLOZOLIN, FLUTAMIDE AND PHENOBARBITAL IN MALE SPRAGUE DAWLEY RATS AND METHOXYCHLOR, ETHINYL ESTRADIOL AND PHENOBARBITAL IN FEMALE SPRAGUE DAWLEY RATS WHEN ADMINISTERED IN CORN OIL BY ORAL GAVAGE

COMMENT REPORT

STUDY ID: 7244-600 STUDY NUMBER: 7244600
SEX: FEMALE GROUP: 8: 50 mg/kg Phenobarbital

Animal ID: 9276 Pathologist: JLQ
Animal Fate: Terminal Kill Days on Test: 43

TISSUE COMMENTS:
Non-Protocol Tissues:
URINARY BLADDER - Multiple calculi were observed in the urinary bladder at gross necropsy. The mixed inflammatory cell infiltrate and hyperplasia of the urinary epithelium is consistent with this observation.

PATHOLOGY ASSOCIATES

PUBERTAL TOXICITY STUDY OF VINCLOZOLIN, FLUTAMIDE AND PHENOBARBITAL IN MALE SPRAGUE DAWLEY RATS AND METHOXYCHLOR, ETHINYL ESTRADIOL AND PHENOBARBITAL IN FEMALE SPRAGUE DAWLEY RATS WHEN ADMINISTERED IN CORN OIL BY ORAL GAVAGE

COMMENT REPORT

STUDY ID: 7244-600 STUDY NUMBER: 7244600

SEX: FEMALE GROUP: 9: 100 mg/kg Phenobarbital

No Comments for any animal in this group

THERIMUNE RESEARCH CORPORATION STUDY NUMBER 7244-600

LABCAT HP4.33 (END OF REPORT) 14-FEB-2003

VI. Quality Assurance Statement

CHARLES RIVER
LABORATORIES
Discovery and Development Services
Pathology Associates Division

Pathology Report

Pubertal Toxicity Study of Vinclozolin, Flutamide and Phenobarbital in Male Sprague Dawley Rats and Methoxychlor, Ethinyl Estradiol and Phenobarbital in Female Sprague Dawley Rats When Administered in Corn Oil by Oral Gavage

TherImmune Research Corporation Study Number: 7244-600

QUALITY ASSURANCE STATEMENT

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

Date of Inspection	Phase Inspected	Date Findings Reported to Study Pathologist/ PAI Management	Date Findings Reported to Study Director/Study Director Management
01/15/03	Tissue Trimming	01/22/03	01/22/03
02/13,14/03	Individual Animal Data	02/14/03	02/14/03
02/13,14/03	Draft Pathology Report	02/14/03	02/14/03
05/27/04	Final Pathology Report	05/27/04	05/27/04

Karen E. Butler
Quality Assurance Manager

5/27/04
Date

Appendix 18
Protocol, Amendments, and Deviations
Pubertal Toxicity Study of Vinclozolin, Flutamide and Phenobarbital in Male Sprague Dawley Rats and Methoxychlor, Ethinyl Estradiol and Phenobarbital in Female Sprague Dawley Rats when Administered in Corn Oil by Oral Gavage

MSR 8·13·02

THERIMMUNE
Research Corporation

NTP/NIEHS CONTRACT NO.:N01-ES-75409

Pubertal Vinclozolin Study	RACB 20104
Pubertal Methoxychlor Study	RACB 20103
Pubertal Flutamide Study	RACB 20105
Pubertal Ethinyl Estradiol Study	RACB 20106
Pubertal Phenobarbital Study	RACB 20203

PUBERTAL TOXICITY STUDY OF VINCLOZOLIN, FLUTAMIDE AND PHENOBARBITAL IN MALE SPRAGUE DAWLEY RATS AND METHOXYCHLOR, ETHINYL ESTRADIOL AND PHENOBARBITAL IN FEMALE SPRAGUE DAWLEY RATS WHEN ADMINISTERED IN CORN OIL BY ORAL GAVAGE

Sponsor: National Toxicology Program (NTP)
National Institute of Environmental Health Sciences (NIEHS)
P.O. Box 12233
Research Triangle Park, North Carolina 27709

Testing Facility: TherImmune Research Corporation (TherImmune)
15 Firstfield Road
Gaithersburg, Maryland 20878

Approved by:

TherImmune:

Meredith S. Rocca 8·13·02
Meredith Rocca, Ph.D.
Study Director/Date

Gary W. Wolfe 8/12/02
Gary W. Wolfe, Ph.D., D.A.B.T.
Principal Investigator/Date

Larissa B. Nehrebeckyj 8/12/02
Larissa B.Nehrebeckyj, B.S.
Reproductive Toxicologist/Date

NIEHS:

Jack B. Bishop 8/7/02
Jack Bishop, Ph.D.
Project Officer/Date

Aug 8·13·02

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Aug 8.13.02

PUBERTAL TOXICITY STUDY OF VINCLOZOLIN, FLUTAMIDE AND PHENOBARBITAL IN MALE SPRAGUE DAWLEY RATS AND METHOXYCHLOR, ETHINYL ESTRADIOL AND PHENOBARBITAL IN FEMALE SPRAGUE DAWLEY RATS WHEN ADMINISTERED IN CORN OIL BY ORAL GAVAGE

1.0 INTRODUCTION

1.1 Proposed Investigations/Rationale for Dose Selection

Puberty is a time of rapid interactive endocrine and morphological changes, and numerous pharmaceutical and environmental agents have been shown to alter the timing of pubertal development in mammals. The purpose of the current study is to provide data on proposed procedures to evaluate the effects of Vinclozolin, Methoxychlor, Flutamide, Estradiol, and Phenobarbital on pubertal development in the intact juvenile/peripubertal male and female rat. The proposed design will detect agents that have antithyroid, estrogenic, androgenic, or antiandrogenic activity, or alter puberty by changing follicle stimulating hormone (FSH), luteinizing hormone (LH), prolactin, growth hormone (GH), or hypothalamic function (Stoker *et al.*, 2000 and Goldman *et al.*, 2000).

Vinclozolin, 3-(3,5-dichlorophenyl)-5-methyl-5-vinylox-azolidine-2,4-dione, is a fungicide used on fruits, vegetables, turfgrass, and ornamental plants (U.S. EPA, 1998). *In vivo*, vinclozolin inhibits androgen receptor (AR)-dependent gene expression (Kelce *et al.*, 1997) and produces a spectrum of anatomical defects. Administration of vinclozolin (400 mg/kg) to rats on gestational day (GD) 14 through postnatal day (PND) 3 resulted in effects similar to those caused by flutamide, a well known AR antagonist. These effects included reduced anogenital distance (AGD); persistent nipples; cleft phallus; hypospadias; reduced weights of the ventral prostate, seminal vesicles, and epididymis; and reduced sperm count (Gray *et al.*, 1994; 1999a). Exposing weanling male rats to the antiandrogenic pesticides p,p'- DDE or vinclozolin delayed pubertal development as indicated by delayed preputial separation and increases body weight at puberty. In contrast to delays associated with exposure to estrogenic substances, antiandrogens do not inhibit food consumption or retard growth (Anderson *et al.*, 1995b).

Flutamide (4'-nitro-3'-trifluoromethyl-isobutyranilide) is a potent nonsteroidal androgen receptor antagonist that has been used therapeutically to treat androgen-dependent prostrate cancer (Delaere and Van Thillo, 1991; Murphy *et al.*, 1991) and as a tool to study male reproductive development. Studies in rats have demonstrated that pre- or postnatal flutamide (6.25 to 50 mg/kg) exposure alters androgen-dependent reproductive development (Imperato-McGinley *et al.*, 1992; Kassim *et al.*, 1997) and has been shown to produce decreased reproductive organ weights, feminization of male external genitalia, altered androgen-dependent testicular descent, and retention of nipples when male offspring are exposed *in utero* (Imperato-McGinley *et al.*, 1992).

Methoxychlor has been used for nearly 50 years for insect and larval control. Its advantage over DDT is that the methoxychlor is more readily metabolized and excreted by mammalian systems (Kapoor *et al.*, 1970) and is therefore less likely to undergo bioconcentration than is DDT. This metabolism also yields mono- and bis-hydroxy metabolites of methoxychlor (Bulger *et al.*, 1978), which helps explain both the uterotrophic effects noted earlier for methoxychlor (Tullner, 1961) and the observations that methoxychlor *in vivo* reduced the uterine uptake of radiolabeled estradiol (Welch *et al.*, 1969). Treatment of rats with methoxychlor at 5, 50, or 150 mg/kg for the week before and the week after birth to PND 7 resulted in unchanged anogenital distance, accelerated vaginal opening, and delayed prepuce separation, and at 50 and 150 mg/kg disrupted adult estrous cyclicity and reduced epididymal sperm counts and testis weights.

As cited in Goodman and Gilman's *Pharmacological Basis of Therapeutics* (1996), estrogens are among the most commonly prescribed drugs in the United States. The two major uses are as components of combination oral contraceptives and hormone replacement therapy. The pharmacological considerations for estrogen use in oral contraceptives as opposed to postmenopausal hormone replacement are substantially different, primarily because of the doses used. Historically, conjugated estrogens have been the most common agents for postmenopausal use, and 625 μg/kg/day is effective in most women. In contrast, most combination oral contraceptives in current use employ 20 to 35 μg/day of ethinyl estradiol. Conjugated estrogens and

ethinyl estradiol differ widely in their oral potencies; for example, a dose of 625 μg of conjugated estrogens generally is considered equivalent to 5 to 10 μg of ethinyl estradiol.

Several authors have demonstrated estrogenic responses to ethinyl estradiol in rodents. Laws *et al.* (2000) showed that *in vivo* studies indicated that the 3-day uterotrophic assay in prepubertal rats was best for detecting estrogenic activity when compared with all other models, based upon the dose-response data for ethinyl estradiol (0.01-0.1 mg/kg, oral), 4-tert-octylphenol (50-200 mg/kg, oral), and 4-nonylphenol (25-100 mg/kg, oral). Although oral doses of ethinyl estradiol (0.01 mg/kg) induced a significant increase in uterine weight in the prepubertal rat, this dose was ineffective for stimulating a similar response in ovariectomized adult rats. The age of vaginal opening was advanced following oral exposure from postnatal days 21-35 to ethinyl estradiol (0.01 mg/kg). Ethinyl estradiol advanced the day of vaginal opening by 6.0 ± 0.18 days (30.6 days in control vs. 24.6 days in treated). In addition, the number of 4-5 day estrous cycles was reduced during a 25 day exposure to ethinyl estradiol (0.01 mg/kg). Advanced vaginal opening was also demonstrated by Odum *et al.* (1997) using doses of 2-400 μg/kg/day, subcutaneous, and Singh and Kamgoj (1980) using doses of 5 μg/kg/day for 5 days. Singh and Kamboj (1980) also showed an advance in the appearance of cornified vaginal cells.

Phenobarbital is a commonly prescribed antiepileptic agent whose hepatotoxicity and effects on the thyroid have been established. Endpoints of reproductive and developmental toxicity have not been as well explored, although some data are available. Gupta *et al.* (1980) evaluated the reproductive and developmental toxicity of phenobarbital in male rats. Males were exposed *in utero* from GD 12-19 (40 mg/kg-day administered to the dam). Treatment-related effects included reduced anogenital distance; delayed testes descent; decreased seminal vesicles weight; and reduced fertility. Both serum testosterone and leutinizing hormone were decreased in the exposed males. However, the age at onset of puberty (i.e., age at preputial separation) was not affected by phenobarbital exposure. In a similar study (Gupta and Yaffe, 1981), female rats were exposed to phenobarbital *in utero* (40 mg/kg-day administered to the dam) or immediately following birth (20 mg/kg-day on PND 1-8). Phenobarbital treatment resulted in delayed vaginal opening (34.6 ± 1.2 days in

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the control vs. 37.5 ± 1.2 days in the treated group); disruptions in the estrous cycle (only 40% of treated rats displayed normal estrous cyclicity, compared to 91% of control rats); and impaired fertility (50% of treated rats were fertile, and 100% of the control rats were fertile). These effects were observed during critical periods for neuroendocrine development: GD 17-20 and PND 1-8 (Gupta and Yaffe, 1981). Whether or not similar effects occur outside of these periods is unknown at this time; data generated in this study should help to address this question.

Based on the above-cited literature, the hypotheses of the current study are: 1. ethinyl estradiol and methoxychlor administration to juvenile female rats will result in advanced vaginal opening, advanced first estrous and onset of estrous cycles, and/or persistent vaginal estrus; 2. administration of vinclozolin and flutamide to juvenile male rats will result in delayed preputial separation, decreased reproductive organ weights, altered external genitalia, and/or retention of nipples; and 3. phenobarbital treatment will result in delayed vaginal opening and irregular estrous cyclicity in juvenile female rats and in reduced reproductive organ weights and possibly delayed preputial separation in juvenile males; phenobarbital treatment may also cause increased thyroid weights and follicular cell hyperplasia, particularly in the males.

1.2 Regulatory Compliance

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

1.3 Quality Assurance

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

1.4 Testing Facility
TherImmune Research Corporation (TherImmune)
15 Firstfield Road
Gaithersburg, MD 20878

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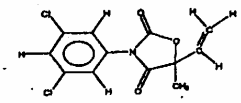
2.0 TEST ARTICLE

2.1 Characterization of Test Articles

2.1.1 Vinclozolin (from MSDS)

Identity:	Vinclozolin
ID No.:	1317B
Source:	Battelle Organic Synthesis Group
CAS No.:	50471-44-8
Lot No.:	102996
Molecular Wt:	286.1g/mol
Formula:	$C_{12}H_9NO_3Cl_2$
Structure:	
Purity:	99.6%

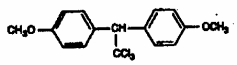
Storage:

Test Article:	Store at room temperature (~25°C) and protected from light.
Formulation:	Stored in sealed amber glass bottles at room temperature (~25°C) and protected from light.

Stability:

Test Article:	Analyze every 24± 2 weeks to verify stability.
Formulation:	Dose formulations (2 mg/mL in corn oil) are stable for 42 days at temperatures of 25°C, 5°C, and -20°C in sealed amber glass bottles and protected from light.

2.1.2 Methoxychlor (from MSDS)

Identity:	Methoxychlor
ID No.:	1321B
Source:	Sigma Chemical Company
CAS No.:	72-43-5
Lot No.:	124F0575
Molecular Wt:	345.7 g/mol
Formula:	$C_{16}H_{15}Cl_3O_2$
Structure:	

Purity: 95%

Storage:

Test Article: Stored in a sealed container under nitrogen and protected from light at ambient temperature (23 to 28°C).

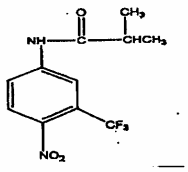
Formulation: Stored in sealed glass vials at refrigerator temperature.

Stability:

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

Formulation: Dose formulations (1.82 mg/mL in corn oil) are stable for 30 days under refrigerated conditions (2 to 5°C) and for 23 days under ambient (23 to 28°C) conditions. Under conditions that simulate animal dosing (room temperature, exposed to air in hood), the dosage formulation showed no appreciable loss.

2.1.3 Flutamide

Identity:	Flutamide
ID No.:	1198E
Source:	Sigma Chemical Company
CAS No.:	13311-84-7
Lot No.:	109H0952
Molecular Wt:	276.2 g/mol
Formula:	$C_{11}H_{11}F_3N_2O_3$
Structure:	
Purity:	99%

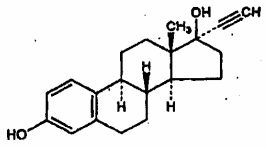
Storage:

Test Article:	Store at room temperature (~25°C) in sealed amber glass bottles.
Formulation:	Store in sealed amber glass bottles at 5°C or –20°C, protected from light.

Stability:

Test Article:	Analyze every 24± 2 weeks to verify stability.
Formulation:	Dose formulations (10 mg/mL in corn oil) are stable for 42 days at 5°C or –20°C, with –20°C being preferable. Under conditions that simulate animal dosing (room temperature, exposed to air in hood), the dosage formulation showed no appreciable loss.

2.1.4 Ethinyl Estradiol

Identity:	Ethinyl estradiol
ID No.:	1318B
Source:	Sigma Chemical Company
CAS No.:	57-63-6
Lot No.:	45H0716
Molecular Wt:	296.44 g/mol
Formula:	$C_{20}H_{24}O_2$
Structure:	
Purity:	99.7%

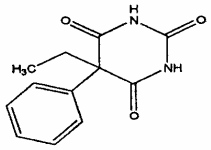
Storage:

Test Article:	Store in sealed amber glass bottles away from light at ambient temperatures (23 to 28°C) under inert headspace (i.e., under nitrogen).
Formulation:	Store in sealed amber glass bottles away from light and refrigerated (~5°C).

Stability:

Test Article:	Analyze every 24± 2 weeks to verify stability.
Formulation:	Dose formulations (1.0 μg/mL in corn oil) are stable for up to 14 days under refrigerated conditions (~5°C). Under conditions that simulate animal dosing (room temperature, exposed to air and light), the dosage formulation showed no appreciable loss.

2.1.5 Phenobarbital

Identity:	Phenobarbital (free acid)
ID No.:	1909A
Source:	Spectrum Chemical Manufacturing Corporation
CAS No.:	50-06-6
Lot No.:	QI0645 (Batch No. 01 – MRI Assigned)
Molecular Wt:	232.23 g/mol
Formula:	$C_{12}H_{12}N_2O_3$
Structure:	
Purity:	>99%
Storage:	
Test Article:	Store in a cool, dry place at ambient temperature. Protect from heat.
Formulation:	Store refrigerated (~5°C) in sealed amber glass bottles protected from light.
Stability:	
Test Article:	Analyze every 24± 2 weeks to verify stability.
Formulation:	Dose formulations (2.5 mg/mL in corn oil) are stable for up to 14 days (refrigerated at ~5°C). Under conditions that simulate animal dosing (room temperature, exposed to air and light), the dosage formulation showed no appreciable loss.

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2.1.6 Certificate of Analysis

Each batch of each test article will be accompanied by a certificate of analysis. The Sponsor will determine for each batch of test article the strength, purity, and composition or other characteristics that appropriately define the test article. A copy of the dose formulation report will be included in the study data.

2.1.7 Bulk Chemical Samples

Prior to use, two 5 g samples of each bulk test article will be collected into a glass bottle with Teflon® coated lids, sealed, and stored in the freezer (~-20°C) protected from light for possible future reanalysis.

A sample of 5 grams will be collected from each bulk test article and sent to a NTP subcontractor for purity and stability testing within 30 days of receipt, and thereafter at 24±2 week intervals. An additional 35 mL aliquot will be sent within 30 days prior to the start of any study.

2.2 Safety and Handling

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

2.2.1 Emergency First Aid Procedures

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

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

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

Ingestion: If the victim is conscious and not convulsing, give 1 or 2 glasses of water to dilute the chemical and IMMEDIATELY call a hospital or poison control center. If the victim is

convulsing or unconscious, ensure that the victim's airway is open and lay the victim on his/her side with the head lower than the body. DO NOT INDUCE VOMITING. IMMEDIATELY TRANSPORT THE VICTIM TO A HOSPITAL.

2.2.2 Protective Equipment

Eye:	Safety glasses/goggles
Gloves:	Two pairs of dissimilar protective gloves shall be worn when handling the neat chemical and dosing solutions.
Clothing:	Minimally, a disposable laboratory suit (e.g., Tyvek ®), bouffant, and shoe covers shall be worn, as specified in the most current NTP Statement of Work or the NTP Health and Safety Minimum Requirements.
Respiratory Protection:	A NIOSH-approved chemical cartridge respirator with an organic vapor, acid gas, and high-efficiency particulate filter cartridge. Use the test materials only in well-ventilated areas.

2.2.3 Spills and Containment

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

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

If the spillage is not containable (at the discretion of the Health and Safety Officer), self-contained breathing apparatus will be used.

2.2.4 Decontamination of Laboratory Equipment

Computer Terminal/Equipment	Whenever feasible, a protective covering (e.g., plastic wrap) shall be placed over the keyboard when in use. Clean work surfaces of general laboratory equipment (i.e., lab carts, portable hoods, and balances) with a 1% T.B.Q (quaternary ammonium) solution before removing them from animal dosing rooms and/or chemical preparation areas.

2.2.5 Disposal Procedures

Waste Disposal:	Securely package and label, in double bags, all waste material. All potentially contaminated material (i.e., carcasses, bedding, soiled disposable clothing) shall be disposed of by incineration in a chemical incinerator equipped with an afterburner and

scrubber in a manner consistent with federal (EPA), state, and local regulations.

2.3 Dose Formulation and Analysis

The required quantity of Vinclozolin, Methoxychlor, Ethinyl Estradiol, Flutamide, and Phenobarbital will be accurately weighed into a volumetric flask. The vehicle (corn oil) will be added to the neat test material to form a solution, and the solution will be stirred for at least 10 minutes to ensure complete dissolution. The formulation for each group will be dispensed into daily aliquots which will be stored in glass bottles with Teflon®-coated lids protected from light at 2-9°C. Each solution will be stirred prior to dosing.

Each formulation will be labeled with the ID No., group, dose level, vehicle, mix number, preparation date, expiration date, storage conditions, and TherImmune Study No. and will be color coded by group. The tray used to hold the daily aliquots will be labeled with the TherImmune Study No., ID No., test article name, group, dose level, vehicle, mix number, preparation date, expiration date, storage conditions, and group color code (see SOP No 506.0 Storage Sampling, and Labeling of Control and Test Diets and Mixtures and SOP No.121.0 Color-Coding for Study Identification and Dose Groups).

Every time a new mix or batch of chemical is prepared, three (3) 35 mL archival samples of each dose level of test article formulation will be collected and stored at TherImmune in glass bottles with Teflon®-coated lids protected from light in the refrigerator. One set of samples from each dose level will be forwarded on ice packs to an NTP analytical chemistry subcontractor for dose concentration analysis at the following times: initial, middle, and final formulations and at other periods specified by the Sponsor and communicated to the Study Director. If the formulations are suspensions, three archival samples (35 mL each) will be collected from the top, middle, and bottom of the low and high dose formulations in glass bottles with Teflon®-coated lids. Archival samples not selected for analysis will be discarded as hazardous waste following requirements in Section 2.2.5 at least ninety days following preparation.

3.0 EXPERIMENTAL DESIGN

3.1 Test System

Species:	Sprague Dawley Crl: CD® (SD) IGS BR
Rationale:	The Sprague Dawley rat was selected as the test system due to its established quality as a breeder and the availability of historical toxicologic data for reference.
Supplier:	Charles River Breeding Laboratories. (Portage, Michigan or Raleigh, North Carolina)
Number/Sex:	Fifty time-mated females plus 5 extras will arrive on Gestation Day (GD) 7-10 (day of mating = GD 0)

Dam Age (at study initiation):	Approximately 12 weeks.

3.2 Animal Husbandry

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

Acclimation period:	At least 7 days
Animal housing (arrival until weaning):	1 dam per cage
Juvenile animal housing following weaning:	2-3 per cage (same sex groups)
Lighting:	14 hours light /10 hour dark cycle
Temperature:	22±2°C
Relative Humidity:	40-50%
Observations:	Twice daily observations for general health and availability of adequate food and water.
Cage changes:	At least twice a week, unless the animals are individually housed in 19" x 10½" x 8" (group-housed) cages which may be changed once a week.
Feeder/bottle changes:	At least once per week
Procedure for Individual Animal Identification:	All animals will be uniquely identified by tail tattoo and by cage cards.

Housing Requirements:	
Cage Type:	Polycarbonate
Cage Measurement:	19" x 10 1/2" x 8" (group housed) 9" x 8 1/2" x 8" (single housed) (20x25x47cm)
Bedding Material:	"Sani Chip" Hardwood Laboratory Bedding (P.J. Murphy Forest Products Corp., Montville, N.J.). **All bedding will be autoclaved prior to use.**
Feed:	**Purina Certified 5002. The same batch/lot of feed will be used for the entire study.**
Frequency:	*ad libitum*
Analysis:	The feed is analyzed for nutrients, aflatoxins, nitrosamines, heavy metals, chlorinated hydrocarbons, organophosphates, PCBs, nitrates, nitrites, BHA, BHT, total bacterial plates, coliforms, *E. coli*, and *Salmonella* by the vendor prior to release. **An analysis of the concentration of genistein, daidzein, glycite, and metabolizable energy content in the batch/lot will be provided from the vendor and included in the final report. Approximately 50 grams of feed from the batch/lot used on study will be collected and stored at room temperature for possible future analytical chemistry work.**
Water:	Deionized water provided in water bottles and/or filtered tap water provided by an automatic watering system.
Frequency:	*ad libitum*
Analysis:	A water quality sample is analyzed for total dissolved solids, heavy metals, chlorinated hydrocarbons, organophosphates, nitrates, nitrites, microbiological content, and total trihalomethanes at least semi-annually to conform with the Safe Drinking Water Act. None of the contaminants is expected to be at levels sufficient to interfere with the study.

Health Screening Requirements:	During the acclimation period, one dam will be sent to AnMed/Biosafe Laboratories (Rockville, MD) for the following serological tests: Pneumonia Virus of Mice (PVM) Respiratory Enteric Orphan III (REO3) Toolan's H-1 (parvovirus) (TH1) Encephalomyelitis (GD7) Sialodacryoadenitis Virus (coronavirus) (SDAV/RCV) Sendai (SEN) Mycoplasma Pulmonis (MYCO) Lymphocytic Choriomeningitis (LCM) Kilham's rat Virus/Rat Orphan Parvovirus (KRV/rOPV)

4.0 STUDY DESIGN

4.1 General Study Design

Fifty time-mated females will be used on study and will produce juvenile animals. One hundred thirty-five juvenile males will be assigned to a male cohort study, and one hundred thirty-five juvenile females will be assigned to a female cohort study.

Definitions: Gestation Day 0 (GD 0) = Day of mating (Sperm positive)

Postnatal Day 0 (PND 0) = Day of Delivery

This will be a blinded study, which requires that personnel who are performing dosing and/or observations should not know which animals are receiving test article and which animals are the control animals.

4.1.1 Mortality

Any animals found dead or killed *in extremis* during the study will be subject to necropsy (following euthanasia with carbon dioxide, if moribund). The following tissues will be retained and placed in Bouin's then transferred into 70% ethanol within 24-48 hours:

liver
kidneys
thymus
adrenals
spleen
both testes and epididymides (male)
prostate (ventral and dorso-lateral lobes) (male)
seminal vesicles/coagulating glands (male)
vagina/uterus/cervix (female)
ovaries (female)
gross lesions
pituitary
brain
stomach
thyroid/parathyroids

4.1.2 Necropsy

All necropsies are performed according to TherImmune Standard Operating Procedures.

4.1.3 Histopathology

Histopathological examination of fixed tissues for animals found dead or killed *in extremis* will not be conducted unless indicated by a protocol amendment.

4.2 Juvenile Animal Production

All dams will be housed individually from the day of receipt to the day of euthanasia. Fifty females will be allowed to deliver litters to be used on study. Litters will be culled to 8-10 pups on PND 4, and approximately equal numbers of male and female pups will be kept in each litter.

On PND 21, all male and female pups will be separated from dams, and one hundred thirty-five males will be assigned to the juvenile male study and one hundred thirty-five females will be assigned to the juvenile female study. Juvenile animals will be housed 2-3 per cage (same sex groups). Selection will be made by weighing all pups and selecting the 135 animals of each sex that are most similar in weight. All extra animals will be removed from study and discarded without necropsy on the day of separation (PND 21).

4.2.3 Allocation

Fifty time-mated females plus 5 extras will be ordered from Charles River and will arrive on Gestation Day 7-10 (day of mating = Gestation Day 0).

4.2.4 Treatment

There is no treatment for time-mated females.

4.2.5 Measurements

Dams

Observations for mortality and clinical signs:	Twice daily
Body Weight:	At littering and on PND 21

Pup Observations

The following observations will be made for the F_1 pups:

PND 0:	Number of live pups
	Number of dead pups
	Number of males
	Total body weight of males
	Number of females
	Total body weight of females

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PND 4: Litters will be culled to 8-10 pups (approximately equal numbers of male and female pups, if possible). Pups not selected will be discarded without necropsy.

Body weights: Weekly

Individual pup weights will also be collected on PND 21 and recorded to the nearest 0.1 gram.

4.2.6 Disposition of Offspring and Dams

Dams: Discarded without necropsy on PND 21 following terminal body weight collection and euthanasia with carbon dioxide.

Neonates: On PND 4, pups not selected will be euthanized with sodium pentobarbital (or equivalent) and discarded without necropsy.

Weanlings: Animals that are not selected for juvenile male or female cohort studies will be euthanized with carbon dioxide and discarded without necropsy on PND 21.

4.3 Juvenile Male Cohort Study:

4.3.1 Number of Animals and dose levels:

Group No.	Test Material	Dose Level (mg/kg/day)	Number of Males
1	Corn Oil Vehicle	0	15
2	Vinclozolin	10	15
3	Vinclozolin	30	15
4	Vinclozolin	100	15
5	Flutamide	25	15
6	Flutamide	50	15
7	Phenobarbital	25	15
8	Phenobarbital	50	15
9	Phenobarbital	100	15

4.3.2 Allocation

After PND 21 body weight collection, the male pups in each litter will be assigned to treatment groups in a randomized block-fashion based upon weight, with 9 males per block. The 15 blocks range from heaviest to lightest. Each treatment group then gets one pup from each of the 15 blocks.

4.3.3 Treatment

Starting on PND 23, animals will be administered the corn oil vehicle or test article in corn oil by oral gavage at 2.5 mL/kg/day (using an 18gauge gavage needle, 1 inch in length, with a 2.25 mm ball and a 1 cc glass disposable tuberculin syringe) once daily at 0700-1000 each day, continuing through necropsy. Control animals will receive the vehicle, corn oil, only. The dose formulations will be stirred before and during dosing. Dose volumes will be calculated daily based on the same-day body weight.

On the day of termination, animals will be dosed between 0700 and 0900.

4.3.4 Measurements

Observations for mortality and signs of toxicity:	Twice daily
Body Weight:	PND 23 and daily thereafter, including the day of preputial separation and termination.
Physical Examination:	PND 23 and weekly thereafter At termination
Preputial Separation Observation:	Preputial separation will be evaluated on all males daily starting on PND 23. A partial separation, complete separation, or persistent thread of tissue between the gland and prepuce will be recorded. However, the day of complete separation is the endpoint used in the analysis for the age of preputial separation.

4.3.5 Termination

Schedule:	On PND 53-54
Groups:	The necropsy will examine all treated males from each dose group.
Procedures:	Care must be taken to remove mesenteric fat from the sex accessory glands with small scissors such that the fluids are retained. Once free from the fat and adnexa, the weight with fluid is recorded. The seminal vesicle with coagulating gland is then placed on a paper towel, pressed so that the fluid is exuded, gently blotted dry, and reweighed. The thyroid/parathyroids will be fixed as described below, then the attached portion of the trachea will be removed, and the fixed thyroid/parathyroids will then be weighed.

Small tissues such as the adrenals and pituitary, as well as tissues that contain fluid, will be weighed immediately to prevent partial drying prior to weighing.

Organ Weights:	The following organs will be weighed to the nearest 0.1 mg: adrenal glands (paired) epididymides (right and paired weights) kidneys (paired) levator ani plus bulbocavernosus liver pituitary seminal vesicle and coagulating gland with and without fluid testes (paired) thyroid/parathyroids (weighed post-fixation) ventral and dorsal-lateral prostate, separately
Tissue Preservation:	After weighing (except the thyroid/parathyroids), the following tissues will be placed in Bouin's and transferred into 70% ethanol within 24-48 hours: adrenal glands (paired) epididymides kidneys (paired) liver pituitary testes thyroid/parathyroids (with attached portion of the trachea) gross lesions
Histopathology:	Tissues will be transferred to Pathology Associates – A Charles River Company (PAI) located in Frederick, MD, under subcontract to TherImmune. The thyroid, testes, and epididymides from all males/group will be embedded in paraffin, sectioned, stained with hematoxylin and eosin, and examined microscopically. All gross lesions from all males/group will be embedded in paraffin, sectioned, and examined microscopically by the study pathologist.

4.4 Juvenile Female Cohort:

4.4.1 Number of Animals and dose levels

Group No.	Test Material	Dose (mg/kg/day)	Number of Females
1	Corn Oil Vehicle	0	15
2	Methoxychlor	12.5	15
3	Methoxychlor	25	15
4	Methoxychlor	50	15
5	Ethinyl Estradiol	0.0025	15
6	Ethinyl Estradiol	0.005	15
7	Phenobarbital	25	15

8	Phenobarbital	50	15
9	Phenobarbital	100	15

4.4.2 Allocation

After PND 21 body weight collection, the female pups in each litter will be assigned to treatment groups in a randomized-block-fashion based upon weight with 9 females per block. The 15 blocks range from heaviest to lightest. Each treatment group then gets one pup from each of the 15 blocks.

4.4.3 Treatment

Starting on PND 22, animals will be administered the corn oil vehicle or test article in corn oil by oral gavage at 2.5 ml/kg/day day (using an 18 gauge gavage needle, 1 inch in length, with a 2.25 mm ball and a 1 cc glass disposable tuberculin syringe), once daily between 0700-1000 each day, continuing through necropsy. Control animals will receive the vehicle, corn oil, only. The dose formulations will be stirred before and during dosing. Dose volumes will be calculated daily based on the same-day body weight.

On the day of termination, animals will be dosed between 0700 and 0900.

4.4.4 Measurements

Observations for mortality and signs of toxicity:	Twice daily
Body Weight:	PND 22 and daily thereafter
Physical Examination:	PND 22 and weekly thereafter At termination
Vaginal Opening Observation:	Vaginal opening will be evaluated on all females daily starting on PND 22. The appearance of a small pin hole, a vaginal thread, or complete vaginal opening are recorded. However, the day of complete vaginal opening is the endpoint used in the analysis for the age of vaginal opening. If vaginal opening does not occur by PND 42, then PND 43 may be used to determine the mean for the age at vaginal opening. In this case, the number of females that did not reach vaginal opening by necropsy within each treatment group should also be included in the data summary.
Estrous Cyclicity:	Beginning on the day of vaginal opening and continuing through day of necropsy, daily vaginal smears are obtained and evaluated under a low-power light microscope for the presence of leukocytes, nucleated epithelial cells, or cornified epithelial cells to determine the age of the first vaginal cycle and/or any effects on estrous cyclicity. Extended estrus shall be defined as exhibiting cornified cells with no

leukocytes for 3 or more days and extended diestrus as the presence of leukocytes for 4 or more days.

4.4.5 Termination

Schedule: On PND 42-43

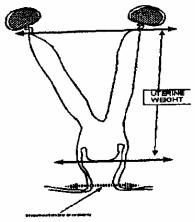
Groups: The necropsy will examine all treated females from each dose group.

Procedures: Care must be taken to remove mesenteric fat from the uterine horns and to avoid damaging the uterus so that the uterine fluid is retained. The uterus plus cervix are separated from the vagina and the weight of the uterus with fluid is recorded. The uterus is then placed on a paper towel, slit to allow the fluid contents to leak out, gently blotted dry, and reweighed.

The thyroid/parathyroids will be fixed as described below, then the attached portion of the trachea will be removed, and the fixed thyroid/parathyroids will then be weighed.

Small tissues such as the adrenals and pituitary, as well as tissues that contain fluid, will be weighed immediately to prevent partial drying prior to weighing.

The uterus (without ovaries) will be carefully dissected and trimmed of fascia and fat to avoid loss of luminal contents. The vagina shall be removed from the uterus at the level of the uterine cervix. See below for further details for the removal and preparation of uterine tissues for weight measurement.



Each ovary and uterine horn is detached from the dorsal abdominal wall. Urinary bladder and ureters are removed from the ventral and lateral sides of the uterus and vagina. Fibrous adhesions between the rectum and the vagina are detached until the junction of vaginal orifice and perineal skin is identified. The uterus and vagina are detached from the body by incising the vaginal wall just above the junction with the perineal skin as shown in the figure. The excess fat and connective tissue are trimmed away. The vagina is removed from the uterus as shown in the figure for uterine weight measurement. Weight with luminal fluid (wet weight) and the without the luminal fluid (blotted weight) are obtained.

Organ Weights: The following tissues will be weighed to the nearest 0.1 mg:

adrenal glands (paired)
kidneys (paired)
liver
ovaries (paired)
pituitary
thyroid/parathyroids (weighed post-fixation)
uterus and cervix with and without fluid

Tissue Preservation:

After weighing (except the thyroid/parathyroids), the following tissues will be placed in Bouin's and transferred into 70% ethanol within 24-48 hours:

adrenal glands (paired)
kidneys (paired)
liver
ovaries (paired)
pituitary
thyroid/parathyroids (attached with the portion of the trachea)
uterus and cervix
gross lesions

Histopathology: Tissues will be transferred to Pathology Associates – A Charles River Company (PAI) located in Frederick, MD, under subcontract to TherImmune. The ovaries, uterus and cervix, and gross lesions from all females/group will be embedded in paraffin, sectioned, stained with hematoxylin and eosin, and examined microscopically by the study pathologist.

5.0 PROPOSED STATISTICAL ANALYSES

All raw data will be sent in Excel spreadsheet (both electronic file and soft copy) to the Project Officer at the same time that the data are sent to the statistical support group.

Statistical analyses of the following will be performed:

Data from the main study will be analyzed by a statistical support group under contract to NTP/NIEHS, RTP, NC. A statistical analysis report will be submitted to TherImmune by the contractor for inclusion in the final study report. All data (age and weight at vaginal opening/preputial separation, body and organ weights at necropsy, food consumption, and energy intake) are analyzed using ANOVA. Organ weights may be analyzed by ANCOVA using the body weight at necropsy as a covariate. When significant treatment effects are observed, treatment means are tested using an appropriate multiple comparison test. Data should be evaluated for heterogeneity of variance by an appropriate statistical test and, if present, data should be transformed or analyzed using a suitable non-parametric test.

6.0 REPORTS

The following reports will be submitted:

Draft Study Report

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

An executive summary will be prepared describing the number and strain of rats observed, the doses used for each chemical tested, and the effects and level of statistical significance for all

required endpoints specified in the protocol for the maternal cohort, neonates, and pubertal female/male cohorts. Raw data from each individual animal should be presented in a spreadsheet format. Data summary tables containing the mean, standard error of the mean (SEM), and sample size for each treatment group should be provided for all endpoints. The mean, SEM and CV values for the control data are examined to determine whether they meet acceptable QA criteria for consistency with historical values. Organ weight data may also be presented after covariance adjustment for body weight, but this should not replace presentation of the unadjusted data. In addition, the data tables are accompanied by summary of histological findings with photomicrographs of significant observations.

Final Study Report

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

7.0 STORAGE OF RECORDS

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

8.0 PERSONNEL

Project Officer:	Jack Bishop, Ph.D. (NTP)
Study Director:	Meredith Rocca, Ph.D.
Principal Investigator:	Gary W. Wolfe, Ph.D., D.A.B.T
Reproductive Toxicologist:	Larissa B Nehrebeckyj, B.S.
Technical Supervisor:	Jennifer Hoeck, B.A.
Health and Safety Officer/ Facility Manager:	Robert Blackford, A.A., LATG
Veterinarian:	Edward Greenstein, D.V.M, ACLAM
Quality Assurance Officer:	David Wilson, B.A., RQAP
Report Manager:	Rita Patel, B.S.
Dose Preparation Supervisor:	Gary Holley, B.S.
Pathologist:	Jerry Quance, D.V.M., D.A.C.V.P.

9.0 SUBCONTRACTORS

Necropsy/Pathology:	PAI, Frederick, MD
Serology:	AnMed/Biosafe, Rockville, MD

10.0 REFERENCES

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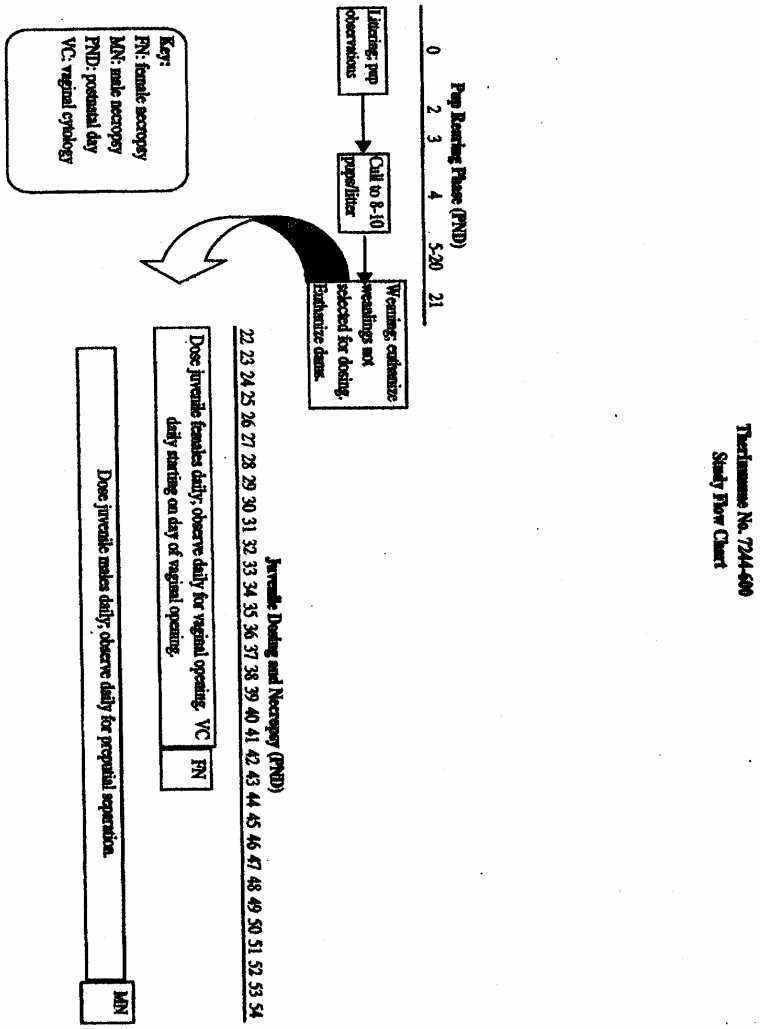
PUBERTAL TOXICITY STUDY OF VINCLOZOLIN, FLUTAMIDE AND PHENOBARBITAL IN MALE SPRAGUE DAWLEY RATS AND METHOXYCHLOR, ETHINYL ESTRADIOL AND PHENOBARBITAL IN FEMALE SPRAGUE DAWLEY RATS WHEN ADMINISTERED IN CORN OIL BY ORAL GAVAGE

Appendix 1: STUDY SCHEDULE


TherImmune No. 7244-600
Study Flow Chart
Key:
FN: female necropsy
MN: male necropsy
PND: postnatal day
VC: vaginal cytology
Littering; pup observations
Chill to 8-10 pups/litter
Weanings; euthanize weanlings not selected for dosing. Euthanize dams.
Pup Rearing Phase (PND)
0 2 3 4 5-20 21
Juvenile Dosing and Necropsy (PND)
22 23 24 25 26 27 28 29 30 31 32 33 34 35 36 37 38 39 40 41 42 43 44 45 46 47 48 49 50 51 52 53 54
Dose juvenile females daily; observe daily for vaginal opening. VC
FN
Dose juvenile males daily; observe daily for preputial separation.
MN

NTP/NIEHS TherImmune No.: 7244-600

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PUBERTAL TOXICITY STUDY OF VINCLOZOLIN, FLUTAMIDE AND PHENOBARBITAL IN MALE SPRAGUE DAWLEY RATS AND METHOXYCHLOR, ETHINYL ESTRADIOL AND PHENOBARBITAL IN FEMALE SPRAGUE DAWLEY RATS WHEN ADMINISTERED IN CORN OIL BY ORAL GAVAGE

Appendix 2: DOSE FORMULATION REPORTS IN STUDY DATA

VINCLOZOLIN
METHOXYCHLOR
FLUTAMIDE
ETHINYL ESTRADIOL
PHENOBARBITAL

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PUBERTAL TOXICITY STUDY OF VINCLOZOLIN, FLUTAMIDE AND PHENOBARBITAL IN MALE SPRAGUE DAWLEY RATS AND METHOXYCHLOR, ETHINYL ESTRADIOL AND PHENOBARBITAL IN FEMALE SPRAGUE DAWLEY RATS WHEN ADMINISTERED IN CORN OIL BY ORAL GAVAGE

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References

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Medical Surveillance	1001.0, CHP, RPP
Eye Protection	132.0, 1003.0, CHP
Personal Protective Equipment	1003.0, CHP, RPP
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Shipping of Study Materials	506.0, 506.1,1005.0, CHP
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Note: CHP = Chemical Hygiene Plan
RPP = Respiratory Protection Program

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NTP/NIEHS TherImmune No.: 7244-600

THERIMMUNE RESEARCH CORPORATION
PROTOCOL AMENDMENT

Study Number: 7244-600
Study Title: Pubertal Toxicity Study Of Vinclozolin, Flutamide And Phenobarbital In Male Sprague Dawley Rats And Methoxychlor, Ethinyl Estradiol And Phenobarbital In Female Sprague Dawley Rats When Administered In Corn Oil By Oral Gavage
Amendment Number: 1

1. **Subject: Bulk Chemical Samples (p. 14)**

   The sentence "An additional 35 mL aliquot will be sent within 30 days prior to the start of any study." is deleted.

2. **Subject: Dose Formulation and Analysis (p. 16)**

   Formulations will be labeled with a unique identification code and not with the test article name or dose level as the study is being performed "blinded". The same unique coding will be used on the cage cards.

   Homogeneity samples will be sent on ice packs to an NTP analytical chemistry subcontractor for dose concentration analysis.

3. **Subject: Test System (p. 16)**

   Time mated females will be 175 to 250 g and 60-70 days old at mating.

4. **Subject: Animal Husbandry (p. 17)**

   F0 females will be identified by tail mark. F1 pups will not be individually identified. F1 animals will be identified by tail tattoo upon randomization and assignment to study.

5. **Subject: Health Screening (p. 19 and 27)**

   AnMed/Biosafe Laboratories has changed its name to Taconic Anmed.

6. **Mortality (p. 19)**

   This section refers to F1 animals after randomization to the Juvenile Male or Female Cohort. F0 females and F1 pups will be discarded without necropsy.

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7. **Subject: Juvenile Animal Production (p. 20)**

   F0 females and their pups that are not delivered on the expected day of parturition will be removed from study and either transferred to TherImmune's training colony or euthanized and discarded without necropsy.

8. **Subject: Treatment (p. 22 and 24)**

   The gavage needle used for dosing will be 1-2 inches long with a 2.25-3 mm ball. This allows for use of larger needles as the animals grow.

9. **Subject: Termination (p. 22 and 25)**

   The method of euthanasia will be carbon dioxide inhalation and exsanguination.

10. **Subject: Tissue Preservation (p. 23 and 26)**

    Thyroid/parathyroid will be fixed in 10% neutral buffered formalin.

11. **Subject: Histopathology (p. 26)**

    Thyroid is added to the list of tissues to be processed and examined microscopically for females.

12. **Subject: Personnel (p. 27)**

    There have been changes in the personnel assigned to this study. Technical Supervisor will be Michael Brunty. Report Manager will be Larissa B. Nehrebeckyj. Any additional changes in personnel will be included in the final report.

13. **Subject: References (p. 29)**

    The following reference is added:

    Laws, SC; Carey, SA; Ferrell, JM; Bodman, GJ; and Cooper, RL (2000). Estrogenic activity of octylphenol, nonylphenol, bisphenol A and methoxychlor in rats. *Toxicol. Sci.* 54: 154-167.

14. **Subject: Standard Operating Procedures (p. 34)**

    The SOP numbers listed in the protocol were correct when the protocol was drafted, but will change as SOPs are revised. The most current SOP will be used for all procedures.

NTP/NIEHS **TherImmune No.: 7244-600**

Justification : Errors and omissions in the original protocol.

Study Director Approval:

Meredith S. Rocca 12.4.02
Meredith S. Rocca, PhD, DABT Date

Sponsor Approval:

Jack B Bishop 12-03-02
Jack Bishop, PhD Date

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NTP/NIEHS **TherImmune No.: 7244-600**

THERIMMUNE RESEARCH CORPORATION
PROTOCOL AMENDMENT

Study Number: 7244-600
Study Title: Pubertal Toxicity Study Of Vinclozolin, Flutamide And Phenobarbital In Male Sprague Dawley Rats And Methoxychlor, Ethinyl Estradiol And Phenobarbital In Female Sprague Dawley Rats When Administered In Corn Oil By Oral Gavage
Amendment Number: 2

Subject: 8.0 Personnel

The Study Director is changed to:

Gary W. Wolfe, PhD, DABT
610 Professional Drive
Gaithersburg, MD 20879
Phone: 301-330-3723
e-mail: gwolfe@genelogic.com

Justification: Change in TherImmune staffing.

Study Director Approval:

Gary W. Wolfe 10/10/03
Gary W. Wolfe, PhD, DABT Date
Vice President, Toxicology

Sponsor Approval:

Jack B Bishop 10/10/03
Jack Bishop, PhD Date

Appendix 18
Protocol, Amendments, and Deviations
Pubertal Toxicity Study of Vinclozolin, Flutamide and Phenobarbital in Male Sprague Dawley Rats and Methoxychlor, Ethinyl Estradiol and Phenobarbital in Female Sprague Dawley Rats when Administered in Corn Oil by Oral Gavage

The following deviations from the protocol were noted:

Dams were aged 9-10 weeks at receipt. The protocol required the dams to be approximately 12 weeks old at receipt.

F_0 dams were single housed in cages designated for group-housed animals, to allow room for littering and rearing of pups.

George Lathrop served as the veterinarian on this study. The protocol listed Edward Greenstein as the veterinarian.

There is no documentation that bulk Methoxychlor and Ethinyl Estradiol were stored under nitrogen or that bulk Phenobarbital was stored protected from heat.

Compounds were used on the study in a different group order than that assigned by protocol.

On the first day of dosing, F_1 females were dosed until 10:37 AM.

F_1 weanlings not selected for the male and female cohort studies were not terminated until PND 24, to allow for possible replacement.

Due to a technical error, the pituitary was lost for animals # 9291 (before weighing) and # 9086 (after weighing), at necropsy.

Due to a technical error, the dorsal-lateral prostate was lost for animals # 9071 (before weighing), at necropsy.

Prostate glands and seminal vesicles with lesions were not preserved.

Appendix 18 (Continued)
Protocol, Amendment and Deviations
Pubertal Toxicity Study of Vinclozolin, Flutamide and Phenobarbital in Male Sprague Dawley Rats and Methoxychlor, Ethinyl Estradiol and Phenobarbital in Female Sprague Dawley Rats when Administered in Corn Oil by Oral Gavage

The following deviations from the protocol were noted:

Temperature (°F) and humidity (%) deviations for Room 10:

November 07, 2002		58%
November 10, 2002		58%
November 11, 2002		64%
November 12, 2002		66%
November 14, 2002		62%
November 15, 2002	67°F	52%
November 16, 2002		60%
November 17, 2002		60%
November 19, 2002		66%
November 20, 2002	66°F	56%
November 21, 2002		52%
November 22, 2002		62%
November 23, 2002		38%
November 25, 2002		34%
November 26, 2002	66°F	34%
November 27, 2002	66°F	

Appendix 18 (Continued)
Protocol, Amendment and Deviations
Pubertal Toxicity Study of Vinclozolin, Flutamide and Phenobarbital in Male Sprague Dawley Rats and Methoxychlor, Ethinyl Estradiol and Phenobarbital in Female Sprague Dawley Rats when Administered in Corn Oil by Oral Gavage

The following deviations from the protocol were noted:

Temperature (°F) and humidity (%) deviations for Room 10:

December 02, 2002	67°F	30%
December 03, 2002		34%
December 05, 2002		32%
December 18, 2002		36%
December 20, 2002		64%
December 21, 2002		36%
December 28, 2002		34%
January 01, 2003		54%
January 05, 2003	66°F	34%
January 06, 2003		35%
January 07, 2003	65°F	32%
January 11, 2003		30%
January 12, 2003		31%

Temperature (°F) and humidity (%) deviations for Room 11:

December 14, 2002	38%
December 18, 2002	36%
December 20, 2002	64%
December 21, 2002	37%

These deviations had no affect on the outcome of the study or the interpretation of the results.