

FINAL REPORT

Title: Assessment of Pubertal Development and Thyroid Function in Juvenile Male CD® (Sprague-Dawley) Rats After Exposure to Selected Chemicals Administered by Gavage on Postnatal Days 23 to 52/53

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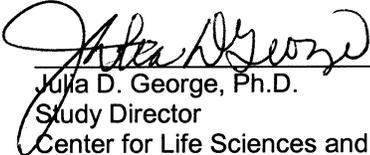
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FINAL REPORT

Assessment of Pubertal Development and Thyroid Function in Juvenile Male CD® (Sprague-Dawley) Rats After Exposure to Selected Chemicals Administered by Gavage on Postnatal Days 23 to 52/53

ABSTRACT

The purpose of this study was to examine the sensitivity of pubertal assays to the effects of a wide variety of chemicals that are known to affect the endocrine system through different pathways and/or mechanisms of action. This assay detects agents that display anti-thyroid, estrogenic, anti-estrogenic [estrogen receptor (ER) or steroid enzyme mediated] activity, or alter luteinizing hormone (LH), follicle stimulating hormone (FSH), prolactin, growth hormone (GH) secretion or hypothalamic function. Therefore, EPA decided to test eight chemicals that have various modes of action [i.e., atrazine, p, p'-dichlorodiphenyldichloroethane (p, p'-DDE), vinclozolin, methoxychlor, propylthiouracil, ketoconazole, and linuron], using the male pubertal assay study design.

The study was conducted in two components. In each component, F1 males, produced from undosed timed-pregnant CD® (Sprague-Dawley) rats (the F0 generation), were tested. On the day of birth [postnatal day (pnd 0)], F1 pups were counted, sexed, weighed, and examined externally. On pnd 4, the litters were standardized to ten pups, maximizing the number of male pups. Natural litters with ten or fewer pups were not culled. The F0 females were allowed to rear their pups to pnd 21. F1 survival, gender identification, gross observations, and body weight were recorded on pnd 4, 7, 14, and 21. On pnd 21, F1 males were weaned and weight ranked across litters, then randomized into the treatment groups based on body weight. Fifteen F1 males were assigned to each treatment group in each component. F1 males were orally dosed with a test compound or the vehicle (Mazola® corn oil) from pnd 23 to pnd 52/53. Dose volume (5 ml/kg/day) was based on daily body weight. In Component 1, animals received atrazine (75 or 150 mg/kg/day), p, p'-DDE (50 or 100 mg/kg/day), vinclozolin (30 or 100 mg/kg/day), or methoxychlor (25 or 50 mg/kg/day) in corn oil. In Component 2, animals received propylthiouracil (2 or 25 mg/kg/day), ketoconazole (50 or 100 mg/kg/day), linuron (50 or 100 mg/kg/day), or phenobarbital (50 or 100 mg/kg/day) in corn oil. A separate vehicle control group dosed with corn oil was run concurrently with each component. Body weights for the F1 males were recorded during the postweaning treatment period to scheduled sacrifice. Clinical signs were recorded twice daily during the treatment period. Beginning with pnd 23, F1 males were examined for preputial separation. The day of complete preputial separation was identified as the age of preputial separation, and body weight was recorded on that day. At necropsy on pnd 52/53, males were euthanized and blood was collected by external cardiac puncture for analysis of thyroxine (T4) and thyroid-stimulating hormone (TSH). Body, liver, paired kidney, pituitary, paired adrenal, thyroid, seminal vesicles plus coagulating glands (with and without fluid), ventral prostate, levator ani plus bulbocavernosus (LABC),

epidymal, and testes weights were recorded. The thyroid, epididymides, and testes were evaluated histopathologically.

Observations

The following observations were made:

- ◆ **Atrazine**. Treatment with atrazine up to 150 mg/kg/day did not significantly alter the day of acquisition of preputial separation. Adjusted organ weights revealed an increasing trend for paired testes weight but no other differences. No differences were noted in T4 or TSH levels, and no treatment-related histopathological changes were observed in the thyroid, testes, or epididymides.
- ◆ **p,p'-DDE**. Treatment with 50 or 100 mg/kg/day p,p'-DDE significantly delayed preputial separation at both treatment levels. Adjusted thyroid, liver, and paired kidney weights were significantly increased at both doses of p,p'-DDE. With respect to reproductive tissues, adjusted paired epididymides weight exhibited a significant decrease at the high dose. Adjusted LABC weight exhibited a decreasing trend. Decreased circulating T4 levels were observed at the high dose, whereas TSH levels exhibited no effect of treatment. No treatment-related histopathological change were observed in the thyroid, testes, or epididymides.
- ◆ **Vinclozolin**. The day of acquisition of preputial separation exhibited significant delays at both the 30 and 100 mg/kg/day dose levels. In addition, three males in the high-dose group failed to achieve preputial separation prior to scheduled necropsy. Adjusted testes (increase), paired epididymides (decrease), and paired seminal vesicles with coagulating glands (decrease) weights exhibited significant treatment-related effects at both dose levels. Adjusted LABC weight was significantly decreased at the high dose, whereas adjusted ventral prostate weight exhibited a significant treatment effect, but no pairwise differences from the control group. Circulating T4 levels were significantly decreased at both doses of vinclozolin, while no effect was observed on TSH. No treatment related histopathological changes were observed in the thyroid, testes, or epididymides.
- ◆ **Methoxychlor**. No effect on preputial separation was noted at either 25 or 50 mg/kg/day methoxychlor. Adjusted paired adrenal weight (increase) and seminal vesicle with coagulating glands weight (decrease) exhibited a significant effect at the high dose. Adjusted thyroid weight exhibited a significant treatment effect, but no pairwise differences from the control group. Thyroid hormone levels were unaffected, and no treatment-related histopathology was observed. These results are in accordance with those observed by Gray et al. (1989), who saw delayed puberty, and altered

reproductive organ weights, TSH levels, and testicular histopathology at doses higher than 50 mg/kg/day, in F1 animals exposed to methoxychlor from gestation through pnd 15.

- ◆ **Propylthiouracil.** As expected, propylthiouracil produced a decrease in the circulating T4 and an increase in the circulating level of TSH, increased adjusted thyroid weight, and thyroid follicular cell hypertrophy/hyperplasia at both 2 and 25 mg/kg/day. Preputial separation was significantly delayed at the high dose. Treatment effects were also observed at both doses as increased adjusted weights of the seminal vesicles with coagulating glands. Adjusted paired epididymides weight exhibited an increasing trend.
- ◆ **Ketoconazole.** The postnatal day of acquisition of preputial separation was delayed at both doses of ketoconazole. Other treatment-related changes observed at both treatment levels included increased adjusted liver and paired adrenal weight, and decreased adjusted seminal vesicles with coagulating glands weight. The high dose of ketoconazole also increased adjusted paired kidney weight and decreased adjusted paired testes weight. No effect of treatment was observed histologically in the testes, epididymides, or thyroid. Thyroid hormone levels were not affected.
- ◆ **Linuron.** Linuron delayed puberty at both the 50 and 100 mg/kg/day dose levels, as evidenced by delayed acquisition of preputial separation. Of the organ weights evaluated at necropsy, only adjusted liver weight exhibited a treatment effect (increase) at both dose levels; adjusted seminal vesicles with coagulating glands weight was decreased at the high dose. Both T4 and TSH levels were decreased at both dose levels, although the decrease in TSH at the high dose of linuron did not reach statistical significance. No treatment-related histopathological changes were observed in the testes, epididymides, or thyroid.
- ◆ **Phenobarbital.** The postnatal day of acquisition of preputial separation was delayed at both doses of phenobarbital. In addition, treatment-related effects were detected at both dose levels in an increase in the adjusted weights of the thyroid and liver. A decreased in adjusted organ weight was observed at the high dose for paired testes and LABC. No effect of treatment was observed for thyroid hormone levels or histologically in the testes, epididymides, or thyroid.

OBJECTIVE

Purpose and Applicability

The purpose of this protocol is to outline procedures to quantify of the effects of environmental compounds on pubertal development and thyroid function in the intact juvenile/peripubertal male rat. This assay detects compounds that display antithyroid, estrogenic, androgenic, antiandrogenic [androgen receptor (AR) or steroid enzyme mediated] activity, or alter follicle stimulating hormone (FSH), luteinizing hormone (LH), prolactin, growth hormone (GH) or hypothalamic function.

Required Endpoints:

- ◆ Growth (daily body weight)
- ◆ Age and weight at preputial separation
- ◆ T4 and TSH
- ◆ Thyroid histology
- ◆ Seminal vesicle plus coagulating gland weight (with and without fluid)
- ◆ Ventral prostate weight
- ◆ Levator ani plus bulbocavernosus (LABC) weight
- ◆ Epididymal and testis weights and histology
- ◆ Liver, kidney, adrenal and pituitary weights

To further define the procedures for this protocol, EPA tested six chemicals that have various modes of action:

- ◆ atrazine (affects the hypothalamus-pituitary axis in male rats)
- ◆ propylthiouracil (affects the thyroid directly, causing hypothyroidism)
- ◆ vinclozolin (metabolites M1 and M2 act as anti-androgen; competitive binding to androgen receptor; M1 also binds weakly to the rat progesterone receptor)
- ◆ linuron (anti-androgen; competitive binding to androgen receptor)
- ◆ p,p'-DDE (stable metabolite of DDT; anti-androgen through competitive binding to the androgen receptor)
- ◆ ketoconazole (inhibits steroidogenesis in both sexes)
- ◆ methoxychlor (a xeno-estrogen through α -estrogen receptor, anti-estrogen through β -estrogen receptor and an anti-androgen through androgen receptor mediated mechanism)
- ◆ phenobarbital (induces P450 isoforms predominantly in the liver, accelerates metabolism of endogenous hormones and exogenous xenobiotics).

The study was conducted in two components. Component 1 consisted of animals dosed with atrazine (75 or 150 mg/kg/day), p,p'-DDE (50 or 100 mg/kg/day), vinclozolin (30 or 100 mg/kg/day), or methoxychlor (25 or 50 mg/kg/day), and a concurrent vehicle control (corn oil) group. Component 2

consisted of animals receiving propylthiouracil (2 or 25 mg/kg/day), ketoconazole (50 or 100 mg/kg/day), linuron (50 or 100 mg/kg/day) or phenobarbital (50 or 100 mg/kg/day), and a concurrent vehicle control (corn oil) group.

MATERIALS AND METHODS

Test Materials and Dose Formulations

The test chemicals used in this study were procured and analyzed for purity by the Sponsor by gas chromatography with flame ionization detection (GC-FID), high pressure liquid chromatography (HPLC), or gravimetric methods. All bulk test chemicals were stored at room temperature. Test chemicals formulated in corn oil were stored at 4°C. Dose formulations were mixed in corn oil for administration at 5 ml/kg. One vehicle formulation was prepared and administered to the control group animals concurrently with Component 1 compounds, whereas a separate vehicle formulation was prepared to be administered to the control group animals concurrently with Component 2 compounds. Formulations were prepared at Battelle, Sequim, and assayed between 91.0% and 109.6% of the target concentration prior to shipping to RTI International.

Animals and Husbandry

For Component 1, 20 timed-pregnant and 2 nonpregnant female outbred albino CD® (Sprague-Dawley) rats (CrI:CD®[SD] IGS BR) were received from Charles River Breeding Laboratories (Raleigh, NC) at gestational day (gd) 13. A separate order of 20 timed-pregnant and 2 nonpregnant female rats were received for use in Component 2. The females were 10 weeks old upon arrival. The F0 animals were individually housed during the quarantine period and during gestation, and with their litters during lactation in solid-bottom polycarbonate cages with stainless-steel wire lids (Laboratory Products, Rochelle Park, NJ) and Sani-Chip® cage litter (P.J. Murphy Forest Products Inc., Montville, NJ). Postwean, retained F1 males were housed under standard conditions until necropsy. All animals were housed in the RTI Animal Research Facility for the duration of the study. All animal rooms were on a 14:10 hour (light:dark) light cycle per day and were air-conditioned. Temperature (22±4°C) and relative humidity (30-70%) were continuously monitored, controlled, and recorded using an automatic system (Siebe/Barber-Colman Network 8000 System, Version 4.4.1, Loves Park, IL). Purina Certified Rodent Chow (No. 5002, PMI Feeds, Inc., St. Louis, MO) and water were available *ad libitum*.

F0 females were individually identified by eartag. F0 females were allowed to give birth and rear their litters. Litters were adjusted on pnd 4 to ten pups, maximizing the number of male pups. A total of 15 F1 males per group was assigned to each component in this study. F1 males were assigned to treatment groups by stratified randomization for body weight on pnd 21, so that mean body weight on pnd 21 did not differ among treatment groups. Selected male F1 weanlings were identified by eartag. They were housed in the study room(s) in polycarbonate solid-bottom cages with bedding and provided feed and water *ad libitum*. They were examined once daily by cage-side observation for morbidity or mortality while clinical observations or morbidity/mortality checks for the study animals. All adult animals assigned to the study were euthanized by CO₂ asphyxiation. F1 pups culled on pnd 4 were sacrificed by decapitation.

Additional Methods. In order to allow for collection of individual feed consumption data, F1 weanling males were singly housed from pnd 21 to pnd 52/53. The feed was also analyzed by the manufacturer for the phytoestrogens daidzein, genistein, and glycitein. The singly housed animals, collection of feed consumption data, and the analysis of phytoestrogens in the feed were additions to the basic male pubertal study design. Additional information regarding these and other data collected during this study are presented in the Final Technical Study Report and its appendices.

Study Design

A graphic representation of the component study design is presented in Figure 1. The study began with 20 timed-mated F0 females in each component.

F0 Females

Beginning on gestational day (gd) 20, each female was examined twice daily (a.m. and p.m.) for evidence of littering. Females who were littering at morning and afternoon checks had this information recorded on the gestational sheet. Signs of dystocia or other signs of difficulty at parturition were also recorded, if observed. Any dams whose whole litters were born dead or died prior to pnd 21 were sacrificed, and the number of uterine implantation scars recorded. On pnd 21 of each F1 litter, each F0 dam was euthanized by CO₂ asphyxiation, and the carcass discarded.

Progeny (F1)

All pups were counted, sexed, weighed, and examined as soon as possible on the day of birth (designated as pnd 0) to determine the number of viable and stillborn members of each litter. Thereafter, litters were evaluated for survival, sex, gross observations, and body weights on pnd 4, 7, 14, and 21. Any pup that appeared moribund or died during lactation was necropsied, when possible, to investigate the cause of death and discarded. No organs were weighed or saved. On pnd 4, the size of each litter was adjusted to ten pups, maximizing the number of male pups retained. Natural litters with ten or fewer pups were not culled. All culled pups were sacrificed by decapitation. The F0 dams were allowed to rear their remaining F1 young to pnd 21. On pnd 21, each litter was weaned.

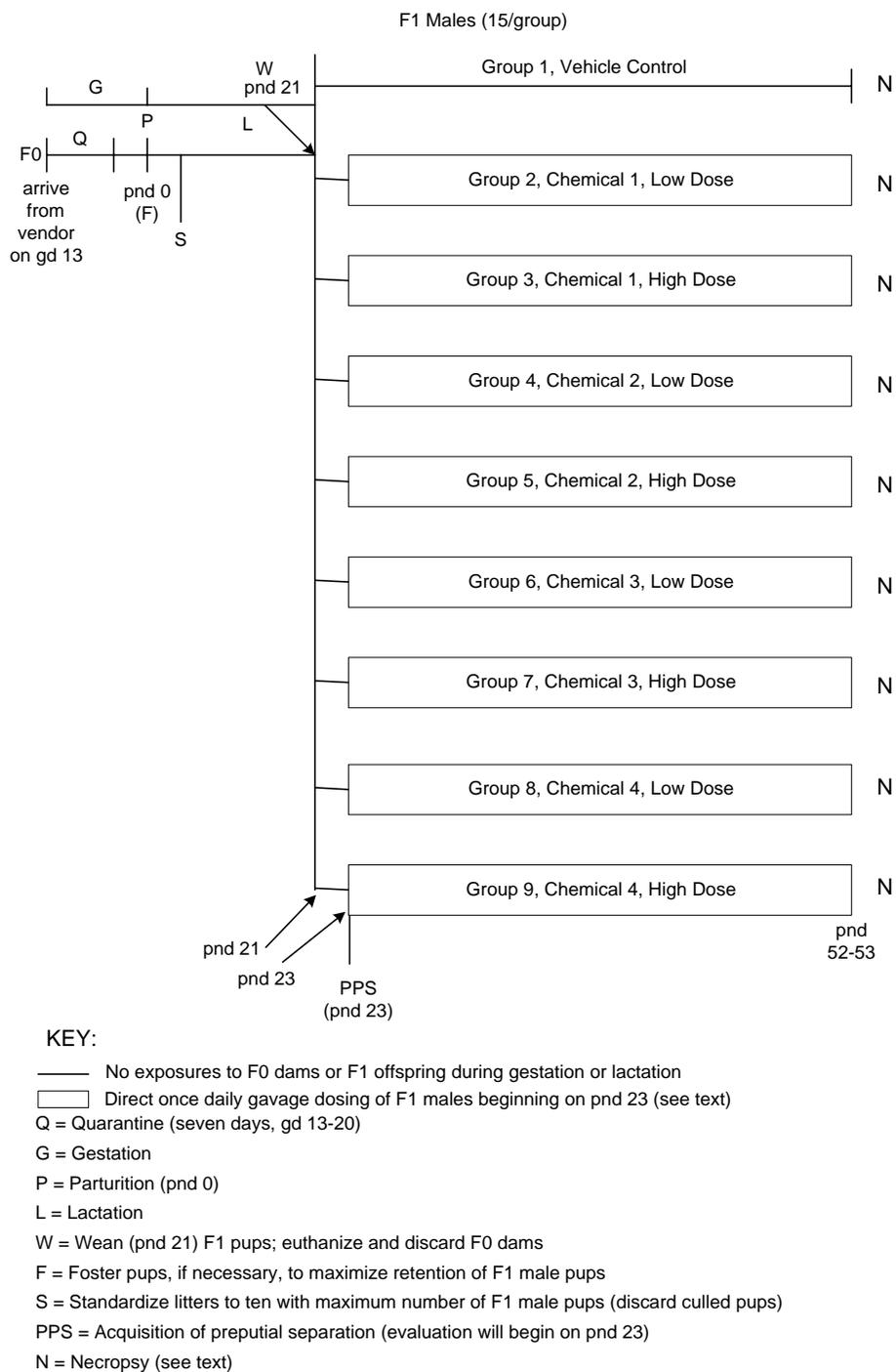


Figure 1. General Component Study Design for the Male Pubertal Assay.

F1 Males

When each F1 litter reached pnd 21, the F1 males from each pnd 21 (wean) date were weight ranked across litters (outliers, i.e., heaviest and lightest pups, were eliminated from selection). The selected males were eartagged and distributed across the seven groups of the component by stratified randomization (e.g., one of the seven heaviest selected males went into each of the nine treatment groups, etc.).

Beginning on pnd 23, each F1 male was dosed with a test material at one of the selected dose levels or the vehicle control (corn oil for all chemicals). EPA selected the eight test chemicals for this evaluation and selected the low and high target doses (in mg/kg/day) for each of them. Each animal was weighed every day prior to treatment and the body weight recorded. Treatments were administered daily by oral gavage from pnd 23 and continuing to pnd 52/53. The treatments were administered on a mg/kg body weight basis, adjusted based on the most recent body weight (Table 1).

Table 1. Study Design, Test Chemicals, and Target Doses

Group No.	No. F1 Males	Chemical	Dose (mg/kg/day)	Concentration (mg/ml)	Dose Volume (ml/kg)
COMPONENT 1					
1	15	– ^a	0	0.0	5
2	15	Atrazine	75	15.0	5
3	15		150	30.0	5
4	15	p,p'-DDE	50	10.0	5
5	15		100	20.0	5
6	15	Vinclozolin	30	6.0	5
7	15		100	20.0	5
8	15	Methoxychlor	25	5.0	5
9	15		50	10.0	5
COMPONENT 2					
10	15	Propylthiouracil	2	0.4	5
11	15		25	5.0	5
12	15	Ketoconazole	50	10.0	5
13	15		100	20.0	5
14	15	Linuron	50	10.0	5
15	15		100	20.0	5
16	15	Phenobarbital	50	10.0	5
17	15		100	20.0	5
18	15	– ^a	0	0.0	5

^a Corn oil, vehicle control

Clinical observations of F1 male study animals were documented at least once daily on pnd 21 and 22 (prior to dosing period) and at least twice daily, at dosing and one to two hours postdosing, throughout the dosing period (pnd 23 to pnd 52 or 53). All F1 males were weighed in the morning on pnd 21 and 22 and every morning during the dosing period on pnd 23 to pnd 52/53, for adjustment of dosing volume based on the most recent body weight. Daily body weights and body weight gains were reported and statistically analyzed.

Beginning on pnd 23, each F1 study male was examined daily for preputial separation. The appearance of partial and complete preputial separation or a persistent thread of tissue between the glans and prepuce was recorded. The day of complete preputial separation was the endpoint used in the analysis for the age of preputial separation. Body weight at acquisition of complete preputial separation was recorded.

Additional In-Life Data

At the request of the sponsor the data for preputial separation and acquisition body weight was rerun excluding control male number 51, age at preputial separation of 48 days. These data are summarized in Table 2-E, and presented in the results section, but are not included in the discussion or abstract.

Feed consumption for the individually-housed F1 weanling males was recorded daily and reported as g/day and as g/kg body weight/day. Calculation of these data were additions to the basic male pubertal study design. Additional information regarding these data and other data collected during this study are available in the Final Technical Study Report and its appendices.

Necropsy for Pnd 52/53 F1 Males

Blood Collection and Hormone Assays

At scheduled necropsy of the F1 males, after terminal anesthesia (CO₂ asphyxiation), the males were weighed and the maximum amount of blood was taken by external cardiac puncture and placed in a labeled tube. The blood was allowed to clot and centrifuged under refrigeration. The resulting serum was analyzed for T4 and TSH. All assays were counted in a Packard Biosciences Cobra II Series Model 5002 gamma counter using RIASMART software. The rat thyroid-stimulating hormone (rTSH) RIA used was a no-extraction, double antibody ¹²⁵I RIA (Amersham Biosciences, Piscataway, NJ) which utilized rTSH antibody, ¹²⁵I-rTSH, rTSH calibrators as the standard curve, and a precipitating solution consisting of donkey anti-rabbit serum coated onto magnetizable polymer particles. The sensitivity of this assay was 0.5 ng/tube. Results were reported as ng/ml.

The T4 RIA used was a no-extraction, solid-phase ¹²⁵I RIA which utilized T4-specific antibody-coated tubes and ¹²⁵I-T4 (DPC, Los Angeles, CA). The T4 (Sigma, St. Louis, MO) standard curve was

prepared in RIA Buffer I (0.01 M sodium phosphate plus 0.85% [w/v] sodium chloride with 0.1% [w/v] sodium azide and 1% [w/v] bovine serum albumin, pH 7.6). T4 controls were prepared in the same matrix as unknown samples by adding known concentrations of T4 to male serum. The sensitivity of this assay was 0.25 µg/dL. Results were reported as µg/dL.

Gross Examination and Histopathology

Once each F1 male was bled, the animal was necropsied and internal thoracic and abdominal organs and cavities examined. Any abnormalities were documented. The following organs were dissected out and weighed: paired testes, paired epididymides, ventral prostate, seminal vesicles with coagulating glands (and fluid), LABC muscle complex, liver, paired kidneys, adrenal glands (paired), pituitary, and thyroid (taken with attached portion of trachea, weighed after fixation and removal of the tracheal portion).

Tissues taken at necropsy were placed in fixative (10% neutral buffered formalin or Bouin's). After 24 hours, tissues placed in Bouin's fixative (testes and epididymides) were rinsed and stored in 70% alcohol until embedded in paraffin. All tissues were transferred to Experimental Pathology Laboratories (EPL) for processing. The thyroid was weighed at EPL, after removal of the trachea. The tissues were sectioned at 3-5 microns and stained with hematoxylin and eosin (H and E) for subsequent histological evaluations. Stained sections were evaluated for pathologic abnormalities and potential treatment-related effects by an AVCP board-certified veterinary pathologist.

Additional Necropsy Data

In addition to ventral prostate weight, the dorsolateral and whole prostate weight were also recorded. Additional information regarding these data and other data collected during this study are available in the Final Technical Study Report and its appendices.

Statistical Analyses

All data for a single chemical (two doses) and a concurrent vehicle control group were analyzed using either parametric ANOVA under the standard assumptions or robust regression methods (Zeger and Liang, 1986; Royall, 1986; Huber, 1967) which do not assume homogeneity of variance or normality. The homogeneity of variance assumption was examined via Levene's test (Levene, 1960). When Levene's test indicated lack of homogeneity of variance ($p < 0.05$), robust regression methods were used to test all treatment effects. The robust regression methods use variance estimators that make no assumptions regarding homogeneity of variance or normality of the data. They were used to test for linear trends across dose as well as overall treatment group differences (via Wald chi-square tests). Significant overall treatment effects were followed by single degree-of-freedom *t*-tests for exposed vs. control group comparisons, if the overall treatment effect was significant. When Levene's test did not reject the hypothesis of homogeneous variances, standard ANOVA techniques were applied for

comparing the treatment groups. The GLM procedure in SAS[®] Version 8 (SAS Institute, Inc., 1999a,b,c,d,e; 2000) was used to test for linear trend, evaluate the overall effect of treatment and, when a significant treatment effect is present, to compare each exposed group to control via Dunnett's test (Dunnett, 1955, 1964). Standard ANOVA methods, as well as Levene's test, are available in the GLM procedure of SAS[®], and the robust regression methods were available in the REGRESS procedure of SUDAAN[®] Release 7.5.4 (Shah *et al.*, 1997) or Release 8.0 (RTI, 2001). Organ weights were also analyzed by Analysis of Covariance (ANCOVA) using the body weight at necropsy as the covariate. When statistically significant effects were observed, treatment means were examined further using LSMeans. The unit of comparison was the weanling F1 male offspring on study.

A test for statistical outliers was performed in the UNIVARIATE procedure of SAS[®] Version 8 (SAS Institute, Inc., 1999a,b,c,d,e; 2000) on F1 male body and organ weights. If examination of pertinent study data did not provide a plausible biologically sound reason for inclusion of the data flagged as "outlier," the data was excluded from summarization and analysis and was designated as outliers. For all statistical tests, $p \leq 0.05$ (one- or two-tailed) was used as the criterion for significance.

Personnel

This study was conducted at RTI International, Research Triangle Park, NC, under contract to Battelle, Columbus, OH. Dr. David P. Houchens, EDSP Program Manager, was the Sponsor's Representative. Dr. R.W. Tyl served as Project Toxicologist. Dr. J. D. George served as Study Director. Reproductive and Developmental Toxicology personnel included Ms. M.C. Marr (Laboratory Supervisor), Ms. C.B. Myers (Reproductive Toxicity Study Supervisor and Data Analyst), Mr. W.P. Ross, Ms. M.C. Rieth, Ms. V.I. Wilson, Ms. L.B. Pelletier, Ms. M.P. Gower, Ms. N.M. Kuney, Ms. R.T. Krebs, Ms. S.W. Pearce, Ms. K.D. Vick, Ms. L. McDonald, Ms. A.J. Parham, Mr. M.D. Crews, Mr. C.G. Leach, Ms. A. B. Goodman, and Mr. T.W. Wiley. Bulk chemical analysis and handling, dose formulation, and dose formulation analysis were provided by the Sponsor through Dr. E.A. Crecelius, PNNL, Battelle Marine Sciences Laboratory, Sequim, WA. Mr. M.M. Veselica (Supervisor, RTI Materials Handling Facility), Mr. D.L. Hubbard, and Mr. R.A. Price provided receipt and disbursement of dose formulations at RTI. Animal care was provided by Dr. D.B. Feldman, DVM, ACLAM, RTI Veterinarian, and Mr. F.N. Ali, Manager of RTI Animal Research Facility. RTI Quality Assurance personnel were Ms. D.A. Drissel, Ms. D.J. Smith, Ms. M.D. Phillips, Ms. T.M. Kenney, Ms. C. Ingalls, and Ms. M. Oh. Ms. K.D. Andrews, QA Consultant, audited the hormone data and analysis.

The final report was prepared by Dr. J.D. George, with assistance from Dr. R.W. Tyl, Ms. B. Hamby, Ms. C.B. Myers, and Ms. M.C. Marr. Ms. C.B. Myers was responsible for data compilation and statistical analyses, and Mr. T.W. Wiley was responsible for data entry. Ms. M.C. Marr was responsible for all activities concerning organization and custody of the study records and for archiving the study records. Ms. D. Bynum and Ms. K. Kehagias provided secretarial assistance.

Compliance

All records, data, biological specimens, and reports will be maintained in storage for the period specified by the contract or for as long as the quality of the preparation affords evaluation, whichever is less. Quality control (QC) and quality assurance (QA) procedures followed those outlined in the Quality Assurance Project Plan (QAPP) prepared for this study and in accordance with the Quality Management Plan (QMP) for this project. The RTI Animal Research Facility is fully accredited by the Association for Assessment and Accreditation of Laboratory Animal Care (AAALAC), International. Animals were housed, handled, and used according to the NRC Guide (NRC, 1996).

RESULTS

Component 1

Control F1 Males

Fifteen untreated F1 males were assigned to the control group for Component 1. These animals served as the concurrent control group for the animals in Component 1 that were treated with atrazine, p,p'-DDE, vinclozolin, and methoxychlor.

In-Life Data From F1 Males Treated With Atrazine

Fifteen untreated F1 males were assigned to the 0, 75, or 150 mg/kg/day atrazine group. One animal assigned to the 75 mg/kg/day atrazine-treated group died prior to scheduled necropsy. An additional eight animals were removed from the study for various reasons including lack of pnd 0 confirmation, (three control, one low dose, and three high dose) and dosing errors (one low dose). Body weights at weaning (pnd 21), on pnd 22, and on the day of initiation of dosing (pnd 23) were equivalent across treatment groups (Figure 2 and Table 2-A). Daily body weights for F1 males were significantly decreased in the high-dose group compared to the control group from pnd 24 to pnd 52. In the low dose group, body weight was decreased compared to the control group from pnd 31 to 52. Body weight change was significantly less in both atrazine-treated groups compared to the control group for pnd 23 to 52 (Table 2-A). On pnd 52, body weight was significantly reduced at both doses of atrazine, with the low and high dose animals weighing 89.6% and 82.8%, respectively, of the control animal weight (Table 2-A).

Clinical observations were noted in the atrazine-treated groups, and included efflux of the dosing solution, rooting postdosing, rooting prior to dosing, rust-colored fur, salivation postdosing, and salivation prior to dosing in 2, 11, 2, 1, 1, and 12 animal(s) in the high-dose group, respectively. Efflux of the dosing solution, rooting postdosing, and salivation prior to dosing were observed in four, six, and eight animals, respectively, in the low-dose group. Two control animals exhibited salivation prior to dosing.

Treatment with atrazine did not affect the day of acquisition of preputial separation (Table 2-A). Body weight at acquisition decreased in a dose-related manner, with the high dose value significantly below the control value. When the data from control male 51 was removed, the age of preputial separation exhibited an increasing trend, with a significant delay in the day of acquisition of preputial separation at the high dose (42.9 days vs. 40.8 for the control group). The low dose group achieved preputial separation at a mean age of 42.0 days, which was also delayed, but did not reach statistical significance (Table 2-E). The average body weight on the day of acquisition of preputial separation still exhibited a decreasing trend and was significantly reduced at the high dose.

Necropsy and Histopathological Data from F1 Males Treated with Atrazine

At necropsy, average body weight exhibited a dose-related decreasing trend, with the low and high dose atrazine-treated groups significantly below the control group (Table 3-A). Absolute pituitary weight was decreased at both the low and high dose, as was liver weight, and seminal vesicles with coagulating glands weight. Absolute paired kidney weight, paired epididymides weight, and LABC weight were decreased at the high dose, whereas ventral prostate weight exhibited a decreasing trend. When organ weights were adjusted with respect to necropsy body weight, no effect of treatment was observed, with the exception of an increasing trend for adjusted paired testes weight. In addition, atrazine had no effect on circulating T4 or TSH levels (Table 3-A).

Gross necropsy findings were minimal, and included one animal each in the control and low-dose group, and three animals in the high-dose group with hydronephrosis, and one animal in the low-dose group with a small, undescended testis and a small left epididymis. No treatment-related histopathology was observed.

In-Life Data from F1 Males Treated with p, p'-DDE

Fifteen untreated F1 males were assigned to the 0, 50, or 100 mg/kg/day p, p'-DDE groups. Three control animals and one low dose animal were removed because their correct pnd 0 could not be determined. One animal in the 50 mg/kg/day group was euthanized on pnd 29 due to a leg injury. Body weight at weaning (pnd 21), prior to initiation of dosing (pnd 22), and on the day of initiation of dosing (pnd 23) was equivalent across treatment groups (Figure 3 and Table 2-B). Daily body weights for F1 males were equivalent across treatment groups, as was body weight on pnd 52. Animals in the low and high dose p,p'-DDE groups weighed 99.9 and 98.7% of the control value on pnd 52 (Table 2-B).

Clinical observations were noted in the p, p'-DDE-treated groups, and consisted of chromodacryorrhea, efflux of the dosing solution, rooting postdosing, and salivation prior to dosing, in one, one, nine, and three, animal(s) in the high-dose group, respectively, and chromodacryorrhea, rooting postdosing, rust-colored fur, and salivation prior to dosing in one each in the low-dose group. Two control animals were observed to salivate prior to dosing.

Treatment with p, p'-DDE significantly delayed preputial separation in the low- and high-dose groups (44.9 and 45.7 days, respectively) compared to the control group (41.4 days, Table 2-B). On the day of acquisition, average body weight was significantly increased at both doses of p,p'-DDE, compared to the control group value. Removal of the data from control male 51 did not alter these results (Table 2-E).

Necropsy and Histopathological Data from F1 Males Treated with p, p'-DDE

At necropsy, average body weight was equivalent across treatment groups (Table 3-B). Absolute weight of the pituitary, paired adrenal glands, paired testes, ventral prostate, seminal vesicles and coagulating glands, and LABC was unaffected by p, p'-DDE treatment. Absolute thyroid, liver, and paired kidney weight were increased at both doses of p, p'-DDE, whereas paired epididymides weight was decreased at the high dose. When organ weights were adjusted with respect to body weight at necropsy, thyroid, liver, and paired kidney weights were significantly increased at both doses of p, p'-DDE. Adjusted paired epididymides weight was decreased at the high dose, whereas the LABC weight exhibited a decreasing linear trend with no significant pairwise comparisons. T4 levels (in microg/dL) exhibited a linear decreasing trend, and were significantly decreased at the high dose (by 17.8% compared to the control value). TSH levels (ng/ml) were slightly increased in a dose-related manner (28.4 and 31.7%, compared to the control group values), but these differences did not reach statistical significance (Table 3-B).

Gross necropsy findings were minimal, and included hydronephrosis in one animal in the control group, one animal in the low-dose group, and four animals in the high-dose group, and one animal each in the high-dose group with chromodacryorrhea or intestines distended with air. No treatment-related histopathology was observed.

In-Life Data from F1 Males Treated with Vinclozolin

Fifteen untreated F1 males were assigned to the 0, 30, or 100 mg/kg/day vinclozolin groups. Three animals in the control and high dose groups, and two animals in the low dose group were removed because their correct pnd 0 could not be determined. Body weight at weaning (pnd 21), pnd 22, and on the day of initiation of dosing (pnd 23) was equivalent across treatment groups (Figure 4 and Table 2-C). In addition, daily body weights for F1 males were unaffected by vinclozolin treatment through pnd 52. Body weight change was equivalent across treatment groups for pnd 23 to 52 (Table 2-C). Final body weight on pnd 52 was 102.2 and 97.4% of the control value in the low and high dose groups, respectively (Table 2-C).

Clinical observations were noted in the vinclozolin-treated groups, and consisted of efflux of dosing solution, piloerection, rooting postdosing, and salivation prior to dosing in three, one, four, and five high-dose animal(s), respectively, and efflux of the dosing solution, rooting postdosing, salivation prior to dosing, and sore(s) in one, four, two, and one low-dose animal(s), respectively. Two animals in the low-dose group were not dosed with vinclozolin on pnd 50, because there was not enough dosing solution. These animals resumed dosing the following day until scheduled necropsy. Two control animals exhibited salivation prior to dosing.

Treatment with vinclozolin resulted in a dose-related delay in preputial separation that was significant in both treated groups (Table 2-C). The average postnatal day of preputial separation was 43.8 and 46.8 for the low- and high-dose groups, respectively, compared to 41.4 days for the control group. Body weight on the day of acquisition of preputial separation was significantly increased for both the low and the high dose vinclozolin-treated groups, likely secondary to the older age of these animals. Two males in the high-dose group failed to acquire preputial separation by pnd 52, and were therefore excluded from the calculation of this parameter. It is not known whether these animals would have exhibited preputial separation if the study had been extended. Removal of the data from control male 51 did not alter these results (Table 2-E).

Necropsy and Histopathological Data from F1 Males Treated with Vinclozolin

At necropsy, average body weight exhibited a decreasing trend that was not dose-related (Table 3-C). Absolute pituitary, thyroid, liver, paired adrenal, paired kidney, and paired testes weights were unaffected by treatment with vinclozolin. However, absolute seminal vesicles with coagulating glands, absolute paired epididymides, and LABC weights were decreased at the high dose. Ventral prostate weight exhibited a decreasing trend. When organ weights were adjusted with respect to necropsy body weight, paired testes, paired epididymides, and seminal vesicles with coagulating gland weights were decreased at both dose levels. Adjusted LABC weight was significantly decreased at the high dose. Adjusted ventral prostate weight exhibited a decreasing trend, but no significant pairwise differences in either vinclozolin-treated group compared to the control group. Vinclozolin treatment had no significant effect on adjusted pituitary, thyroid, liver, adrenal, or kidney weight. T4 levels were significantly decreased at both doses of vinclozolin, whereas TSH levels were unaffected (Table 3-C).

Gross necropsy findings were minimal, and included one to four animals in each group with hydronephrosis, and one animal in the high-dose group that had small seminal vesicles. No treatment-related histopathology was observed.

In-Life Data from F1 Males Treated with Methoxychlor

Fifteen untreated F1 males were assigned to the 0, 25, or 50 mg/kg/day methoxychlor groups. Three animals in the control group and two animals in the high dose group were removed because their correct pnd 0 could not be determined. Body weights at weaning (pnd 21), pnd 22, and on the day of initiation of dosing (pnd 23) were equivalent across treatment groups (Figure 5 and Table 2-D). In addition, daily body weights for F1 males were unaffected by methoxychlor treatment through pnd 52. A significant decreasing trend was observed for body weight gain for pnd 23 to 52 (Table 2-D). On pnd 52, body weight in the low and high dose groups was 96.1 and 94.6% of the control value, respectively (Table 2-D).

Clinical observations were noted in the methoxychlor-treated groups and consisted of chromodacryorrhea in one animal at the low dose, efflux of dosing solution in two animals at the low dose and one animal at the high-dose, and rooting postdosing in two animals at the high-dose. Two control animals exhibited salivation prior to dosing.

Treatment with methoxychlor had no significant effect on the average day of preputial separation (Table 2-D). The average postnatal day of preputial separation was 41.4, 41.8 and 41.8 for the control, low- and high-dose groups, respectively. One animal at the mid dose failed to achieve preputial separation prior to necropsy. Body weight on the day of acquisition of preputial separation was equivalent across the treatment groups (Table 2-D). Removal of the data from control male 51 did not alter these results (Table 2-E).

Necropsy and Histopathological Data from F1 Males Treated with Methoxychlor

At necropsy, average body weight did not significantly differ among dose groups (Table 3-D). Absolute pituitary, thyroid, liver, paired kidney, paired testes, paired epididymides, ventral prostate, and LABC weights were not significantly affected by treatment with methoxychlor. A dose-related increasing trend was observed for absolute paired adrenal weight, with the high dose significantly increased compared to the control group. Absolute seminal vesicles with coagulating glands weights exhibited a decreasing trend, and was significantly decreased at the high-dose of methoxychlor. When organ weights were adjusted with respect to necropsy body weight, paired adrenal gland weight was significantly increased at the high dose. In addition, seminal vesicles and coagulating glands weight was decreased at the high dose. T4 and TSH levels were unaffected by treatment with methoxychlor (Table 3-D).

Gross necropsy findings were minimal, and included one animal each in the control and high-dose group with hydronephrosis. No treatment-related histopathology was observed.

Component 2

Control F1 Males

Fifteen untreated F1 males were assigned to the control group for Component 2. These animals served as the concurrent control group for the animals in Component 2 that were treated with propylthiouracil, ketoconazole, linuron, and phenobarbital. One animal in the control group was found dead on pnd 31 due to a misdirected dose, and was removed from further evaluation in the study.

In-Life Data from F1 Males Treated with Propylthiouracil

Fifteen untreated F1 males were assigned to the 0, 2, or 25 mg/kg/day propylthiouracil groups. One animal in the control group was found dead on pnd 31 due to a misdirected dose, and was removed from the study, and one animal in the high-dose group was found dead on pnd 50. Body weight at

weaning (pnd 21), pnd 22, and on the day of initiation of dosing (pnd 23) was equivalent across treatment groups (Figure 6 and Table 2-F). In addition, daily body weights for F1 males were unaffected by propylthiouracil treatment through pnd 33. A significant dose-related decreasing trend was observed for all time points from pnd 32 through pnd 52, with the high-dose group significantly decreased compared to the control group at all time points beginning with pnd 33. Overall weight change during the treatment period until pnd 52 was significantly reduced in both the low (by 9.6%) and high (by 58.1%) dose groups, compared to the control group value (Table 2-F). On pnd 52, the low and high dose groups weighed 92.3 and 53.7% of the control group, respectively (Table 2-F).

Clinical observations were noted in the propylthiouracil-treated groups, and consisted of efflux of the dosing solution, rooting postdosing, rough coat, and salivation prior to dosing in 1, 2, 1, and 1 animal(s) in the low-dose group, and efflux of the dosing solution, rooting postdosing, and salivation prior to dosing in 1, 14, and 1 animal(s) in the high-dose group. The control animals had no distinctive clinical signs. As noted previously, one animal each in the control and high-dose group was found dead prior to necropsy.

Treatment with propylthiouracil significantly delayed acquisition of preputial separation in the high-dose group (Table 2-F). The average postnatal day of preputial separation was 40.4 and 43.3 for the low- and high-dose groups, respectively, compared to 39.6 days for the control group. Body weight on the day of acquisition of preputial separation was significantly decreased at the high dose (Table 2-F).

Necropsy and Histopathological Data from F1 Males Treated with Propylthiouracil

At necropsy, average body weight exhibited a decreasing trend that was dose-related, and was significantly decreased at both the low and high doses, compared to the control group (Table 3-E). Both the low and high-dose animals exhibited a dose-related increase in absolute thyroid, liver, adrenal, and kidney weight significantly increased over the control group value. All other organ weights exhibited a dose-related decreasing trend, with all absolute organ weights except seminal vesicles with coagulating gland weight was significantly reduced at the high-dose. When organ weights were adjusted with respect to necropsy body weight, both the thyroid and the seminal vesicles with coagulating glands exhibited increased adjusted organ weight for both treatment groups. The adjusted weights of the paired adrenal glands and the paired kidneys were significantly decreased at the low dose, although there was not statistically significant change at the high-dose. Adjusted paired liver and epididymides weight exhibited an increasing trend. As anticipated, T4 levels were significantly reduced at both dose levels, in a dose-related manner, and TSH levels were significantly increased in a dose-related manner at both doses (Table 3-E).

Gross necropsy findings included one animal in the control group with urinary calculi and thickened bladder wall, one animal each in the low-dose group exhibiting hydronephrosis, pulmonary

foci, reduced dorsolateral or ventral prostate, or reduced seminal vesicles, and nine animals with enlarged and/or otherwise thyroid glands. In the high-dose group, 4 animals had reduced adrenal glands, 2 animals had hydronephrosis, 1 animal had small kidneys, pituitary, dorsolateral prostate, or ventral prostate, 6 animals had hepatic abnormalities (mottled or reduced in size), and 14 animals had thyroid observations, including enlargement, reddening, or darkening. All animals in the high-dose group exhibited thyroid follicular cell hypertrophy/hyperplasia, characterized by increased size and apparent number of follicular cells, and reduction of follicular lumen size. The severity of these changes were scored as:

- ◆ minimal = multifocal follicles affected, with size and number of follicular cells slightly enlarged and increased;
- ◆ mild = diffuse change with further increased cell size and hyperplasia;
- ◆ moderate = enhanced severity with the presence of notable numbers of follicular cell mitoses; and
- ◆ marked = increased mitotic rate, some degenerative cells within the follicular epithelium, and obvious enlargement of the thyroid shape and size.

Based on these criteria, no animals were scored as minimal, one animal was scored as mild, nine moderate, and four marked at the high dose. Based on these results, the low dose thyroids were also examined, revealing that all 15 animals at the low dose also exhibited thyroid hypertrophy/hyperplasia. As would be expected, the severity was somewhat less, with scores of 0 minimal, 10 mild, 5 moderate, and no markedly affected animals. These changes were reflected in the dose-related increases in adjusted thyroid weight (for body weight at necropsy) at both dose levels.

In-Life Data from F1 Males Treated with Ketoconazole

Fifteen untreated F1 males were assigned to the 0, 50, or 100 mg/kg/day ketoconazole group. One animal each in the control and high-dose group died of a misdirected dose on pnd 31 and 37, respectively, and was removed from further evaluation in the study. Daily body weight from pnd 23 to pnd 52 was largely unaffected by treatment with ketoconazole (Figure 7 and Table 2-G). Body weight was equivalent across treatment groups on pnd 23 and 52. A significant decreasing trend was observed for body weight on pnd 46, 48, and 49, but with no pairwise difference from controls. Body weight gain (pnd 23 to 52) exhibited a decreasing trend (Table 2-G). On pnd 52, animals in the low and high dose groups weighed 100.0 and 94.4% of the control value, respectively.

Clinical observations noted in the ketoconazole-treated groups consisted of efflux of dosing solution, rooting postdosing, rooting prior to dosing, rough coat, and salivation prior to dosing in 1, 14, 1, 1, and 4 animal(s) in the low-dose group, respectively, and efflux of dosing solution, piloerection, rooting postdosing, rough coat, and salivation prior to dosing in 1, 1, 15, 2, and 13 animal(s) in the high-dose group, respectively.

Treatment with ketoconazole resulted in a significant delay in the average postnatal day of preputial separation at both treatment levels (Table 2-G). Acquisition of preputial separation occurred at 42.3 days in the low-dose group and 44.1 days in the high-dose group, compared to 39.6 days for the control group. Body weight on the day of acquisition was increased at both treatment levels in a dose-related manner, likely reflecting the older age of the ketoconazole-treated animals.

Necropsy and Histopathological Data from F1 Males Treated with Ketoconazole

At necropsy, average body weight was equivalent across treatment groups (Table 3-F). Absolute paired adrenal weights were increased in a dose-related manner that was significant at both treatment levels of ketoconazole. Paired testes, epididymides, ventral prostate, seminal vesicles with coagulating gland, and LABC weight exhibited dose-related decreasing trends. Seminal vesicles with coagulating glands weight was significantly decreased at both treatment levels, whereas, paired testes weight was decreased at the high dose. When organ weights were adjusted with respect to necropsy body weight, liver weight and paired adrenal glands weight were significantly increased at both treatment levels, whereas seminal vesicles with coagulating glands weight was decreased at both treatment levels. Adjusted paired kidney weight increased in a dose-related manner that was significant at the high dose of ketoconazole, whereas adjusted paired testes weight was significantly decreased at the high dose. Ketoconazole treatment had no significant effect on T4 or TSH levels (Table 3-F).

Gross necropsy findings included abnormal adrenal gland, hydronephrosis, small dorsolateral prostate, and small seminal vesicles in four, one, one, and one animal(s) in the low-dose group, respectively, and abnormal adrenal gland, hydronephrosis, pulmonary foci, small dorsolateral prostate, small ventral prostate, and small seminal vesicles in six, two, one, one, three, and four animal(s) in the high-dose group. One control animal had urinary calculi and thickened bladder wall. No treatment-related histopathology was observed.

In-Life Data from F1 Males Treated with Linuron

Fifteen untreated F1 males were assigned to the 0, 50, or 100 mg/kg/day linuron group. One animal assigned to the control group was found dead on pnd 31 due to a misdirected gavage dose, and was removed from further evaluation in the study. Body weights were equivalent across treatment groups from pnd 21 to pnd 23 (Figure 8 and Table 2-H). Thereafter, a significant decreasing trend was observed for daily body weights until pnd 52. The high-dose group weighed significantly less than the control group every day from pnd 25 to pnd 52. Daily body weight in the low-dose group was significantly less than the control group during the middle portion of the dosing period, i.e., pnd 32 to pnd 43, with the exception of pnd 36. Body weight change for the entire treatment period from pnd 23 to pnd 52 was significantly reduced at both dose levels of linuron (Table 2-H). On pnd 52, the low and the high dose animals weighed 94.0 and 84.9% of the control animals.

Clinical observations were noted in the linuron-treated groups, and consisted of rooting postdosing, salivation postdosing, and salivation prior to dosing in 15, 1, and 6 animal(s) in the low-dose group, and difficult (resisting) to dose, efflux of dosing solution, rooting postdosing, and salivation prior to dosing in 1, 1, 15, and 14 animal(s) at the high-dose.

Treatment with linuron significantly delayed preputial separation at both dose levels (Table 2-H). Preputial separation occurred at 43.6 days and 45.5 days in the low- and high-dose groups, respectively, compared to 39.6 days for the control animals. Average body weight at acquisition was significantly increased in both linuron-treated groups, likely reflecting the older age of these animals.

Necropsy and Histopathological Data from F1 Males Treated with Linuron

At necropsy, average body weight exhibited a dose-related decreasing trend, with the low- and high-dose linuron-treated group values significantly below the control group value (Table 3-G). Absolute pituitary, paired kidney, ventral prostate, seminal vesicles with coagulating glands and LABC weights decreased in a dose-related manner, with significant reductions at both doses of linuron. Thyroid, liver, paired testes, and paired epididymides weights were decreased only at the high dose. Paired adrenal gland weight was not affected by linuron treatment. When organ weights were adjusted with respect to necropsy body weight, adjusted liver weight exhibited a significant dose-related increase at both the low- and high-dose of linuron. Adjusted ventral prostate weight was decreased at the low, but not the high-dose of linuron, whereas adjusted seminal vesicles with coagulating glands weight was decreased at the high dose. T4 levels decreased in a dose-related manner with the values in both linuron-treated groups significantly decreased compared to control values (by 20.4% and 41.2%, low- and high-dose, respectively). TSH levels were significantly decreased at the low dose (by 22.2%) compared to the control animals. The high-dose animals exhibited a 17.8% decrease in TSH levels, but this did not reach statistical significance (Table 3-G).

Gross necropsy findings included two, two, and three animal(s) in the low-dose group with small dorsolateral prostate, small ventral prostate, and small seminal vesicles, respectively, one, one, one, three, four, six, and one animal(s) in the high-dose group with hydronephrosis, reddened lungs, small pituitary, small dorsolateral prostate, small ventral prostate, small seminal vesicles, and enlarged spleen with white foci, respectively. One animal in the control group had urinary calculi and a thickened bladder wall. No treatment-related histopathology was observed.

In-Life Data from F1 Males Treated with Phenobarbital

Fifteen untreated F1 males were assigned to the 0, 50, or 100 mg/kg/day phenobarbital group. One animal assigned to the control group was found dead on pnd 31 due to a misdirected gavage dose, and was removed from further evaluation in the study. Body weights were generally equivalent across treatment groups from pnd 21 to pnd 53, with the exception of decreasing trends on pnd 32, 33, 34, 37,

38, 39, 49, 50, and 52 with no significant pairwise comparisons (Figure 9 and Table 2-I). Body weight change for the entire treatment period from pnd 23 to pnd 52 exhibited a decreasing trend and treatment effect with no significant pairwise comparisons. On pnd 52, the low and high dose animals weighed 99.8 and 93.4% of the control value, respectively (Table 2-I).

Clinical observations were noted in the phenobarbital-treated groups, and consisted of ataxia postdosing, efflux of the dosing solution, prone postdosing, rooting postdose, rough coat, and salivation prior to dosing in 6, 1, 4, 12, 4, and 4 animal(s) in the low-dose group, and ataxia postdosing, efflux of the dosing solution, prone postdosing, rooting postdosing, rough coat, and salivation prior to dosing in 5, 1, 15, 15, 3, and 6 animal(s) at the high dose.

Treatment with phenobarbital significantly delayed preputial separation at both dose levels (Table 2-I). Preputial separation was observed at 41.3 days and 43.0 days in the low- and high-dose groups, respectively, compared to 39.6 days for the control animals. Average body weight at acquisition was significantly increased in the high-dose group, and also increased (by 6.25%, $p>0.05$) at the low dose of phenobarbital, compared to the control group, likely reflecting the older age of these animals.

Necropsy and Histopathological Data from F1 Males Treated with Phenobarbital

At necropsy, average body weight exhibited a dose-related decreasing trend with no significant pairwise comparisons (Table 3-H). Absolute thyroid and liver weight increased in a dose-related manner that was significant at both doses of phenobarbital. Absolute paired testes, paired epididymides, seminal vesicles with coagulating glands, and LABC weight each exhibited a decreasing trend and a significant decrease at the high dose compared to the control group value. When organ weights were adjusted with respect to necropsy body weight, the effects on the thyroid, liver, paired testis, and LABC were the same as observed for the absolute organ weights; paired epididymides weight exhibited a decreasing trend, only. T4 and TSH levels were not affected by phenobarbital treatment (Table 3-H).

Gross necropsy findings included two, one, one, and one, animal(s) in the low-dose group with hydronephrosis, small dorsolateral prostate, small ventral prostate, and small seminal vesicles, respectively, two, two, two, and three animal(s) in the high-dose group with hydronephrosis, small dorsolateral prostate, small ventral prostate, and small seminal vesicles, respectively. One animal in the control group had urinary calculi and a thickened bladder wall. No treatment-related histopathology was observed.

DISCUSSION AND CONCLUSION

This study was designed to gather information describing the conduct and usefulness of the male pubertal assay, that has been designated as an optional Endocrine Disruptor Tier I screening protocol (EDSTAC, 1998), using compounds selected to aid in the optimization of the protocol. Currently in the

prevalidation stage, the male pubertal assay provides a means of screening apical effects of endocrine disruptors that may alter a number of endocrine-dependent mechanisms, including estrogenic-, androgenic-, and thyroid hormone-related processes (Goldman *et al.*, 2000). As summarized in Goldman *et al.* (2000), the male pubertal protocol should be able to detect alterations in sexual maturation and thyroid function. The endpoints in the current version of this protocol were chosen to reflect specific changes in pubertal development, thyroid function, or general toxicity. In an effort to evaluate the ability of this protocol to detect alterations each of these areas, the following compounds were tested:

- ◆ atrazine (affects the hypothalamus-pituitary axis); p,p'-DDE (stable metabolite of DDT; anti-androgen through competitive binding to the androgen receptor);
- ◆ vinclozolin (metabolites M1 and M2 act as anti-androgen; competitive binding to androgen receptor; M1 also binds weakly to the rat progesterone receptor);
- ◆ methoxychlor (a xeno-estrogen through α -estrogen receptor, anti-estrogen through β -estrogen receptor and an anti-androgen through androgen receptor mediated mechanism);
- ◆ propylthiouracil (affects the thyroid directly, causing hypothyroidism); linuron (anti-androgen; competitive binding to androgen receptor);
- ◆ ketoconazole (inhibits steroidogenesis in both sexes); and
- ◆ phenobarbital (induces P450 isoforms predominantly in the liver, accelerates metabolism of endogenous hormones and exogenous xenobiotics).

The results of this study are discussed below with respect to how the protocol performed with respect to the test compounds.

Atrazine. Treatment with atrazine up to 150 mg/kg/day did not affect the day of acquisition of preputial separation. Adjusted organ weights revealed an increasing trend for paired testes weight but no other differences. No differences were noted in T4 or TSH levels, and no treatment-related histopathological changes were observed in the thyroid, testes, or epididymides.

p,p'-DDE. Treatment with 50 or 100 mg/kg/day p,p'-DDE significantly delayed preputial separation at both treatment levels. Adjusted thyroid, liver, and paired kidney weights were significantly increased at both doses of p,p'-DDE. With respect to reproductive tissues, adjusted paired epididymides weight exhibited a significant decrease at the high dose. Decreased circulating T4 levels were observed

at the high dose, whereas TSH levels exhibited no effect of treatment. No treatment-related histopathological change were observed in the thyroid, testes, or epididymides.

Vinclozolin. The day of acquisition of preputial separation exhibited significant delays at both the 30 and 100 mg/kg/day dose levels. In addition, three males in the high-dose group failed to achieve preputial separation prior to scheduled necropsy. Adjusted testes (increase), paired epididymides (decrease), and paired seminal vesicles with coagulating glands (decrease) weights exhibited significant treatment-related effects at both dose levels. Adjusted LABC weight was significantly decreased at the high dose, whereas adjusted ventral prostate weight exhibited a significant treatment effect. Circulating T4 levels were significantly decreased at both doses of vinclozolin, while no effect was observed on TSH. No treatment related histopathological changes were observed in the thyroid, testes, or epididymides.

Methoxychlor. No effect on preputial separation was noted at either 25 or 50 mg/kg/day methoxychlor. Adjusted paired adrenal weight (increase) and seminal vesicle with coagulating glands weight (decrease) exhibited a significant effect at the high dose. Thyroid hormone levels were unaffected, and no treatment-related histopathology was observed. These results are in accordance with those observed by Gray *et al.* (1989), who saw delayed puberty, and altered reproductive organ weights, TSH levels, and testicular histopathology at doses higher than 50 mg/kg/day, in F1 animals exposed to methoxychlor from gestation through pnd 15.

Propylthiouracil. As expected, propylthiouracil produced a decrease in the circulating T4 and an increase in the circulating level of TSH, increased adjusted thyroid weight, and thyroid follicular cell hypertrophy/hyperplasia at both 2 and 25 mg/kg/day. Preputial separation was significantly delayed at the high dose. Treatment effects were also observed at both doses as increased adjusted weights of the seminal vesicles with coagulating glands. Adjusted paired epididymides weight exhibited an increasing trend.

Ketoconazole. The postnatal day of acquisition of preputial separation was delayed at both doses of ketoconazole. Other treatment-related changes observed at both treatment levels included increased adjusted liver and paired adrenal weight, and decreased adjusted seminal vesicles with coagulating glands weight. The high dose of ketoconazole also increased adjusted paired kidney weight and decreased adjusted paired testes weight. No effect of treatment was observed histologically in the testes, epididymides, or thyroid. Thyroid hormone levels were not affected.

Linuron. Linuron delayed puberty at both the 50 and 100 mg/kg/day dose levels, as evidenced by delayed acquisition of preputial separation. Of the organ weights evaluated at necropsy, only adjusted liver weight exhibited a treatment effect (increase) at both dose levels; adjusted seminal vesicles with coagulating glands weight was decreased at the high dose. Both T4 and TSH levels were decreased at both dose levels, although the decrease in TSH at the high dose of linuron did not reach statistical

significance. No treatment-related histopathological changes were observed in the testes, epididymides, or thyroid.

Phenobarbital. The postnatal day of acquisition of preputial separation was delayed at both doses of phenobarbital. In addition, treatment-related effects were detected at both dose levels in an increase in the adjusted weights of the thyroid and liver. A decreased in adjusted organ weight was observed at the high dose for paired testes and LABC. No effect of treatment was observed for thyroid hormone levels or histologically in the testes, epididymides, or thyroid.

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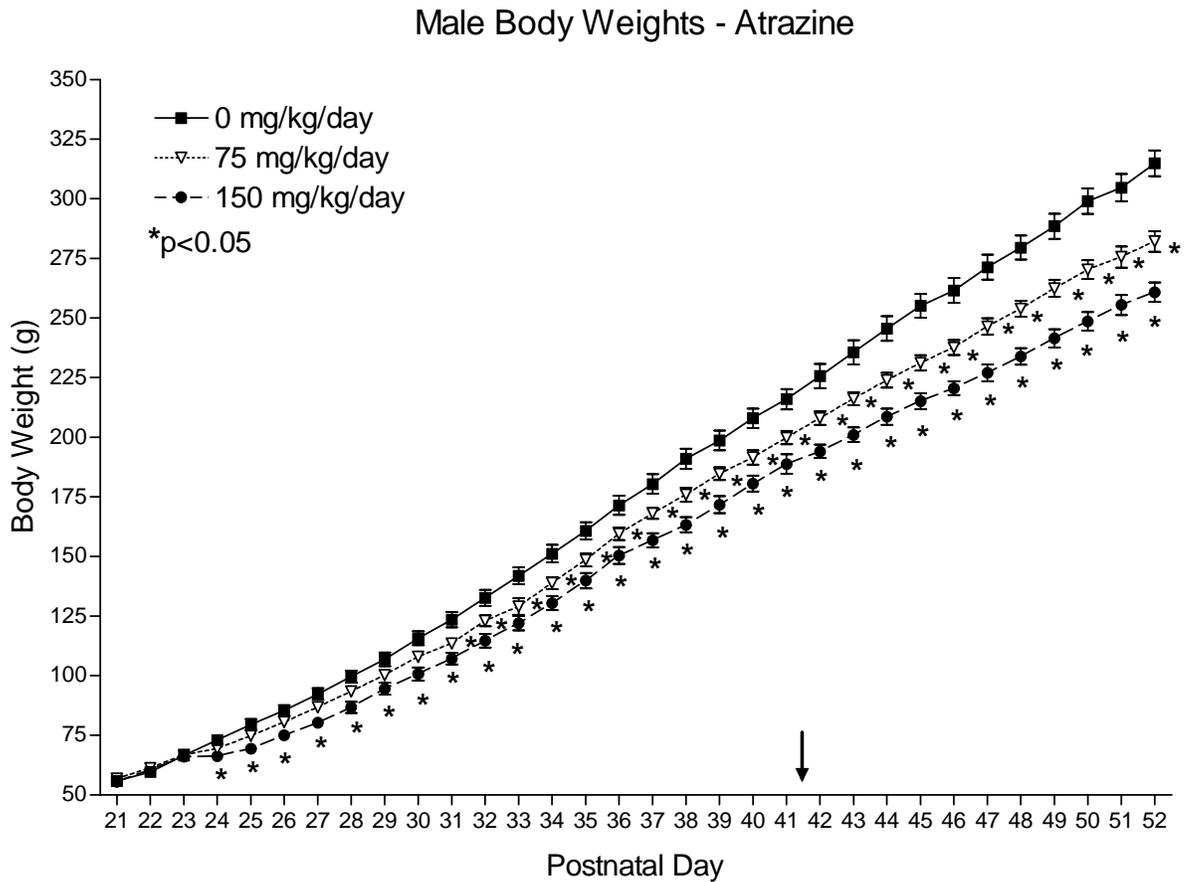


Figure 2. Mean Daily Body Weight in Pubertal Males Treated with 0, 75, or 150 mg/kg/day Atrazine. The mean day of preputial separation (pps) for the control group is indicated by the arrow. Data are presented as mean \pm S.E.M. (n = 12-15 animals per group).

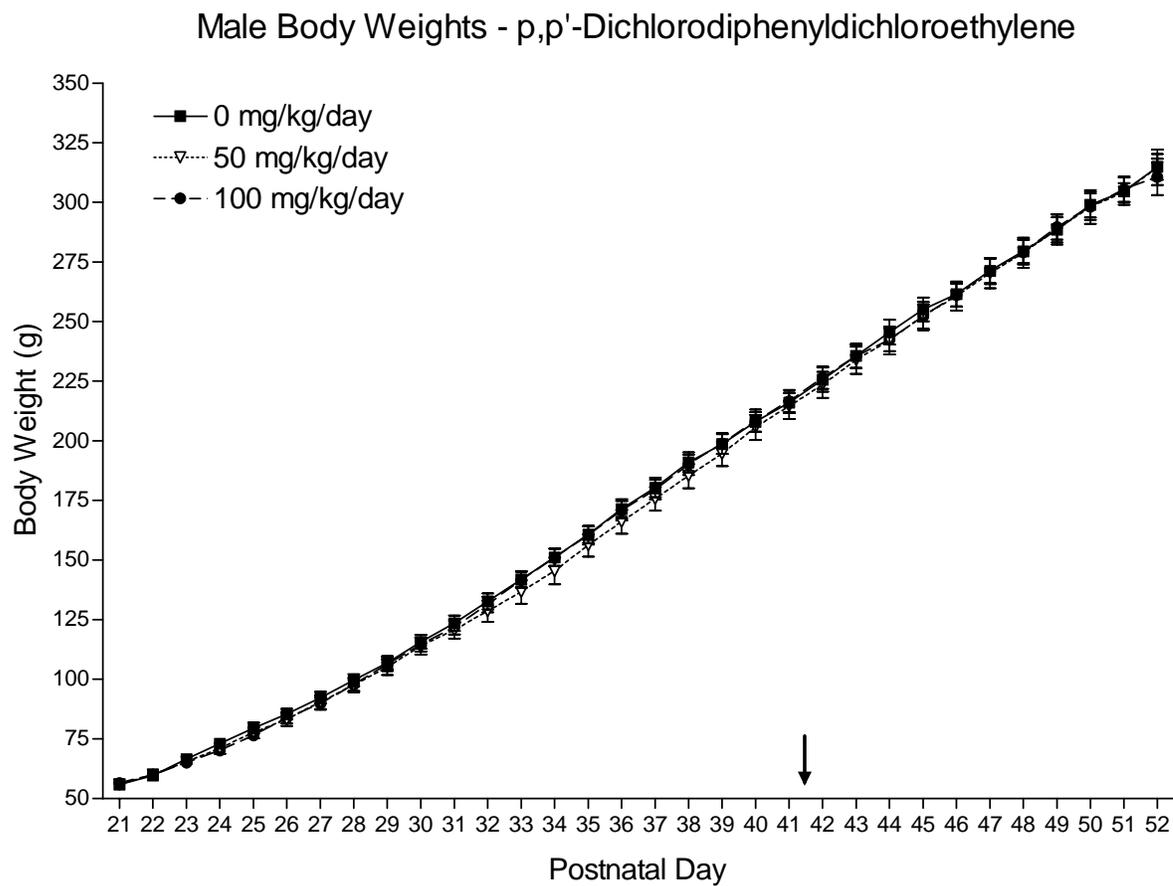


Figure 3. Mean Daily Body Weight in Pubertal Males Treated with 0, 50, or 100 mg/kg/day p,p'-DDE. The mean day of preputial separation (pps) for the control group is indicated by the arrow. Data are presented as mean \pm S.E.M. (n = 12-15 animals per group).

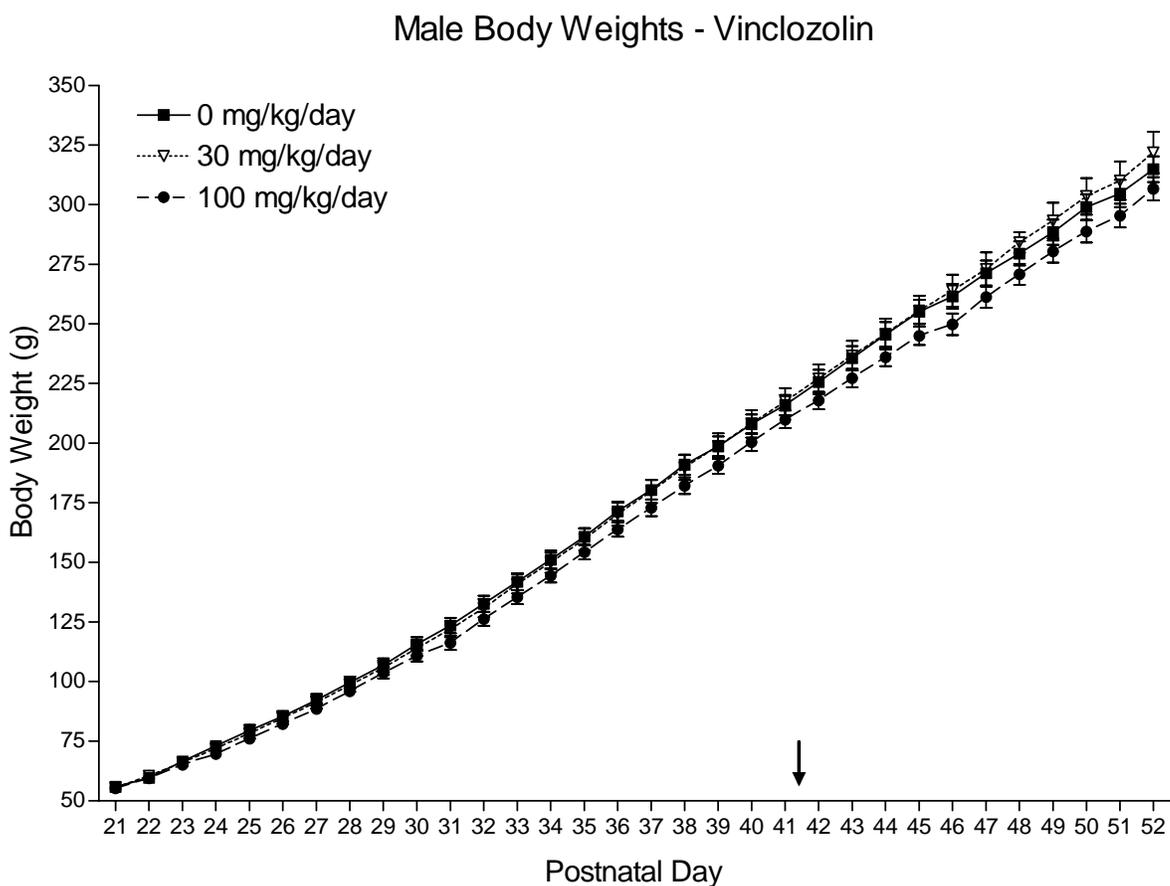


Figure 4. Mean Daily Body Weight in Pubertal Males Treated with 0, 30, or 100 mg/kg/day Vinclozolin. The mean day of preputial separation (pps) for the control group is indicated by the arrow. Data are presented as mean \pm S.E.M. (n = 12-13 animals per group).

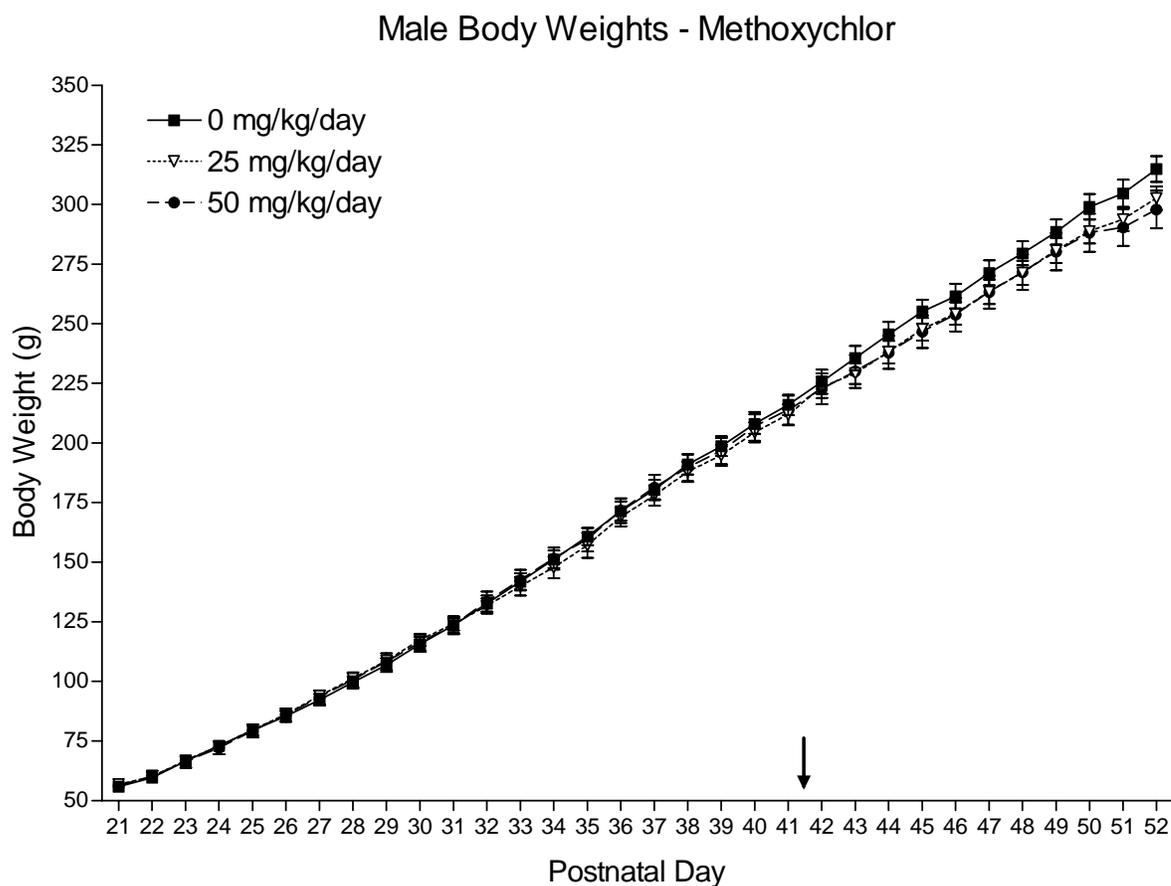


Figure 5. Mean Daily Body Weight in Pubertal Males Treated with 0, 25, or 50 mg/kg/day Methoxychlor. The mean day of preputial separation (pps) for the control group is indicated by the arrow. Data are presented as mean \pm S.E.M. (n = 12-15 animals per group).

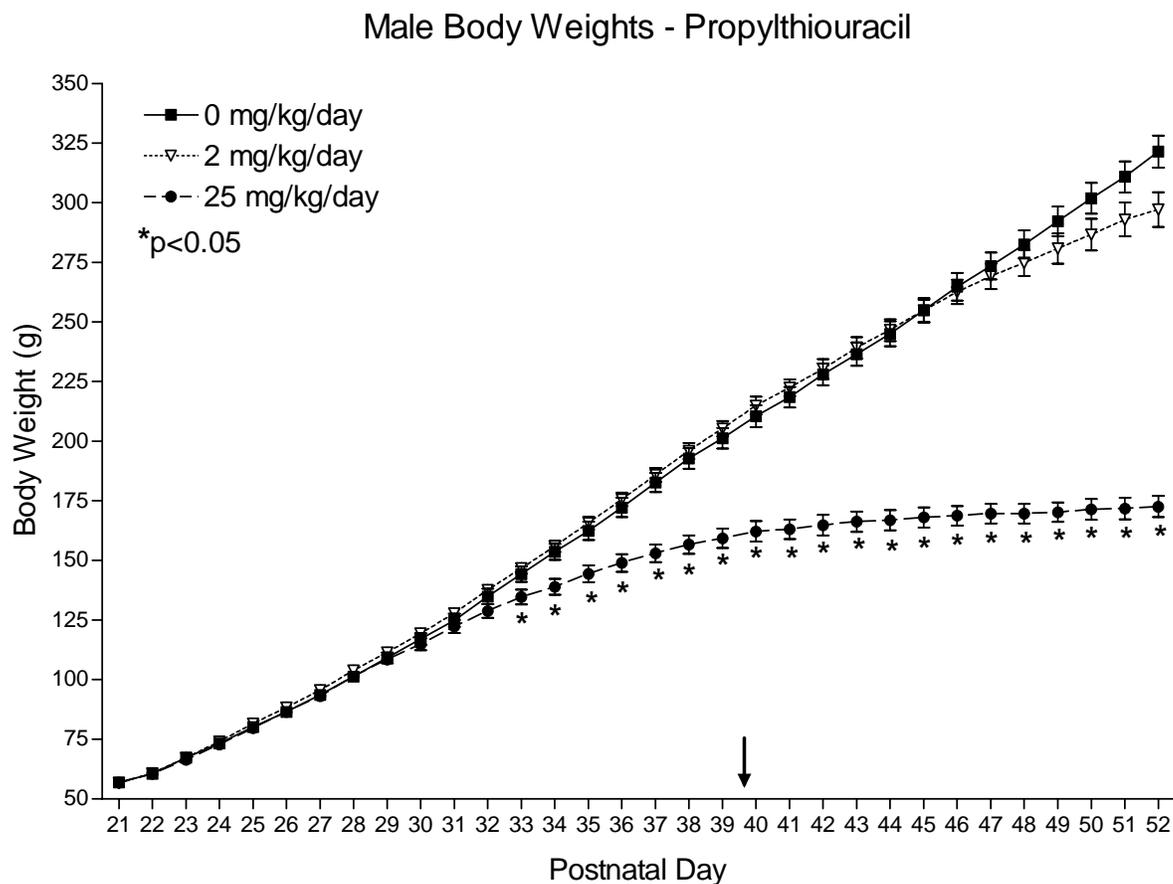


Figure 6. Mean Daily Body Weight in Pubertal Males Treated with 0, 2, or 25 mg/kg/day Propylthiouracil. The mean day of preputial separation (pps) for the control group is indicated by the arrow. Data are presented as mean \pm S.E.M. (n = 14-15 animals per group). * = p<0.05, Dunnett's Test.

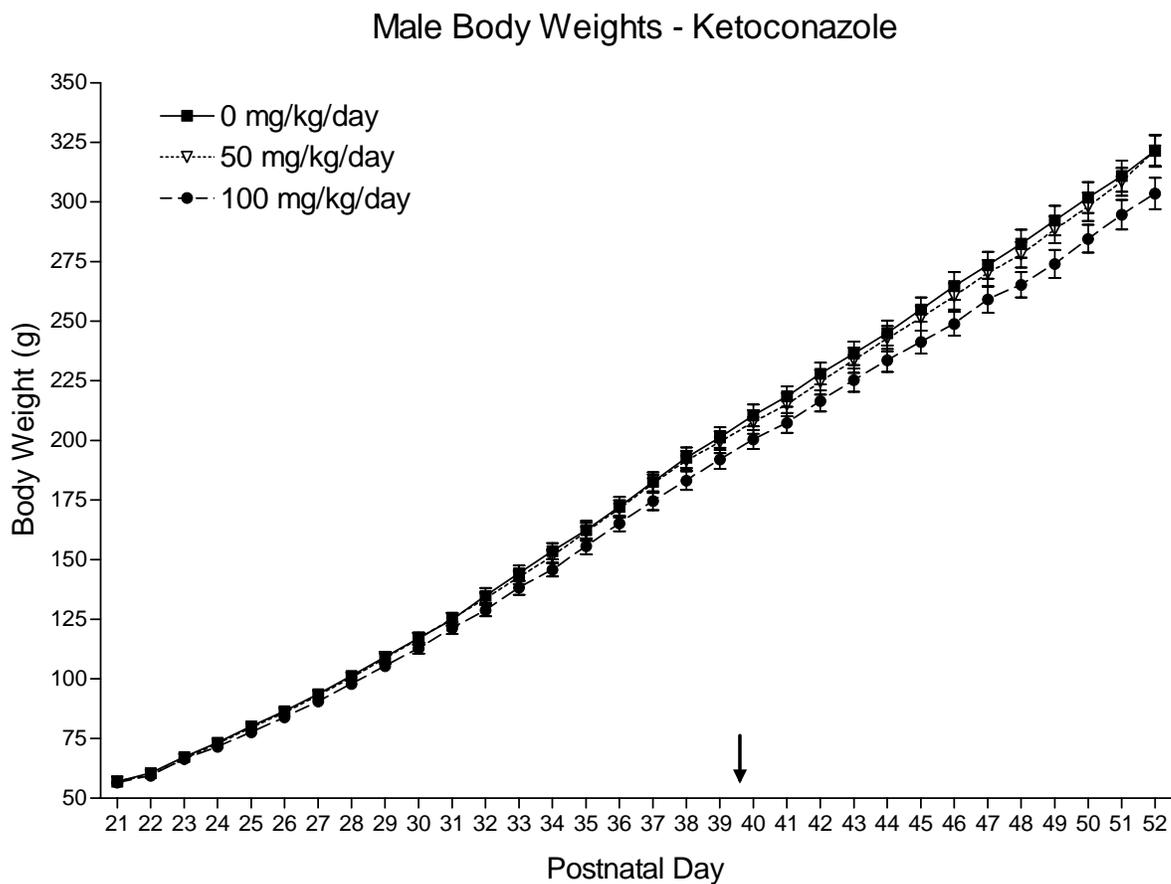


Figure 7. Mean Daily Body Weight in Pubertal Males Treated with 0, 50, or 100 mg/kg/day Ketoconazole. The mean day of preputial separation (pps) for the control group is indicated by the arrow. Data are presented as mean \pm S.E.M. (n = 14-15 animals per group).

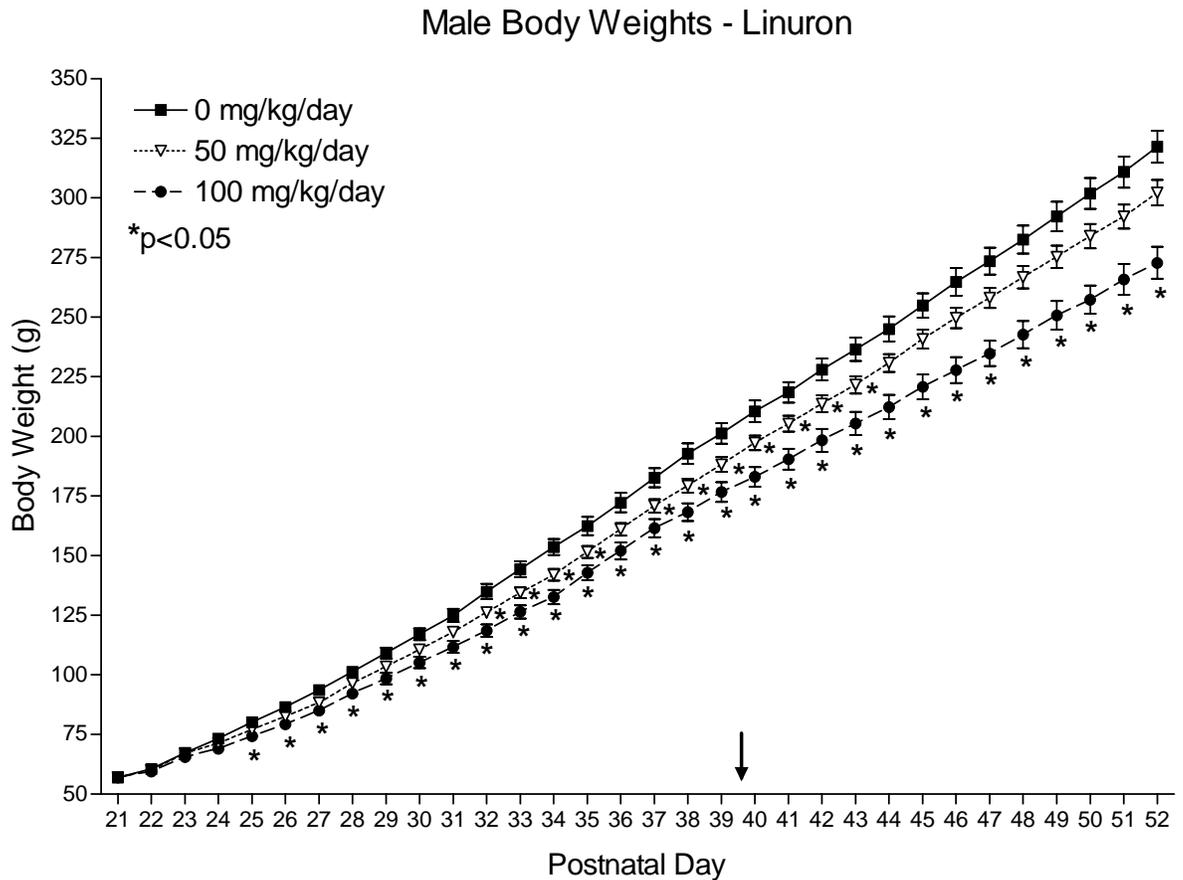


Figure 8. Mean Daily Body Weight in Pubertal Males Treated with 0, 50, or 100 mg/kg/day Linuron. The mean day of preputial separation (pps) for the control group is indicated by the arrow. Data are presented as mean \pm S.E.M. (n = 14-15 animals per group). * = $p < 0.05$, Dunnett's Test.

Table 2-A. Preputial Separation and Body Weight in Atrazine-Treated F1 Males^a

Parameter	Atrazine (mg/kg/day, po)		
	0	75	150
Age at PPS (days)	41.4 ±0.7 (12)	42.0 ±0.5 (12)	42.9 ±0.4 (12)
BW at PPS (grams)	219.50 ^{b,c} ±1.93 (12)	208.84 ±5.57 (12)	200.26* ±2.64 (12)
Initial BW (pnd 23, grams)	66.55 ±5.79 (12)	66.85 ±1.22 (13)	66.05 ±1.83 (12)
Final BW (pnd 52, grams)	314.88 ^{b,c} ±5.38 (12)	282.14* ±4.28 (12)	260.85* ±4.10 (12)
Final BW as % of control (pnd 52)	---	89.6	82.8
BW gain (pnd 23 to 52, grams)	248.32 ^{b,c} ±4.47 (12)	215.13* ±3.56 (12)	194.80* ±3.48 (12)

^a Mean ± SE (n = 15 for each treatment group unless otherwise noted in parentheses).

^b Significant Test for Linear Trend (p<0.05).

^c Significant treatment effect by ANOVA (p<0.05).

*Significantly different from control by Dunnett's Test (p<0.05).

Table 2-B. Preputial Separation and Body Weight in p,p'-DDE-Treated F1 Males^a

Parameter	DDE (mg/kg/day, po)		
	0	50	100
Age at PPS (days)	41.4 ^{b,c} ±0.7 (12)	44.9* ±0.3 (13)	45.7* ±0.4
BW at PPS (grams)	219.50 ^{b,c} ±5.79 (12)	251.10* ±6.35 (13)	259.19* ±4.58
Initial BW (pnd 23, grams)	66.55 ±1.93 (12)	65.35 ±2.06 (14)	65.02 ±1.88
Final BW (pnd 52, grams)	314.88 ±5.38 (12)	314.73 ±7.42 (13)	310.70 ±7.56
Final BW as % of control (pnd 52)	---	99.9	98.7
BW gain (pnd 23 to 52, grams)	248.32 ±4.47 (12)	248.94 ±5.73 (13)	245.69 ±6.17

^a Mean ± SE (n = 15 for each treatment group unless otherwise noted in parentheses).

^b Significant Test for Linear Trend (p<0.05).

^c Significant treatment effect by ANOVA (p<0.05).

*Significantly different from control by Dunnett's Test (p<0.05).

Table 2-C. Preputial Separation and Body Weight in Vinclozolin-Treated F1 Males^a

Parameter	Vinclozolin (mg/kg/day, po)		
	0	30	100
Age at PPS (days)	41.4 ^{b,c} ±0.7 (12)	43.8* ±0.3 (13)	46.8* ±0.3 (10) ^e
BW at PPS (grams)	219.50 ^{b,c} ±5.79 (12)	242.86* ±4.97 (13)	259.64* ±5.98 (10)
Initial BW (pnd 23, grams)	66.55 ±1.93 (12)	66.01 ±1.91 (13)	65.21 ±1.82 (12)
Final BW (pnd 52, grams)	314.88 ^d ±5.38 (12)	321.93 ±8.73 (13)	306.71 ±4.90 (12)
Final BW as % of control (pnd 52)	---	102.2	97.4
BW gain (pnd 23 to 52, grams)	248.32 ^d ±4.47 (12)	255.92 ±7.47 (13)	241.50 ±3.92 (12)

^a Mean ± SE (n = 15 for each treatment group unless otherwise noted in parentheses).

^b Significant Test for Linear Trend (p<0.05).

^c Significant treatment effect by ANOVA (p<0.05).

^d Significant Levene's test for homogeneity of variances (p<0.05).

^e Two males (2/12) failed to achieve preputial separation prior to necropsy.

* Significantly different from control by Dunnett's Test (p<0.05).

Table 2-D. Preputial Separation and Body Weight in Methoxychlor-Treated F1 Males^a

Parameter	Methoxychlor (mg/kg/day, po)		
	0	25	50
Age at PPS (days)	41.4 ±0.7 (12)	41.8 ±0.7 (14) ^c	41.8 ±0.6 (13)
BW at PPS (grams)	219.50 ±5.79 (12)	218.70 ±4.33 (14)	220.46 ±6.32 (13)
Initial BW (pnd 23, grams)	66.55 ±1.93 (12)	66.82 ±1.63	66.17 ±2.34 (13)
Final BW (pnd 52, grams)	314.88 ±5.38 (12)	302.69 ±4.91	297.93 ±7.88 (13)
Final BW as % of control (pnd 52)	---	96.1	94.6
BW gain (pnd 23 to 52, grams)	248.32 ^b ±4.47 (12)	235.87 ±4.20	231.76 ±5.82 (13)

^a Mean ± SE (n = 15 for each treatment group unless otherwise noted in parentheses).

^b Significant Test for Linear Trend (p<0.05).

^c One animal (1/15) failed to achieve preputial separation prior to necropsy.

Table 2-E. Summary of Preputial Separation and Acquisition Body Weight Data for Component 1 (Male 51, Control Group Removed) in Atrazine, p,p'-DDE, Vinclozolin, and Methoxychlor-Treated F1 Males^a

Atrazine	0 (mg/kg/day, po)	75 (mg/kg/day, po)	150 (mg/kg/day, po)
Age at PPS (days)	40.8 ^{b,c,d} ±0.7 (11)	42.0 ±0.5 (12)	42.9* ±0.4 (12)
BW at PPS (grams)	214.87 ^{b,c,d} ±3.83 (11)	208.84 ±5.57 (12)	200.26* ±2.64 (12)
p,p'-DDE	0 (mg/kg/day, po)	50 (mg/kg/day, po)	100 (mg/kg/day, po)
Age at PPS (days)	40.8 ^{b,c} ±0.4 (11)	44.9* ±0.3 (13)	45.7* ±0.4
BW at PPS (grams)	214.87 ^{b,c} ±3.83 (11)	251.10* ±6.35 (13)	259.19* ±4.58
Vinclozolin	0 (mg/kg/day, po)	30 (mg/kg/day, po)	100 (mg/kg/day, po)
Age at PPS (days)	40.8 ^{b,c} ±0.9 (11)	43.8* ±0.3 (13)	46.8* ±0.3 (10) ^e
BW at PPS (grams)	214.87 ^{b,c} ±3.83 (11)	242.86* ±4.97 (13)	259.64* ±5.98 (10)
Methoxychlor	0 (mg/kg/day, po)	25 (mg/kg/day, po)	50 (mg/kg/day, po)
Age at PPS (days)	40.8 ^c ±0.4 (11)	41.8 ±0.7 (14) ^f	41.8 ±0.6 (13)
BW at PPS (grams)	214.87 ±3.83 (11)	218.70 ±4.33 (14)	220.46 ±6.32 (13)

^a Mean ± SE (n = 15 for each treatment group unless otherwise noted in parentheses).

^b Significant Test for Linear Trend (p<0.05).

^c Significant treatment effect by ANOVA or Wald Chi-Square Test (p<0.05).

^d Significant Levene's test for homogeneity of variances (p<0.05).

^e Two males (2/12) failed to achieve preputial separation prior to necropsy.

^f One male (1/15) failed to achieve preputial separation prior to necropsy.

*Significantly different from control by Dunnett's Test or individual t-test (p<0.05).

Table 2-F. Preputial Separation and Body Weight in Propylthiouracil-Treated F1 Males^a

Parameter	Propylthiouracil (mg/kg/day, po)		
	0	2	25
Age at PPS (days)	39.6 ^{b,c} ±0.4 (14)	40.4 ±0.4	43.3* ±0.5
BW at PPS (grams)	207.50 ^{b,c} ±4.20 (14)	216.75 ±4.75	166.54* ±3.95
Initial BW (pnd 23, grams)	67.30 ±1.41	67.16 ±1.46	66.43 ±1.55
Final BW (pnd 52, grams)	321.52 ^{b,c} ±6.65 (14)	297.22* ±7.24	172.60* 4.46 (14)
Final BW as % of control (pnd 52)	---	92.4	53.7
BW gain (pnd 23 to 52, grams)	254.36 ^{b,c} ±5.91 (14)	230.05* ±6.48	106.67* ±3.35 (14)

^a Mean ± SE (n = 15 for each treatment group unless otherwise noted in parentheses).

^b Significant Test for Linear Trend (p<0.05).

^c Significant treatment effect by ANOVA (p<0.05).

*Significantly different from control by Dunnett's Test (p<0.05).

Table 2-G. Preputial Separation and Body Weight in Ketoconazole–Treated F1 Males^a

Parameter	Ketoconazole (mg/kg/day, po)		
	0	50	100
Age at PPS (days)	39.6 ^{b,c,d} ±0.4 (14)	42.3* ±0.4	44.1* ±0.2 (14)
BW at PPS (grams)	207.47 ^{b,c} ±4.20 (14)	227.15* ±7.26	234.76* ±4.98 (14)
Initial BW (pnd 23, grams)	67.30 ±1.41	66.39 ±1.64	66.39 ±1.63
Final BW (pnd 52, grams)	321.52 ±6.65 (14)	321.62 ±6.46	303.57 ±6.56 (14)
Final BW as % of control (pnd 52)	---	100.0	94.4
BW gain (pnd 23 to 52, grams)	254.36 ^b ±5.91 (14)	255.23 ±5.45	237.51 ±5.32 (14)

^a Mean ± SE (n = 15 for each treatment group unless otherwise noted in parentheses).

^b Significant Test for Linear Trend (p<0.05).

^c Significant treatment effect by ANOVA or Wald Chi-Square Test (p<0.05).

^d Significant Levene's test for homogeneity of variances (p<0.05).

*Significantly different from control by Dunnett's Test or individual t-test (p<0.05).

Table 2-H. Preputial Separation and Body Weight in Linuron-Treated F1 Males^a

Parameter	Linuron (mg/kg/day, po)		
	0	50	100
Age at PPS (days)	39.6 ^{b,c} ±0.4 (14)	43.6* ±0.5	45.5* ±0.5
BW at PPS (grams)	207.47 ^{b,c} ±4.20 (14)	226.64* ±3.41	223.97* ±6.41
Initial BW (pnd 23, grams)	67.30 ±1.41	67.11 ±1.32	65.60 ±1.28
Final BW (pnd 52, grams)	321.52 ^{b,c} ±6.65 (14)	302.24 ±5.36	272.85* ±6.68
Final BW as % of control (pnd 52)	---	94.0	84.9
BW gain (pnd 23 to 52, grams)	254.36 ^{b,c} ±5.91 (14)	235.13* ±4.51	207.24* ±5.92

^a Mean ± SE (n = 15 for each treatment group unless otherwise noted in parentheses).

^b Significant Test for Linear Trend (p<0.05).

^c Significant treatment effect by ANOVA (p<0.05).

*Significantly different from control by Dunnett's Test (p<0.05).

Table 2-I. Preputial Separation and Body Weight in Phenobarbital-Treated F1 Males^a

Parameter	Phenobarbital (mg/kg/day, po)		
	0	50	100
Age at PPS (days)	39.6 ^{b,c,d} ±0.4 (14)	41.3* ±0.3	43.0* ±0.5
BW at PPS (grams)	207.47 ^{b,c} ±4.20 (14)	220.48 ±5.27	224.17* ±4.83
Initial BW (pnd 23, grams)	67.30 ±1.41	67.20 ±1.53	66.82 ±1.62
Final BW (pnd 52, grams)	321.52 ^b ±6.65 (14)	320.85 ±8.08	300.43 ±6.82
Final BW as % of control (pnd 52)	---	99.8	93.4
BW gain (pnd 23 to 52, grams)	254.36 ^{b,c} ±5.91 (14)	253.65 ±7.64	233.61 ±5.83

^a Mean ± SE (n = 15 for each treatment group unless otherwise noted in parentheses).

^b Significant Test for Linear Trend (p<0.05).

^c Significant treatment effect by ANOVA or Wald Chi-Square Test (p<0.05).

^d Significant Levene's Test for homogeneity of variances (p < 0.05).

*Significantly different from control by Dunnett's Test or individual t-test (p<0.05).

Table 3-A. Necropsy and Hormone Data for the Atrazine-Treated F₁ Males

	Atrazine (mg/kg/day, po)		
	0	50	100
Sacrifice Body Weight (g) ^a	316.31 ^{c,d} ± 6.46 (11)	279.00* ± 4.52 (12)	256.67* ± 4.35 (12)
Pituitary Weight (g) ^a	0.0113 ^{c,d} ± 0.0003 (12)	0.0100* ± 0.0005 (12)	0.0079* ± 0.0005 (12)
Thyroid Weight (g) ^a	0.0185 ^e ± 0.0012 (12)	0.0205 ± 0.0008 (12)	0.0186 ± 0.0007 (12)
Liver Weight (g) ^a	16.4133 ^{c,d,e} ± 0.6404 (12)	14.8414* ± 0.3419 (12)	13.9548* ± 0.3022 (12)
Paired Adrenal Gland Weight (g) ^a	0.0534 ± 0.0025 (12)	0.0497 ± 0.0019 (12)	0.0538 ± 0.0021 (12)
Paired Kidney Weight (g) ^a	2.8524 ^{c,d} ± 0.0865 (12)	2.7231 ± 0.0537 (12)	2.5378* ± 0.0527 (12)
Paired Testis Weight (g) ^a	2.7402 ± 0.0497 (12)	2.8174 ± 0.0580 (12)	2.8196 ± 0.0664 (12)
Paired Epididymis Weight (g) ^a	0.4856 ^{c,d} ± 0.0129 (12)	0.4478 ± 0.0133 (12)	0.4207* ± 0.0126 (12)
Ventral Prostate Weight (g) ^a	0.2407 ^c ± 0.0113 (12)	0.2092 ± 0.0145 (12)	0.1969 ± 0.0127 (12)
Seminal Vesicles with Coagulating Glands Weight (g) ^a	0.5529 ^{c,d} ± 0.0332 (12)	0.4528* 0.0260 (12)	0.4204* 0.0215 (12)
Levator Ani plus Bulbcavernosus Muscle Complex Weight (g) ^a	0.7060 ^{c,d} ± 0.0259 (12)	0.5961 ± 0.0406 (12)	0.5601* ± 0.0335 (12)

(continued)

Table 3-A. Necropsy and Hormone Data for the Atrazine-Treated F₁ Males (Continued)

	Atrazine (mg/kg/day, po)		
	0	50	100
Adjusted Pituitary Weight (g)^b	0.0105 ± 0.0006 (11)	0.0101 ± 0.0004 (12)	0.0087 ± 0.0005 (12)
Adjusted Thyroid Weight (g)^b	0.0195 ± 0.0014 (11)	0.0204 ± 0.0007 (12)	0.0183 ± 0.0008 (12)
Adjusted Liver Weight (g)^b	14.7885 ± 0.6006 (11)	15.0751 ± 0.2601 (12)	15.4710 ± 0.3320 (12)
Adjusted Paired Adrenal Gland Weight (g)^b	0.0493 ± 0.0033 (11)	0.0502 ± 0.0022 (12)	0.0570 ± 0.0029 (12)
Adjusted Paired Kidney Weight (g)^b	2.5828 ± 0.0744 (11)	2.7595 ± 0.0481 (12)	2.7744 ± 0.0648 (12)
Adjusted Paired Testis Weight (g)^b	2.6125 ^c ± 0.0812 (11)	2.8368 ± 0.0525 (12)	2.9456 ± 0.0707 (12)
Adjusted Paired Epididymides Weight (g)^b	0.4526 ± 0.0189 (11)	0.4522 ± 0.0122 (12)	0.4490 ± 0.0165 (12)
Adjusted Ventral Prostate Weight (g)^b	0.2254 ± 0.0204 (11)	0.2112 ± 0.0132 (12)	0.2097 ± 0.0178 (12)
Adjusted Seminal Vesicles with Coagulating Glands Weight (g)^b	0.5288 ± 0.0418 (11)	0.4572 ± 0.0270 (12)	0.4491 ± 0.0364 (12)
Adjusted Levator Ani plus Bulbocavernosus Muscle Complex Weight (g)^b	0.6639 ± 0.0516 (11)	0.6028 ± 0.0333 (12)	0.6040 ± 0.0449 (12)
Thyroxine Hormone (T4) (µg/dL) ^a	6.46 ± 0.16 (12)	6.35 ± 0.26 (12)	6.28 ± 0.25 (12)

(continued)

Table 3-A. Necropsy and Hormone Data for the Atrazine-Treated F₁ Males (Continued)

	Atrazine (mg/kg/day, po)		
	0	50	100
Thyroid Stimulating Hormone (TSH) (ng/ml) ^a	10.95	10.89	12.66
	± 1.06	± 1.31	± 1.81
	(12)	(12)	(12)

^a Reported as the mean ± S.E.M. pnd = postnatal day; N = 15 unless otherwise noted in parenthesis.

^b Reported as the adjusted mean ± S.E.M. (Sacrifice weight as covariate). N = 15 unless otherwise noted in parenthesis.

^c Significant Test for Linear Trend or Linear Trend of Covariance ($p < 0.05$).

^d Significant treatment effect by ANOVA or Wald Chi-square Test ($p < 0.05$).

^e Significant Levene's test for homogeneity of variances ($p < 0.05$).

* Significantly different from control by Dunnett's Test ($p < 0.05$).

Table 3-B. Necropsy and Hormone Data for the p,p'-DDE-Treated F₁ Males

	p,p-DDE (mg/kg/day, po)		
	0	50	100
Sacrifice Body Weight (g) ^a	316.31 ± 6.46 (11)	316.80 ± 7.41 (13)	311.09 ± 7.88 (15)
Pituitary Weight (g) ^a	0.0113 ± 0.0003 (12)	0.0110 ± 0.0005 (13)	0.0116 ± 0.0004 (14)
Thyroid Weight (g) ^a	0.0185 ^{c,d,e} ± 0.0012 (12)	0.0220* ± 0.0007 (13)	0.0243* ± 0.0016 (15)
Liver Weight (g) ^a	16.4133 ^{c,d} ± 0.6404 (12)	22.4886* ± 0.6320 (13)	23.8107* ± 0.7765 (15)
Paired Adrenal Gland Weight (g) ^a	0.0534 ± 0.0025 (12)	0.0536 ± 0.0023 (12)	0.0510 ± 0.0033 (15)
Paired Kidney Weight (g) ^a	2.8524 ^{c,d} ± 0.0865 (12)	3.1923* ± 0.0776 (13)	3.2025* ± 0.1025 (15)
Paired Testis Weight (g) ^a	2.7402 ± 0.0497 (12)	2.7960 ± 0.0465 (13)	2.7760 ± 0.0604 (15)
Paired Epididymides Weight (g) ^a	0.4856 ^{c,d} ± 0.0129 (12)	0.4670 ± 0.0140 (13)	0.4356* ± 0.0144 (15)
Ventral Prostate Weight (g) ^a	0.2407 ± 0.0113 (12)	0.2576 ± 0.0195 (13)	0.2285 ± 0.0159 (15)
Seminal Vesicles with Coagulating Glands Weight (g) ^a	0.5529 ± 0.0332 (12)	0.5648 0.0261 (13)	0.4891 0.0348 (15)
Levator Ani plus Bulbcavernosus Muscle Complex Weight (g) ^a	0.7060 ± 0.0259 (12)	0.6814 ± 0.0420 (13)	0.6217 ± 0.0291 (15)

(continued)

Table 3-B. Necropsy and Hormone Data for the p,p'-DDE-Treated F₁ Males (Continued)

	p,p'-DDE (mg/kg/day, po)		
	0	50	100
Adjusted Pituitary Weight (g) ^b	0.0115 ± 0.0004 (11)	0.0111 ± 0.0004 (13)	0.0116 ± 0.0004 (14)
Adjusted Thyroid Weight (g) ^b	0.0190 ^{c,d} ± 0.0010 (11)	0.0218* ± 0.0007 (13)	0.0245* ± 0.0014 (15)
Adjusted Liver Weight (g) ^b	16.5695 ^{c,d} ± 0.5246 (11)	22.3261* ± 0.4828 (13)	24.0453* ± 0.4503 (15)
Adjusted Paired Adrenal Gland Weight (g) ^b	0.0532 ± 0.0031 (11)	0.0534 ± 0.0030 (12)	0.0513 ± 0.0027 (15)
Adjusted Paired Kidney Weight (g) ^b	2.8625 ^{c,d} ± 0.0616 (11)	3.1691* ± 0.0567 (13)	3.2359* ± 0.0529 (15)
Adjusted Paired Testis Weight (g) ^b	2.7646 ± 0.0504 (11)	2.7878 ± 0.0464 (13)	2.7879 ± 0.0433 (15)
Adjusted Paired Epididymides Weight (g) ^b	0.4868 ^{c,d} ± 0.0145 (11)	0.4653 ± 0.0133 (13)	0.4381* ± 0.0124 (15)
Adjusted Ventral Prostate Weight (g) ^b	0.2398 ± 0.0165 (11)	0.2553 ± 0.0151 (13)	0.2319 ± 0.0141 (15)
Adjusted Seminal Vesicles with Coagulating Glands Weight (g) ^b	0.5620 ± 0.0330 (11)	0.5610 ± 0.0304 (13)	0.4945 ± 0.0284 (15)
Adjusted Levator Ani plus Bulbocavernosus Muscle Complex Weight (g) ^b	0.7155 ^c ± 0.0328 (11)	0.6767 ± 0.0302 (13)	0.6285 ± 0.0281 (15)
Thyroxine Hormone (T4) (µg/dL) ^a	6.46 ^{c,d} ± 0.16 (12)	5.90 ± 0.23 (13)	5.31* ± 0.26 (15)

(continued)

Table 3-B. Necropsy and Hormone Data for the p,p'-DDE-Treated F₁ Males (Continued)

	p,p'-DDE (mg/kg/day, po)		
	0	50	100
Thyroid Stimulating Hormone (TSH) (ng/ml) ^a	10.95	14.06	14.42
	± 1.06	± 1.58	± 1.81
	(12)	(13)	

^a Reported as the mean ± S.E.M. pnd = postnatal day; N = 15 unless otherwise noted in parenthesis.

^b Reported as the adjusted mean ± S.E.M. (Sacrifice weight as covariate). N = 15 unless otherwise noted in parenthesis.

^c Significant Test for Linear Trend or Linear Trend of Covariance (p < 0.05).

^d Significant treatment effect by ANOVA, ANCOVA, or Wald Chi-square Test (p < 0.05).

^e Significant Levene's test for homogeneity of variances (p < 0.05).

* Significantly different from control by Dunnett's Test or individual t-test (p < 0.05).

Table 3-C. Necropsy and Hormone Data for the Vinclozolin-Treated F₁ Males

	Vinclozolin (mg/kg/day, po)		
	0	30	100
Sacrifice Body Weight (g) ^a	316.31 ^{c,e} ± 6.46 (11)	321.16 ± 8.86 (13)	302.40 ± 5.64 (11)
Pituitary Weight (g) ^a	0.0113 ± 0.0003 (12)	0.0114 ± 0.0004 (13)	0.0108 ± 0.0004 (12)
Thyroid Weight (g) ^a	0.0185 ^e ± 0.0012 (12)	0.0198 ± 0.0007 (13)	0.0205 ± 0.0006 (12)
Liver Weight (g) ^a	16.4133 ± 0.6404 (12)	17.6915 ± 0.7660 (13)	17.3103 ± 0.6718 (12)
Paired Adrenal Gland Weight (g) ^a	0.0534 ± 0.0025 (12)	0.0577 ± 0.0021 (13)	0.0572 ± 0.0039 (11)
Paired Kidney Weight (g) ^a	2.8524 ± 0.0865 (12)	2.9890 ± 0.1054 (13)	2.8058 ± 0.0608 (12)
Paired Testis Weight (g) ^a	2.7402 ± 0.0497 (12)	2.9605 ± 0.0688 (13)	2.9348 ± 0.0777 (12)
Paired Epididymides Weight (g) ^a	0.4856 ^{c,d} ± 0.0129 (12)	0.4561 ± 0.0144 (13)	0.4001* ± 0.0157 (12)
Ventral Prostate Weight (g) ^a	0.2407 ^{c,d} ± 0.0113 (12)	0.2582 ± 0.0169 (13)	0.2064 ± 0.0117 (12)
Seminal Vesicles with Coagulating Glands Weight (g) ^a	0.5529 ^{c,d} ± 0.0332 (12)	0.4655 0.0355 (13)	0.3044* 0.0200 (12)
Levator Ani plus Bulbcavernosus Muscle Complex Weight (g) ^a	0.7060 ^{c,d} ± 0.0259 (12)	0.6857 ± 0.0441 (13)	0.5443* ± 0.0273 (12)

(continued)

Table 3-C. Necropsy and Hormone Data for the Vinclozolin-Treated F₁ Males (Continued)

	Vinclozolin (mg/kg/day, po)		
	0	30	100
Adjusted Pituitary Weight (g) ^b	0.0114 ± 0.0003 (11)	0.0113 ± 0.0003 (13)	0.0112 ± 0.0004 (11)
Adjusted Thyroid Weight (g) ^b	0.0191 ± 0.0011 (11)	0.0198 ± 0.0007 (13)	0.0204 ± 0.0007 (11)
Adjusted Liver Weight (g) ^b	16.5142 ± 0.4631 (11)	17.1621 ± 0.4613 (13)	17.8317 ± 0.4903 (11)
Adjusted Paired Adrenal Gland Weight (g) ^b	0.0529 ± 0.0026 (11)	0.0563 ± 0.0016 (13)	0.0598 ± 0.0039 (10)
Adjusted Paired Kidney Weight (g) ^b	2.8566 ± 0.0556 (11)	2.9193 ± 0.0669 (13)	2.9180 ± 0.0385 (11)
Adjusted Paired Testis Weight (g) ^b	2.7618 ^{c,d} ± 0.0425 (11)	2.9337* ± 0.0534 (13)	2.9357* ± 0.0607 (11)
Adjusted Paired Epididymides Weight (g) ^b	0.4848 ^{c,d} ± 0.0112 (11)	0.4463* ± 0.0104 (13)	0.4177* ± 0.0127 (11)
Adjusted Ventral Prostate Weight (g) ^b	0.2396 ^d ± 0.0107 (11)	0.2524 ± 0.0127 (13)	0.2100 ± 0.0131 (11)
Adjusted Seminal Vesicles with Coagulating Glands Weight (g) ^b	0.5613 ^{c,d} ± 0.0331 (11)	0.4551* ± 0.0307 (13)	0.3188* ± 0.0194 (11)
Adjusted Levator Ani plus Bulbocavernosus Muscle Complex Weight (g) ^b	0.7158 ^{c,d} ± 0.0234 (11)	0.6760 ± 0.0424 (13)	0.5504* ± 0.0264 (11)
Thyroxine Hormone (T4) (µg/dL) ^a	6.46 ^{c,d} ± 0.16 (12)	4.87* ± 0.24 (13)	4.28* ± 0.23 (12)

(continued)

Table 3-C. Necropsy and Hormone Data for the Vinclozolin-Treated F₁ Males (Continued)

	Vinclozolin (mg/kg/day, po)		
	0	30	100
Thyroid Stimulating Hormone (TSH) (ng/ml) ^a	10.95	10.57	12.86
	± 1.06	± 0.62	1.16
	(12)	(13)	(12)

^a Reported as the mean ± S.E.M. pnd = postnatal day; N = 15 unless otherwise noted in parenthesis.

^b Reported as the adjusted mean ± S.E.M. (Sacrifice weight as covariate). N = 15 unless otherwise noted in parenthesis.

^c Significant Test for Linear Trend or Linear Trend of Covariance ($p < 0.05$).

^d Significant treatment effect by ANOVA or ANCOVA ($p < 0.05$).

^e Significant Levene's test for homogeneity of variances ($p < 0.05$).

* Significantly different from control by Dunnett's Test ($p < 0.05$).

Table 3-D. Necropsy and Hormone Data for the Methoxychlor-Treated F₁ Males

	Methoxychlor (mg/kg/day, po)		
	0	25	50
Sacrifice Body Weight (g) ^a	316.31 ± 6.46 (11)	301.97 ± 5.46	297.66 ± 8.19 (13)
Pituitary Weight (g) ^a	0.0113 ± 0.0003 (12)	0.0109 ± 0.0004	0.0106 ± 0.0003 (12)
Thyroid Weight (g) ^a	0.0185 ^d ± 0.0012 (12)	0.0210 ± 0.0010	0.0168 ± 0.0009 (13)
Liver Weight (g) ^a	16.4133 ± 0.6404 (12)	15.4169 ± 0.5109	15.1498 ± 0.5405 (13)
Paired Adrenal Gland Weight (g) ^a	0.0534 ^{c,d} ± 0.0025 (12)	0.0576 ± 0.0025	0.0647* ± 0.0035 (12)
Paired Kidney Weight (g) ^a	2.8524 ± 0.0865 (12)	2.7784 ± 0.1042	2.8244 ± 0.1104 (13)
Paired Testis Weight (g) ^a	2.7402 ± 0.0470 (12)	2.7707 ± 0.0570	2.7534 ± 0.0753 (13)
Paired Epididymides Weight (g) ^a	0.4856 ± 0.0129 (12)	0.4933 ± 0.0123	0.4669 ± 0.0169 (13)
Ventral Prostate Weight (g) ^a	0.2407 ± 0.0113 (12)	0.2377 ± 0.0143	0.2432 ± 0.0149 (13)
Seminal Vesicles with Coagulating Glands Weight (g) ^a	0.5529 ^{c,d} ± 0.0322 (12)	0.5013 ± 0.0242	0.4043* ± 0.0374 (13)
Levator Ani plus Bulbocavernosus Muscle Complex Weight (g) ^a	0.7060 ± 0.0259 (12)	0.6840 ± 0.0381	0.6095 ± 0.0370 (12)
Adjusted Pituitary Weight (g) ^b	0.0113 ± 0.0004 (11)	0.0109 ± 0.0003	0.0107 ± 0.0004 (12)

(continued)

Table 3-D. Necropsy and Hormone Data for the Methoxychlor-Treated F₁ Males (Continued)

	Methoxychlor (mg/kg/day, po)		
	0	25	50
Adjusted Thyroid Weight (g) ^b	0.0184 ^d ± 0.0010 (11)	0.0212 ± 0.0008 (13)	0.0172 ± 0.0009 (13)
Adjusted Liver Weight (g) ^b	15.8920 ± 0.3471 (11)	15.5956 ± 0.2879 (13)	15.6251 ± 0.3130 (13)
Adjusted Paired Adrenal Gland Weight (g) ^b	0.0505 ^{c,d} ± 0.0029 (11)	0.0581 ± 0.0025 (13)	0.0668* ± 0.0027 (12)
Adjusted Paired Kidney Weight (g) ^b	2.7527 ± 0.0837 (11)	2.8068 ± 0.0694 (13)	2.8999 ± 0.0755 (13)
Adjusted Paired Testis Weight (g) ^b	2.7131 ± 0.0588 (11)	2.7835 ± 0.0488 (13)	2.7875 ± 0.0531 (13)
Adjusted Paired Epididymides Weight (g) ^b	0.4746 ± 0.0139 (11)	0.4963 ± 0.0115 (13)	0.4749 ± 0.0125 (13)
Adjusted Ventral Prostate Weight (g) ^b	0.2307 ± 0.0145 (11)	0.2401 ± 0.0120 (13)	0.2496 ± 0.0131 (13)
Adjusted Seminal Vesicles with Coagulating Glands Weight (g) ^b	0.5446 ^{c,d} ± 0.0335 (11)	0.5066 ± 0.0287 (14)	0.4158* ± 0.0301 (13)
Adjusted Levator Ani plus Bulbcavernosus Muscle Complex Weight (g) ^b	0.6935 ± 0.0352 (11)	0.6952 ± 0.0295 (13)	0.6191 ± 0.0329 (12)
Thyroxine Hormone (T4) (µg/dL) ^a	6.46 ± 0.16 (12)	6.11 ± 0.24 (15)	6.66 ± 0.25 (13)
Thyroid Stimulating Hormone (TSH) (ng/ml) ^a	10.95 ± 1.06 (12)	10.98 ± 1.65 (13)	10.84 ± 0.74 (13)

^a Reported as the mean ± S.E.M. pnd = postnatal day; N = 15 unless otherwise noted in parenthesis.

^b Reported as the adjusted mean ± S.E.M. (Sacrifice weight as covariate). N = 15 unless otherwise noted in parenthesis.

^c Significant Test for Linear Trend or Linear Trend of Covariance (p < 0.05).

^d Significant treatment effect by ANOVA or ANCOVA (p < 0.05).

* Significantly different from control by Dunnett's Test (p < 0.05).

Table 3-E. Necropsy and Hormone Data for the Propylthiouracil-Treated F₁ Males

	Propylthiouracil (mg/kg/day, po)		
	0	2	25
Sacrifice Body Weight (g) ^a	320.70 ^{c,d} ± 6.74 (14)	294.04* ± 7.18	173.91* ± 3.95 (13)
Pituitary Weight (g) ^a	0.0117 ^{c,d} ± 0.0006 (14)	0.0116 ± 0.0003 (14)	0.0096* ± 0.0005 (14)
Thyroid Weight (g) ^a	0.0273 ^{c,d} ± 0.0011 (14)	0.0770* ± 0.0044	0.0886* ± 0.0067 (14)
Liver Weight (g) ^a	17.6196 ^{c,d} ± 0.5792 (14)	15.2176* ± 0.6546	7.7000* ± 0.3260 (14)
Paired Adrenal Gland Weight (g) ^a	0.0480 ^{c,d} ± 0.0016 (14)	0.0385* ± 0.0014	0.0254* ± 0.0011 (14)
Paired Kidney Weight (g) ^a	3.1147 ^{c,d} ± 0.0800 (14)	2.4769* ± 0.0858	1.4226* ± 0.0411 (14)
Paired Testis Weight (g) ^a	2.8678 ^{c,d} ± 0.0299 (14)	2.8014 ± 0.0447	2.6496* ± 0.0539 (14)
Paired Epididymides Weight (g) ^a	0.4564 ^{c,d} ± 0.0142 (14)	0.4386 ± 0.0160	0.3791* ± 0.0138 (14)
Ventral Prostate Weight (g) ^a	0.2657 ^{c,d} ± 0.0168 (14)	0.2410 ± 0.0147	0.1888* ± 0.0157 (14)
Seminal Vesicles with Coagulating Glands Weight (g) ^a	0.6396 ^{c,d} ± 0.0462 (13)	0.6910 ± 0.0431	0.5244 ± 0.0372 (14)
Levator Ani plus Bulbcavernosus Muscle Complex Weight (g) ^a	0.6384 ^{c,d} ± 0.0279 (14)	0.6539 ± 0.0326	0.3702* ± 0.0308 (14)
Adjusted Pituitary Weight (g)^b	0.0106 ± 0.0008 (14)	0.0111 ± 0.0006 (14)	0.0115 ± 0.0012 (13)

(continued)

Table 3-E. Necropsy and Hormone Data for the Propylthiouracil-Treated F₁ Males (Continued)

	Propylthiouracil (mg/kg/day, po)		
	0	2	25
Adjusted Thyroid Weight (g) ^b	0.0140 ^d ± 0.0071 (14)	0.0701* ± 0.0050	0.1076* ± 0.0106 (13)
Adjusted Liver Weight (g) ^b	13.2806 ^{c,d} ± 0.4405 (14)	12.9385 ± 0.3085	15.0343 ± 0.6553 (13)
Adjusted Paired Adrenal Gland Weight (g) ^b	0.0437 ^d ± 0.0023 (14)	0.0363* ± 0.0016	0.0324 ± 0.0034 (13)
Adjusted Paired Kidney Weight (g) ^b	2.6024 ^d ± 0.0769 (14)	2.2132* ± 0.0539	2.3002 ± 0.1145 (13)
Adjusted Paired Testis Weight (g) ^b	2.7938 ± 0.0706 (14)	2.7633 ± 0.0495	2.8045 ± 0.1051 (13)
Adjusted Paired Epididymides Weight (g) ^b	0.3881 ^c ± 0.0224 (14)	0.4035 ± 0.0157	0.4958 ± 0.0334 (13)
Adjusted Ventral Prostate Weight (g) ^b	0.2045 ± 0.0253 (14)	0.2094 ± 0.0177	0.2924 ± 0.0376 (13)
Adjusted Seminal Vesicles with Coagulating Glands Weight (g) ^b	0.4696 ^d ± 0.0640 (13)	0.6022* ± 0.0441	0.8212* ± 0.0912 (13)
Adjusted Levator Ani plus Bulbocavernosus Muscle Complex Weight (g) ^b	0.5227 ± 0.0495 (14)	0.5943 ± 0.0347	0.5669 ± 0.0737 (13)
Thyroxine Hormone (T4) (µg/dL) ^a	5.78 ^{c,d,e} ± 0.27 (14)	0.53* ± 0.08 (9)	0.37* ± 0.01 (6)
Thyroid Stimulating Hormone (TSH) (ng/ml) ^a	11.54 ^{c,d,e} ± 0.90 (14)	82.13* ± 4.60	134.63* ± 7.89 (14)

^a Reported as the mean ± S.E.M. pnd = postnatal day; N = 15 unless otherwise noted in parenthesis.

^b Reported as the adjusted mean ± S.E.M. (Sacrifice weight as covariate). N = 15 unless otherwise noted in parenthesis.

^c Significant Test for Linear Trend or Linear Trend of Covariance (p < 0.05).

^d Significant treatment effect by ANOVA, ANCOVA, or Wald Chi-square Test (p < 0.05).

^e Significant Levene's test for homogeneity of variances (p < 0.05).

* Significantly different from control by Dunnett's Test or individual t-test (p < 0.05).

Table 3-F. Necropsy and Hormone Data for the Ketoconazole-Treated F₁ Males

	Ketoconazole (mg/kg/day, po)		
	0	50	100
Sacrifice Body Weight (g) ^a	320.70 ± 6.74 (14)	315.68 ± 6.57	301.50 ± 7.51 (14)
Pituitary Weight (g) ^a	0.0117 ± 0.0006 (14)	0.0107 ± 0.0004	0.0117 ± 0.0005 (14)
Thyroid Weight (g) ^a	0.0273 ± 0.0011 (14)	0.0266 ± 0.0011	0.0267 ± 0.0006 (14)
Liver Weight (g) ^a	17.6196 ± 0.5792 (14)	19.1954 ± 0.5408	19.1401 ± 0.8105 (14)
Paired Adrenal Gland Weight (g) ^a	0.0480 ^{c,d,e} ± 0.0016 (14)	0.0704* ± 0.0036	0.0908* ± 0.0047 (14)
Paired Kidney Weight (g) ^a	3.1147 ± 0.0800 (14)	3.1021 ± 0.0708	3.1597 ± 0.1181 (14)
Paired Testis Weight (g) ^a	2.8678 ^{c,d,e} ± 0.0299 (14)	2.8051 ± 0.0563	2.7212* ± 0.0223 (14)
Paired Epididymides Weight (g) ^a	0.4564 ^c ± 0.0142 (14)	0.4294 ± 0.0139	0.4118 ± 0.0109 (14)
Ventral Prostate Weight (g) ^a	0.2657 ^{c,e} ± 0.0168 (14)	0.2372 ± 0.0116	0.2058 ± 0.0243 (14)
Seminal Vesicles with Coagulating Glands Weight (g) ^a	0.6396 ^{c,d} ± 0.0462 (13)	0.4789* ± 0.0215	0.4193* 0.0372 (14)
Levator Ani plus Bulbcavernosus Muscle Complex Weight (g) ^a	0.6384 ^c ± 0.0279 (14)	0.5757 ± 0.0262	0.5425 ± 0.0306 (14)
Adjusted Pituitary Weight (g) ^b	0.0114 ± 0.0005 (14)	0.0106 ± 0.0004	0.0120 ± 0.0005 (14)

(continued)

Table 3-F. Necropsy and Hormone Data for the Ketoconazole-Treated F₁ Males (Continued)

	Ketoconazole (mg/kg/day, po)		
	0	50	100
Adjusted Thyroid Weight (g) ^b	0.0271 ± 0.0010 (14)	0.0265 ± 0.0010	0.0270 ± 0.0010 (14)
Adjusted Liver Weight (g) ^b	16.9686 ^{c,d} ± 0.3347 (14)	18.9523* ± 0.3190	20.0516* ± 0.3397 (14)
Adjusted Paired Adrenal Gland Weight (g) ^b	0.0464 ^{c,d} ± 0.0020 (14)	0.0698* ± 0.0029	0.0930* ± 0.0042 (14)
Adjusted Paired Kidney Weight (g) ^b	3.0337 ^{c,d} ± 0.0604 (14)	3.0718 ± 0.0575	3.2730* ± 0.0613 (14)
Adjusted Paired Testis Weight (g) ^b	2.8591 ^{c,d} ± 0.0287 (14)	2.8019 ± 0.0537	2.7334* ± 0.0238 (14)
Adjusted Paired Epididymides Weight (g) ^b	0.4505 ± 0.0126 (14)	0.4272 ± 0.0120	0.4201 ± 0.0128 (14)
Adjusted Ventral Prostate Weight (g) ^b	0.2616 ± 0.0162 (14)	0.2356 ± 0.0109	0.2117 ± 0.0231 (14)
Adjusted Seminal Vesicles with Coagulating Glands Weight (g) ^b	0.6282 ^{c,d} ± 0.0363 (13)	0.4747* ± 0.0334	0.4344* ± 0.0355 (14)
Adjusted Levator Ani plus Bulbcavernosus Muscle Complex Weight (g) ^b	0.6287 ± 0.0280 (14)	0.5721 ± 0.0267	0.5561 ± 0.0285 (14)
Thyroxine Hormone (T4) (µg/dL) ^a	5.78 ± 0.27 (14)	5.58 ± 0.21	5.65 ± 0.20 (14)
Thyroid Stimulating Hormone (TSH) (ng/ml) ^a	11.54 ± 0.90 (14)	10.02 ± 0.64	11.15 ± 0.88 (14)

^a Reported as the mean ± S.E.M. pnd = postnatal day; N = 15 unless otherwise noted in parenthesis.

^b Reported as the adjusted mean ± S.E.M. (Sacrifice weight as covariate). N = 15 unless otherwise noted in parenthesis.

^c Significant Test for Linear Trend or Linear Trend of Covariance (p < 0.05).

^d Significant treatment effect by ANOVA, ANCOVA, or Wald Chi-square Test (p < 0.05).

^e Significant Levene's test for homogeneity of variances (p < 0.05).

* Significantly different from control by Dunnett's Test or individual t-test (p < 0.05).

Table 3-G. Necropsy and Hormone Data for the Linuron-Treated F₁ Males

	Linuron (mg/kg/day, po)		
	0	50	100
Sacrifice Body Weight (g) ^a	320.70 ^{c,d} ± 6.74 (14)	298.66* ± 5.69	268.89* ± 6.87
Pituitary Weight (g) ^a	0.0117 ^{c,d} ± 0.0006 (14)	0.0097* ± 0.0003	0.0092* ± 0.0003
Thyroid Weight (g) ^a	0.0273 ^{c,d} ± 0.0011 (14)	0.0294 ± 0.0009	0.0242* ± 0.0008
Liver Weight (g) ^a	17.6196 ^{c,d} ± 0.5792 (14)	16.8580 ± 0.6195	14.9885* ± 0.5601
Paired Adrenal Gland Weight (g) ^a	0.0480 ± 0.0016 (14)	0.0483 ± 0.0016	0.0464 ± 0.0025
Paired Kidney Weight (g) ^a	3.1147 ^{c,d,e} ± 0.0800 (14)	2.9232* ± 0.0549	2.7279* ± 0.1179
Paired Testis Weight (g) ^a	2.8678 ^{c,d} ± 0.0299 (14)	2.7752 ± 0.0454	2.6670* ± 0.0552
Paired Epididymides Weight (g) ^a	0.4564 ^{c,d} ± 0.0142 (14)	0.4186 ± 0.0134	0.3809* ± 0.0135
Ventral Prostate Weight (g) ^a	0.2657 ^{c,d} ± 0.0168 (14)	0.1943* ± 0.0090 (14)	0.1845* ± 0.0143
Seminal Vesicles with Coagulating Glands Weight (g) ^a	0.6396 ^{c,d} ± 0.0462 (13)	0.4660* 0.0344	0.3549* 0.0370 (13)
Levator Ani plus Bulbcavernosus Muscle Complex Weight (g) ^a	0.6384 ^{c,d} ± 0.0279 (14)	0.5437* ± 0.0320	0.4559* ± 0.0224
Adjusted Pituitary Weight (g)^b	0.0109 ± 0.0005 (14)	0.0096 ± 0.0004	0.0100 ± 0.0005

(continued)

Table 3-G. Necropsy and Hormone Data for the Linuron-Treated F₁ Males (Continued)

	Linuron (mg/kg/day, po)		
	0	50	100
Adjusted Thyroid Weight (g) ^b	0.0268 ^d ± 0.0011 (14)	0.0293 ± 0.0009	0.0248 ± 0.0011
Adjusted Liver Weight (g) ^b	15.6059 ^{c,d} ± 0.3403 (14)	16.6074* ± 0.2832	17.1187* ± 0.3373
Adjusted Paired Adrenal Gland Weight (g) ^b	0.0462 ± 0.0023 (14)	0.0481 ± 0.0019	0.0483 ± 0.0023
Adjusted Paired Kidney Weight (g) ^b	2.8363 ± 0.0549 (14)	2.8885 ± 0.0505	3.0223 ± 0.0707
Adjusted Paired Testis Weight (g) ^b	2.8254 ± 0.0531 (14)	2.7699 ± 0.0442	2.7118 ± 0.0526
Adjusted Paired Epididymides Weight (g) ^b	0.4372 ± 0.0154 (14)	0.4162 ± 0.0128	0.4011 ± 0.0153
Adjusted Ventral Prostate Weight (g) ^b	0.2501 ^d ± 0.0157 (14)	0.1925* ± 0.0135 (14)	0.2008 ± 0.0155
Adjusted Seminal Vesicles with Coagulating Glands Weight (g) ^b	0.5873 ^{c,d} ± 0.0442 (13)	0.4618 ± 0.0357	0.4081* ± 0.0432 (14)
Adjusted Levator Ani plus Bulbcavernosus Muscle Complex Weight (g) ^b	0.5780 ± 0.0277 (14)	0.5362 ± 0.0231	0.5199 ± 0.0275
Thyroxine Hormone (T4) (µg/dL) ^a	5.78 ^{c,d} ± 0.27 (14)	4.60* ± 0.30 (14)	3.40* ± 0.15 (15)
Thyroid Stimulating Hormone (TSH) (ng/ml) ^a	11.54 ^d ± 0.90 (14)	8.98* ± 0.52 (14)	9.49 ± 0.74 (15)

^a Reported as the mean ± S.E.M. pnd = postnatal day; N = 15 unless otherwise noted in parenthesis.

^b Reported as the adjusted mean ± S.E.M. (Sacrifice weight as covariate). N = 15 unless otherwise noted in parenthesis.

^c Significant Test for Linear Trend or Linear Trend of Covariance (p < 0.05).

^d Significant treatment effect by ANOVA, ANCOVA, or Wald Chi-square Test (p < 0.05).

^e Significant Levene's test for homogeneity of variances (p < 0.05).

* Significantly different from control by Dunnett's Test or individual t-test (p < 0.05).

Table 3-H. Necropsy and Hormone Data for the Phenobarbital-Treated F₁ Males

	Phenobarbital (mg/kg/day, po)		
	0	50	100
Sacrifice Body Weight (g) ^a	320.70 ^c ± 6.74 (14)	312.07 ± 7.63	296.96 ± 7.23
Pituitary Weight (g) ^a	0.0117 ± 0.0006 (14)	0.0105 ± 0.0006 (14)	0.0104 ± 0.0002 (13)
Thyroid Weight (g) ^a	0.0273 ^{c,d} ± 0.0011 (14)	0.0319* ± 0.0014	0.0325* ± 0.0014
Liver Weight (g) ^a	17.6196 ^{c,d} ± 0.5792 (14)	20.3434* ± 0.6582	21.8401* ± 0.7753
Paired Adrenal Gland Weight (g) ^a	0.0480 ± 0.0016 (14)	0.0529 ± 0.0024	0.0483 ± 0.0028
Paired Kidney Weight (g) ^a	3.1147 ± 0.0800 (14)	3.0568 ± 0.0934	2.9835 ± 0.0821
Paired Testis Weight (g) ^a	2.8678 ^{c,d} ± 0.0299 (14)	2.7704 ± 0.0438	2.6432* ± 0.0580
Paired Epididymides Weight (g) ^a	0.4564 ^{c,d} ± 0.0142 (14)	0.4508 ± 0.0127	0.4026* ± 0.0145
Ventral Prostate Weight (g) ^a	0.2657 ^d ± 0.0168 (14)	0.2772 ± 0.0220	0.2154 ± 0.0147
Seminal Vesicles with Coagulating Glands Weight (g) ^a	0.6396 ^{c,d} ± 0.0462 (13)	0.6410 0.0428	0.4873* 0.0344
Levator Ani plus Bulbcavernosus Muscle Complex Weight (g) ^a	0.6384 ^{c,d} ± 0.0279	0.6065 ± 0.0190	0.5252* ± 0.0237
Adjusted Pituitary Weight (g) ^b	0.0116 ± 0.0005 (14)	0.0105 ± 0.0005 (14)	0.0105 ± 0.0006 (13)

(continued)

Table 3-H. Necropsy and Hormone Data for the Phenobarbital-Treated F₁ Males (Continued)

	Phenobarbital (mg/kg/day, po)		
	0	50	100
Adjusted Thyroid Weight (g) ^b	0.0268 ^{c,d} ± 0.0013 (14)	0.0317* ± 0.0013	0.0332* ± 0.0013
Adjusted Liver Weight (g) ^b	16.6809 ^{c,d} ± 0.3085 (14)	20.1385* ± 0.2907	22.9211* ± 0.3012
Adjusted Paired Adrenal Gland Weight (g) ^b	0.0474 ± 0.0025 (14)	0.0528 ± 0.0023	0.0490 ± 0.0024
Adjusted Paired Kidney Weight (g) ^b	3.0108 ± 0.0553 (14)	3.0341 ± 0.0521	3.1031 ± 0.0540
Adjusted Paired Testis Weight (g) ^b	2.8482 ^{c,d} ± 0.0470 (14)	2.7661 ± 0.0443	2.6658* ± 0.0459
Adjusted Paired Epididymides Weight (g) ^b	0.4507 ^c ± 0.0142 (14)	0.4495 ± 0.0133	0.4091 ± 0.0138
Adjusted Ventral Prostate Weight (g) ^b	0.2572 ± 0.0184 (14)	0.2753 ± 0.0173	0.2252 ± 0.0179
Adjusted Seminal Vesicles with Coagulating Glands Weight (g) ^b	0.6114 ± 0.0402 (13)	0.6347 ± 0.0365	0.5180 ± 0.0378
Adjusted Levator Ani plus Bulbcavernosus Muscle Complex Weight (g) ^b	0.6226 ^{c,d} ± 0.0226 (14)	0.6030 ± 0.0213	0.5434* ± 0.0221
Thyroxine Hormone (T4) Epididymides ^a	5.78 ± 0.27 (14)	5.66 ± 0.26	5.73 ± 0.16
Thyroid Stimulating Hormone (TSH) (µg/dL) ^a	11.54 ± 0.90 (14)	16.37 ± 1.58	14.47 ± 1.61

^a Reported as the mean ± S.E.M. pnd = postnatal day; N = 15 unless otherwise noted in parenthesis.

^b Reported as the adjusted mean ± S.E.M. (Sacrifice weight as covariate). N = 15 unless otherwise noted in parenthesis.

^c Significant Test for Linear Trend or Linear Trend of Covariance (p < 0.05).

^d Significant treatment effect by ANOVA or ANCOVA (p < 0.05)

* Significantly different from control by Dunnett's Test (p < 0.05).