FINAL REPORT

Volume 1 of 2
(Text, Figures 1-4, Tables 1-12 and Appendices A-H)

STUDY TITLE

VALIDATION OF THE PUBERTAL MALE ASSAY IN RATS (WA 4-15)

STUDY NUMBER

WIL-431005

EPA CONTRACT NUMBER

68-W-01-023

STUDY DIRECTOR

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STUDY INITIATION DATE

5 January 2005

STUDY COMPLETION DATE

13 December 2005

PERFORMING LABORATORY

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COMPLIANCE STATEMENT

This study, designated WIL-431005, was conducted in compliance with the United States Environmental Protection Agency (EPA) Good Laboratory Practice Standards (40 CFR Part 792, 18 September 1989 and 40 CFR Part 160, 16 October 1989); the standard operating procedures of WIL Research Laboratories, LLC, and the protocol as approved by the Sponsor.

13 DEC 2005

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Date

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VOLUME 2

1. EXECUTIVE SUMMARY

1.1. PURPOSE AND OBJECTIVE

The purpose of the study was to participate in an interlaboratory validation of the male pubertal assay and present results from WIL Research Laboratories, LLC to be used for the subsequent determination of whether independent laboratories arrive at the same conclusion about the ability of substances to interact with the endocrine system using this study design.

The objective of this study was to quantify the effects of test substances on pubertal development and thyroid function in the juvenile/peripubertal male rat.

1.2. STUDY DESIGN

Breeder females (experimentally naive time-mated female Crl:CD®(SD)[1] rats) that were between gestation days 7 and 10 were obtained for this study from Charles River Laboratories, Inc., Portage, Michigan. The breeder females were housed in plastic maternity cages containing nesting material (heat-treated laboratory-grade pine shavings) in a controlled environment. Breeder females were observed twice daily for changes in general appearance and behavior and were allowed to deliver naturally. Litters were standardized to 8 to 10 pups per litter on postnatal day (PND) 4. Offspring clinical observations, body weights, viability and sex were recorded at appropriate intervals. Any unthrifty litters or runted pups were excluded from the study on PND 4 (prior to culling) or on PND 21 (prior to randomization). Breeder females were euthanized and discarded following weaning of the offspring.

On PND 21, all surviving male pups (female pups were used for a pubertal female assay; Bowman, 2005) were weaned and randomly assigned to one of nine groups, with subsequent selection of 15 rats/group with body weights in the middle of the distribution per group and across groups. Selected juvenile rats were housed 2 to 3 animals per cage

[1] Prior to 1 January 2005, this strain of rat was designated the Crl:CD®(SD)IGS BR rat.

in plastic maternity cages in an environmentally controlled room. Beginning on PND 23, the juvenile rats were administered the vehicle (corn oil) or one of 4 test substances [DE-71 (30 or 60 mg/kg), 2-chloronitrobenzene (25 or 100 mg/kg), dibutyl phthalate (500 or 1000 mg/kg) or vinclozolin (30 or 100 mg/kg)], once daily by oral gavage until PND 53. The dosage volume for all groups was 2.5 mL/kg, and dosing occurred between 7:00 a.m. and 8:55 a.m. All animals were observed twice daily for appearance, behavior, mortality and moribundity. Detailed physical examinations, post-dose observations and body weights were recorded daily from PND 21 to PND 53. Each male pup was observed daily for balanopreputial separation beginning on PND 30 and continuing until balanopreputial separation was complete. Approximately 2 hours following dosing on PND 53, all males were euthanized by exposure to carbon dioxide for up to 60 seconds followed by decapitation. Trunk blood was collected from each male and centrifuged; serum levels of thyroxine (T_4), thyroid stimulating hormone (TSH) and testosterone were determined. A complete necropsy was conducted on all males; selected tissues were weighed and examined microscopically. All in-life and post mortem activities were conducted blind to treatment group.

1.3. RESULTS

1.3.1. DE-71

Increased follicular epithelial cell height and decreased colloid area of the thyroid, together with the coordinate decrease in mean serum T_4 and increase in mean serum TSH in both DE-71 dose groups observed in the current study are consistent with previous observations (Stoker, 2004). Mean absolute and PND 21 body weight-adjusted liver weights were also significantly increased in a dose-related manner in the DE-71 treated groups, possibly due to the reported induction of hepatic biotransformation enzymes by DE-71.

The slight delay in the absolute mean age of complete preputial separation observed in the 60 mg/kg/day group was not considered a true delay since the PND 21 body weight-adjusted difference between the control and DE-71 groups was only 0.6 days.

1.3.2. 2-CHLORONITROBENZENE

There was a significant delay (2 days) in the mean age of complete preputial separation in the 100 mg/kg/day 2-chloronitrobenzene group compared to control. There was a slightly increased (2.8%, not statistically significant) mean body weight on the day of preputial separation in the 100 mg/kg/day group compared to control. Post mortem, mean liver weights in both dose groups were significantly increased compared to the control group. Consistent with the observed 39% decrease in mean serum testosterone, the seminal vesicles, ventral prostate and levator ani plus bulbocavernosus muscle in the 100 mg/kg/day dose group were significantly decreased compared to the control group. The dorsal/lateral prostate weight was also slightly decreased in this group. Mean pituitary weight was also significantly decreased in the 100 mg/kg/day group males. Although testes weights were not affected by 2-chloronitrobenzene, there was some unexpected histopathology at 100 mg/kg/day. Specifically, degeneration of round and elongated spermatids and decreased elongating spermatids were observed in addition to retention of spermatids in late stage seminiferous tubules. These testicular lesions were generally subtle. There were no significant differences in mean serum T_4 or TSH values in either dose group compared to the control group.

1.3.3. DIBUTYL PHTHALATE

Dibutyl phthalate significantly decreased mean weights of epididymides, both testes, seminal vesicles and levator ani plus bulbocavernosus muscles at 500 and 1000 mg/kg/day. At 1000 mg/kg/day, dorsal/lateral prostate was significantly decreased, and there were also slight decreases in mean weights of dorsal/lateral prostate at 500 mg/kg/day and of ventral prostate at both dosage levels. The growth and development of the aforementioned organs are dependent on androgens; therefore, the

decreased weights of these organs are not surprising considering serum testosterone was significantly decreased by 53% and 60% in the 500 and 1000 mg/kg/day dibutyl phthalate groups, respectively, compared to the control group. In addition, mean liver weights were significantly increased in the high dose group.

The organ weight effects on the testes and epididymides corresponded convincingly with the histopathology observed at both dosage levels. Specifically, minimal to severe degeneration of the seminiferous tubules, characterized by loss of germinal epithelium as well as reduction and absence of elongated spermatids and minimal to mild interstitial cell hypertrophy were observed. In the epididymis, there was an increased incidence and severity of hypospermia, as well as increased severity of cellular luminal debris within the epididymal ducts. These epididymal changes were considered secondary to the lesions in the testes, and were consistent with the decreased mean epididymis weights.

There was also a slight delay of 1.4 and 1.6 days in the mean age of preputial separation the animals dosed with 500 and 1000 mg/kg/day dibutyl phthalate from PND 23 to 53, respectively. It is unclear whether or not the 1.4- or 1.6-day difference in the 2 dose groups represents a true delay in preputial separation. Previous reports in the literature support a slight delay in preputial separation following 500 mg/kg/day dibutyl phthalate exposure to weaning rats (Ashby, 2000; Gray, 1999).

A significant decrease in body weight gain from PND 23 to 53 was observed in the 1000 mg/kg/day group compared to the control group (229.8 ± 2.92 g vs. 248.4 ± 5.76 g, respectively). This decreased mean body weight gain resulted in a statistically significant decrease (approximately 6.5%) in the mean final body weight compared to the control group.

The decrease in mean T_4 levels described in the current study have also been reported in other male assays with dibutyl phthalate (O'Connor, 2002). However, without supportive changes in thyroid weights or histopathology, the relationship and significance of the decreased T_4 and TSH to the administration of dibutyl phthalate in this study is uncertain.

1.3.4. VINCLOZOLIN

Vinclozolin at 30 and 100 mg/kg/day significantly delayed the mean age of preputial separation by approximately 2 and 5 days, respectively. This is consistent with previous studies that reported a 2.4- and 5.4-day delay (George, 2003) or a 2.8- and 6.6-day delay (Rocca, 2003), respectively, compared to controls at the same dosage levels under the same general pubertal study design. Secondary to the older age at preputial separation in these animals, mean body weights were significantly increased (8.4% and 21.2%, respectively) in the 30 and 100 mg/kg/day groups at the time of preputial separation compared to controls.

With 100 mg/kg/day vinclozolin, there were significantly decreased epididymides, levator ani plus bulbocavernosus muscle, and paired seminal vesicle weights; mean weights of these organs were also decreased at 30 mg/kg/day, but only the mean epididymal weight was significant at this lower dosage. There were no correlating histopathologic changes in the epididymides with vinclozolin in this study. There was also a 10% and 19.8% decrease in mean absolute ventral prostate weights (30 and 100 mg/kg/day groups, respectively) and a 9.2% decrease in mean absolute dorsal/lateral prostate weight (100 mg/kg/day group), but these decreases were not statistically significant.

Mean testosterone levels were increased in both treatment groups, although the changes were not statistically significant. The changes in reproductive organ weights described above were expected results based on the known mechanism of action. Mean liver and adrenal gland weights were also significantly increased in the 100 mg/kg/day group in the current study, which is consistent with previous reports (George, 2003; Kelce, 1997).

In the 100 mg/kg/day group, the mean T4 level was decreased (statistically significant) and the mean TSH level was slightly increased (not statistically significant) compared to the control group. There were no correlating effects on thyroid gland weights or

histopathology. Decreased T_4 in the absence of changes in TSH and thyroid histopathology have been noted previously (George, 2003; O'Connor, 2002).

1.4. CONCLUSIONS

In general, the results of this male pubertal study successfully identified the sensitive features for each the chemicals tested. Specifically, the thyroid toxicity induced by DE-71 was confirmed at both 30 and 60 mg/kg/day by changes in T_4 and TSH, and effects on thyroid histopathology. 2-Chloronitrobenzene at 100 mg/kg/day was identified to have some unexpected antiandrogen-like effects characterized by a slight delay in preputial separation, testicular histopathology, slightly reduced testosterone, and decreased reproductive organ weights (not including the testis or epididymis). Dibutyl phthalate at 500 and 1000 mg/kg/day produced the expected effects of reduced serum testosterone, decreased reproductive organ weights (including testes and epididymides) and histopathology of the testis and epididymis (and possibly a delay in preputial separation at the high dose). Vinclozolin produced effects consistent with previous male pubertal reports, specifically the marked delay in preputial separation (30 and 100 mg/kg/day), reduced reproductive organ weights (not including the testis) in the absence of any testicular or epididymal histopathology, and slightly elevated serum testosterone. Both dibutyl phthalate and vinclozolin produced alteration in T_4 and TSH, but the significance of these findings in the absence of thyroid histopathology is unknown. When considered collectively, the results of this study were considered acceptable for interlaboratory comparisons for the purposes of validating this male pubertal bioassay.

2. INTRODUCTION

2.1. GENERAL STUDY INFORMATION

This report presents the data from "Validation of the Pubertal Male Assay in Rats (WA 4-15)".

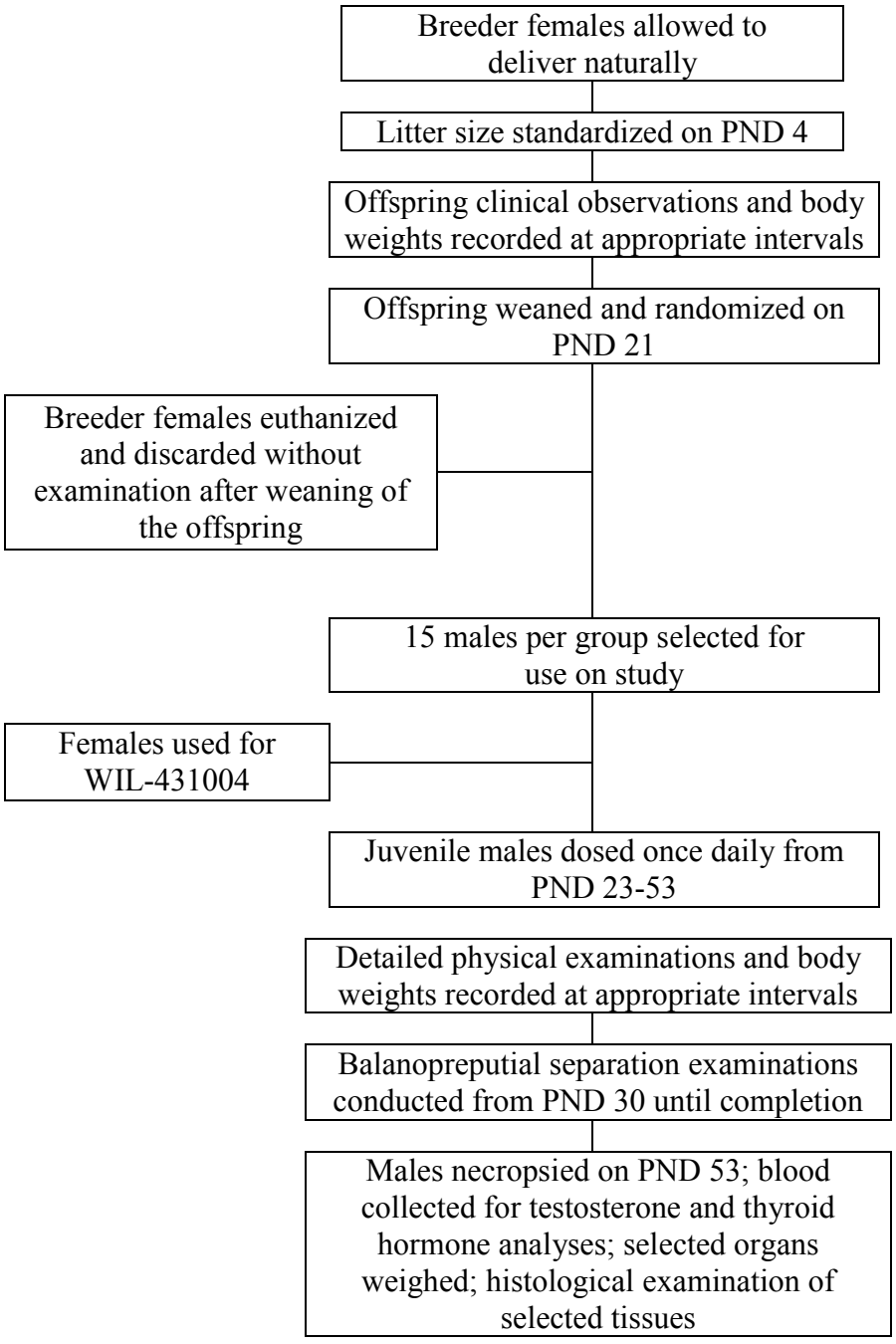
The following computer protocols were used for data collection during the study:

Computer Protocol	Type of Data Collected
WIL-431004P	Breeder female and pretest offspring data
WIL-431005	Main study data

2.2. KEY STUDY DATES

Date(s)	Event(s)
6 January 2005	Experimental starting date (breeder female receipt)
17-21 January 2005	Breeder female parturition
10 February - 14 March 2005	Dosing period (PND 23-53)
17 February - 12 March 2005	Developmental indices (balanopreputial separation)
12-14 March 2005	Necropsies (PND 53)
19 July 2005	Experimental termination date (last TSH analysis)

3. STUDY DESIGN



4. EXPERIMENTAL PROCEDURES - MATERIALS AND METHODS

4.1. TEST SUBSTANCES AND VEHICLE

4.1.1. TEST AND VEHICLE CONTROL SUBSTANCES

The test substances, DE-71, 2-chloronitrobenzene, dibutyl phthalate and vinclozolin, were received from Marine Sciences Laboratory of Battelle Northwest, Sequim, Washington, as follows:

Identification	Quantity Received	Physical Description	Date of Receipt
DE-71[1] Lot no. 4550OD23D Exp. date: 3 Nov 10 CAS no. Mixed [WIL log no. 6385A]	1 bottle Total gross weight: 572.4 g	Clear, amber, viscous liquid	15 Dec 04
2-Chloronitrobenzene[2] Lot no. 09019MC Exp. date: 1 Nov 10 CAS no. 88-73-3 [WIL log no. 6390A]	1 bottle Total gross weight: 1023.3 g	Clear, yellow liquid	17 Dec 04
Dibutyl Phthalate[3] Lot no. 00323PU Exp. date: 11 Oct 10 CAS no. 84-74-2 [WIL log no. 6388A]	1 jar Total gross weight: 4022.0 g	Clear, colorless liquid	16 Dec 04
Vinclozolin[4] Lot no. 329-72-B Exp. date: Oct 07 CAS no. 50471-44-8 [WIL log no. 6386A]	1 bottle[5] Total gross weight: 328.3 g	Fine, white powder	15 Dec 04
Vinclozolin[4] Lot no. 329-72-B Exp. date: Oct 07 CAS no. 50471-44-8 [WIL log no. 6386B]	1 bottle Total gross weight: 53.2 g	Fine, white powder	01 Feb 05

[1] = Sponsor-determined purity: 100.0% bromodiphenylether (BDE; 48.7% tetraBDE, 49.5% pentaBDE, 1.8% hexaBDE)
[2] = Sponsor-determined (manufacturer) purity: 100.34% (99.80%)
[3] = Sponsor-determined (manufacturer) purity: 100.67% (99.90%)
[4] = Sponsor-determined (manufacturer) purity: 100.29% (99.50%)
[5] = This bottle was found broken inside the original packaging material on 2 January 2005. The broken container and vinclozolin were put through an 80 sieve to remove the container material. A 0.5-g sample of the sieved test substance was sent to the Sponsor for purity analysis according to the Sponsor's SOPs. The analyzed concentration of this sample was 99.58% of target (Appendix B). A low-dose pre-initiation solution was prepared and examined for the presence of container material; no container material was noted.

Certificates of Analysis for the test substances were provided by the Sponsor and are presented in Appendix A. The dosing formulations were not adjusted for purity. The test substances were stored at room temperature, and were considered stable under this condition. A reserve sample of dibutyl phthalate (approximately 1 g) was collected on 11 January 2005. Reserve samples of vinclozolin were collected on 17 January 2005 (approximately 1 g) and 3 February 2005 (approximately 0.5 grams). Reserve samples of DE-71 and 2-chloronitrobenzene (approximately 2.5 g and 1.6 g, respectively) were collected on 14 January 2005. All reserve samples were stored in the Archives of WIL Research Laboratories, LLC.

4.1.2. VEHICLE CONTROL SUBSTANCE IDENTIFICATION

The vehicle used in preparation of the test substance formulations and for administration to the control group was corn oil, received from Marine Sciences Laboratory of Battelle Northwest, Sequim, Washington, as follows:

Identification	Quantity Received	Physical Description	Date of Receipt
Corn oil[1] Lot no. AO-001 Exp. date: 28 Aug 05 CAS no. 8001-30-7 WIL Log no. 6389A	14 bottles[2]	Yellow liquid	16 Dec 04
Corn oil Lot no. AO-002 Exp. date: 28 Aug 05 CAS no. 8001-30-7 WIL Log no. 6389B	8 bottles	Clear yellow liquid	26 Jan 05

[1] = Peroxide content less than 3 mEq/mL as determined by WIL Research Laboratories, LLC (Appendix C).

[2] = Eight bottles used for pre-initiation method development. The remaining six bottles were mixed with lot no. AO-002. This new batch was designated 6389A/B-S1 and was used in formulations for dose administration. Peroxide content of this combined batch was less than 3 mEq/mL as determined by WIL Research Laboratories, LLC (Appendix C).

Reserve samples of the vehicle were collected on 17 January 2005 (log no. 6389A, approximately 1 g) and 2 February 2005 (log nos. 6389B and 6389A/B-S1, approximately 1 mL each) and stored in the Archives of WIL Research Laboratories, LLC.

4.1.3. PREPARATION

The bottles of corn oil from the 2 lots remaining after pre-initiation method development (lot nos. AO-001 and AO-002) were combined in a 20-L carboy that had previously been washed, rinsed with acetone and dried. The combined batch (designated 6389A/B-S1) was then stirred for at least 15 minutes. For the control group (Group 1), a sufficient amount of this corn oil was dispensed into four 250-mL amber glass bottles. Each bottle was used for approximately 1 week and stirred continuously throughout preparation, sampling, dispensation and dose administration. The vehicle was stored refrigerated.

All test substance formulations were weight/volume (test substance/vehicle) mixtures and were prepared once for pre-initiation method development and once for dosing animals on study. The formulations prepared as described below were poured into four 250-mL amber glass bottles per group, which were capped tightly with screw-cap lids and refrigerated. Each 250-mL bottle of formulation prepared for use on study was used for dose administration for approximately 1 week. The formulations were mixed using a magnetic stirrer throughout the sampling and dose administration procedures. The formulations were stored refrigerated.

For the DE-71 groups (Groups 2 and 3), the test substance was warmed to approximately 40°C until it was liquefied and then agitated for approximately 2 minutes by inverting. The test substance was then heated to approximately 50°C and an appropriate amount of DE-71 for each group was weighed into a calibrated glass jar. Vehicle was added to each container to bring the formulations nearly to the calibration mark. The formulations were mixed using a magnetic stirrer until a solution was formed. Vehicle was then added to

each container to bring the formulations to the calibration mark. The formulations were then stirred vigorously with an overhead stirrer for approximately 30 minutes.

For the 2-chloronitrobenzene groups (Groups 4 and 5), the test substance was warmed to approximately 40°C until it was liquefied. An appropriate amount of 2-chloronitrobenzene for each group was weighed into a tared, 1-L volumetric flask. Approximately half of the final volume of the vehicle was added and the contents were immediately agitated in order to dissolve the test substance. Vehicle was then added to each container to bring the formulations to the calibration mark.

For the dibutyl phthalate groups (Groups 6 and 7), an appropriate amount of test substance was weighed into a tared 1-L volumetric flask. Vehicle was added to each flask to bring the formulations to the calibration mark. The contents were agitated to dissolve the test substance.

For the vinclozolin groups (Groups 8 and 9), an appropriate amount of test substance was weighed into a tared 1-L volumetric flask. Vehicle was added to each flask to bring the formulations to the calibration mark. The formulations were sonicated for up to 90 minutes, without exceeding 40°C, in order to dissolve the test substance. After sonicating, the solutions were manually agitated to ensure that the contents were uniform.

The vehicle was visually inspected by the study director on 3 February 2005 and found to be visibly homogeneous and acceptable for administration. The pre-initiation and/or dosing formulations were visually inspected by the study director on 3 February 2005 (DE-71 and 2-CNB dosing formulations), 21 January 2005 (dibutyl phthalate and vinclozolin pre-initiation formulations) and 3 and 4 February 2005 (dibutyl phthalate and vinclozolin dosing formulations, respectively) and were found to be visibly homogeneous. The test substance formulations were analytically confirmed to contain the amounts of test substance specified in the protocol (see Section 4.1.5.).

4.1.4. ADMINISTRATION

Dose administration was performed blind to treatment group by assigning each group a letter designation. The test and vehicle control substance formulations were administered orally by gavage, via an appropriately-sized stainless steel ball-tipped dosing cannula (Popper and Sons, Inc., New Hyde Park, New York), once daily from PND 23-53. A dosage volume of 2.5 mL/kg was used. Individual dosages were based on the body weights recorded before dosing on each day to provide the correct mg/kg/day dose. All animals were dosed between 7:00 am and 8:55 am each day; the time of dose administration was recorded for each animal. The juvenile males were assigned to study groups as follows:

Group Number	Letter Code	Test Substance	Dosage Level (mg/kg/day)	Dosage Concentration (mg/mL)	Dosage Volume (mL/kg)	Number of Females
1	A	Corn Oil	0	0	2.5	15
2	B	DE-71	30	12	2.5	15
3	C	DE-71	60	24	2.5	15
4	D	2-Chloronitrobenzene	25	10	2.5	15
5	E	2-Chloronitrobenzene	100	40	2.5	15
6	F	Dibutyl Phthalate	500	200	2.5	15
7	G	Dibutyl Phthalate	1000	400	2.5	15
8	H	Vinclozolin	30	12	2.5	15
9	I	Vinclozolin	100	40	2.5	15

Dosage levels were selected based on the results of previous studies and were provided by the Sponsor representative.

The animal model selected, the Crl:CD®(SD) rat, is recognized as appropriate for prepubertal studies and has been proven to be susceptible to the effects of reproductive toxicants. WIL Research Laboratories, LLC has historical control data for the Crl:CD®(SD) rat.

4.1.5. SAMPLING AND ANALYSIS

Stability of the test substance solutions bracketing the dosage concentrations used on study were provided by the Sponsor and are presented in Appendix B; the test substance

formulations were considered to be stable for 42 days under refrigerated conditions. All test substance formulations except for the 40 mg/mL vinclozolin formulation were considered solutions; therefore, homogeneity was not assessed. The Sponsor provided documentation of homogeneity for the 40 mg/mL vinclozolin formulation. Samples (1 mL each) for concentration analysis were collected from approximately 1 inch below the surface of the pre-initiation dibutyl phthalate formulations and the DE-71, 2-chloronitrobenzene and dibutyl phthalate dosing formulations. Samples (1 mL each) for concentration analysis were collected from the top and bottom 25% of a representative 250-mL aliquot of the 40 mg/mL vinclozolin formulation. In addition, 5 gram samples of the vehicle (corn oil) were analyzed in triplicate for peroxide content from the first lot (pre-initiation formulations) and from the combination of lots (dose administration formulations).

Concentration and peroxide content analyses were conducted by the Analytical Chemistry Department, WIL Research Laboratories, LLC. The methodology and results of these analyses are presented in Appendix C. The test substance formulations contained the amounts of test substance specified in the protocol and the vehicle control contained less than 3 mEq/mL peroxide.

4.2. BREEDER FEMALE RECEIPT AND QUARANTINE

Forty-three experimentally naive, time-mated Crl:CD®(SD) rats were received in good health from Charles River Laboratories, Inc., Portage, Michigan, on 6 January 2005 (gestation days 7-10). Each female was examined by a qualified technician on the day of receipt and weighed the day after receipt. Each rat was uniquely identified by a Monel® metal eartag displaying the animal number and placed in quarantine for the duration of the gestation period. During the quarantine period, the rats were observed twice daily for changes in general appearance and behavior.

4.3. ANIMAL HOUSING

Upon arrival all breeder dams were housed individually in plastic maternity cages (16" x 7 3/8" x 8 1/8") with nesting material (heat-treated laboratory-grade pine shavings, Northeastern Products Corp., Warrensburg, New York). The females were housed in these cages until lactation day 21, the scheduled day of euthanasia. Following weaning on PND 21, pups selected for use on study were housed together (2-3 pups) by litter in plastic maternity cages with bedding material (heat-treated, laboratory-grade pine shavings, as described above), which was changed at least three times each week. Animals were maintained in accordance with the *Guide for the Care and Use of Laboratory Animals* (National Research Council, 1996). The animal facilities at WIL Research Laboratories, LLC are accredited by the Association for Assessment and Accreditation of Laboratory Animal Care International (AAALAC International).

4.4. DIET, DRINKING WATER AND MAINTENANCE

The basal diet used in this study, PMI Nutrition International, LLC, Certified Rodent LabDiet® 5002, is a certified feed with appropriate analyses performed by the manufacturer (under cGMPs) and provided to WIL Research Laboratories, LLC. The batch of feed used on this study was analyzed for isoflavone content and had a total genistein-equivalent content (aglycone) of approximately 319 ppm (Owens et al., 2003). The batch of feed was approved for use on study by the Sponsor, as the genistein-equivalent content (aglycone) content was higher than the protocol-specified 300 ppm. The analytical method summary, results and total genistein-equivalent content are presented in Appendix D. Feeders were changed and sanitized once per week. Feed samples were retained at ≤10°C for possible future analysis. These samples will be discarded after issuance of the final report. Municipal water supplying the facility is sampled for contaminants according to the standard operating procedures. The results of the feed lot analysis were placed in the study records and the results of the water analyses are maintained at WIL Research Laboratories, LLC. No contaminants were thought to be present in animal feed or water at concentrations sufficient to interfere with the objectives

of this study. Reverse osmosis-purified (on-site) drinking water, delivered by an automatic watering system, and the basal diet were provided ad libitum during the study.

4.5. ENVIRONMENTAL CONDITIONS

Animals were housed throughout the quarantine period, pre-test procedures and during the study in an environmentally controlled room. The room temperature and humidity controls were set to maintain daily averages of 71 ± 3°F (22 ± 2°C) and 45 ± 5% relative humidity. Room temperature and relative humidity were monitored using the Metasys DDC Electronic Environmental control system and were recorded approximately hourly. These data are summarized in Appendix E. Actual mean daily temperature ranged from 70.4°F to 70.7°F (21.3°C to 21.5°C) and mean daily relative humidity ranged from 30.8% to 48.8% during the study. Light timers were calibrated to provide a 14-hour light (5 a.m. to 7 p.m.)/10-hour dark photoperiod. Air handling units were set to provide approximately 10 fresh air changes per hour.

4.6. PRETEST PROCEDURES

The breeder dams were allowed to deliver naturally. Cages were checked daily in the morning for new births until parturition was complete. The day parturition was initiated was designated PND 0. All pups were individually identified by application of tattoo markings on the tail following completion of parturition. To maximize uniformity in growth rates, the litters were standardized to 8-10 pups per litter on PND 4. Offspring clinical observations and body weights were recorded weekly. Offspring viability was recorded daily and offspring sex was recorded at birth and on PND 1, 4, 7, 14 and 21. Any unthrifty litters or runted pups were excluded from the study on either PND 4 (prior to culling) or on PND 21 (prior to randomization).

4.7. ASSIGNMENT OF ANIMALS TO TREATMENT GROUPS

Once pretest deliveries were complete, the number of projected PND 21 calendar days was reduced to 3 days, whilst still allowing for adequate numbers of animals available for randomization onto study. The litters and corresponding maternal females not selected

for the projected PND 21 calendar day randomization procedures were transferred to the stock colony or euthanized by carbon dioxide inhalation after PND 10. Male offspring were weaned on PND 21; female rats were also weaned on PND 21 and used for a female pubertal assay (Bowman, 2005). On PND 21, all surviving eligible male pups were individually weighed to the nearest 0.1 g. At the conclusion of the pretest period, all male pups judged to be suitable test subjects were assigned to one of nine groups at random using a WIL Toxicology Data Management System (WTDMS^{TM}) computer program, which randomized the animals based on stratification of the PND 21 body weights in a block design with the condition that litter mates were not placed in the same group. All eligible animals were assigned to groups for each day of randomization and then, on the last PND 21 randomization calendar day, the overall PND 21 mean body weight for each group was determined. In order to keep the mean PND 21 body weight and variances across groups as similar as possible, the animals with PND 21 body weights at the heavy and light end of the distribution were removed from the study on the last PND 21 randomization calendar day (euthanized by carbon dioxide inhalation and discarded without examination). Therefore, the experimental design for WIL-431005 consisted of nine test substance-treated groups (4 test substances at 2 dosage levels each) and 1 vehicle control group composed of 15 rats each. Body weight values for the selected study males ranged from 40.4 g to 55.8 g on PND 21.

5. PARAMETERS EVALUATED

5.1. CLINICAL OBSERVATIONS AND SURVIVAL

All juvenile rats selected for use on study were observed twice daily, once in the morning and once in the afternoon, for appearance, behavior, moribundity and mortality. A detailed physical examination was conducted on the day of randomization and daily prior to dose administration. All animals were also observed for signs of toxicity approximately 1 hour following dose administration each day (except on the day of necropsy). All significant clinical findings were recorded at these observation periods.

5.2. BODY WEIGHTS

Individual body weights (to the nearest 0.1 g) were recorded daily from PND 21-53, inclusively. Group mean body weights were calculated for each of these days. Group mean body weight changes were calculated for each daily interval, and also for PND 23-53.

5.3. BALANOPREPUTIAL SEPARATION

Each male pup was observed for balanopreputial separation beginning on PND 30 (Korenbrot et al., 1977). The day on which balanopreputial separation was first observed was recorded for each pup. Examination of the pups continued daily, and the appearance of partial and complete balanopreputial separation or a persistent thread of tissue between the glans and prepuce was recorded on the days observed. Individual body weights were recorded on the day of complete balanopreputial separation.

5.4. SCHEDULED NECROPSY

On PND 53, all males were euthanized by exposure to carbon dioxide (up to 60 seconds) followed by decapitation; all males were euthanized by 1:00 p.m. to minimize variability in thyroid hormone levels due to normal diurnal fluctuation. A complete necropsy was conducted on all animals starting approximately 2 hours following dose administration. The necropsy included examination of the external surface, all orifices, the cranial cavity, the external surfaces of the brain and spinal cord, and the thoracic, abdominal and pelvic

cavities, including viscera. Selected tissues were retained for all animals as described in Section 5.2.3.

5.4.1. HORMONE ANALYSES (T_4, TSH AND TESTOSTERONE)

Immediately following euthanasia, trunk blood (minimum of 2 mL) was collected for testosterone and thyroid hormone analysis. Serum was isolated by centrifugation (4°C for approximately 10 minutes) and each serum sample volume was divided approximately equally into 2 siliconized microcentrifuge tubes and stored frozen at ≤-20°C until analysis of thyroid-stimulating hormone (TSH), thyroxine (T_4) and testosterone. The Clinical Pathology Department, WIL Research Laboratories, LLC conducted T_4 hormone analyses by a solid-phase chemiluminescent enzyme immunoassay (Immulite®, Diagnostic Products Corporation, Los Angeles, California) and the Metabolism Department, WIL Research Laboratories, LLC conducted TSH and testosterone analyses using a radioimmunoassay (RIA) procedure (Amersham BioSciences, Piscataway, New Jersey). Multiple quality control samples were run dispersed within each assay. For the TSH RIA assay, rat TSH standard (RP3) from the National Institute of Diabetes and Digestive and Kidney Diseases was diluted in assay buffer to 3, 6 and 9 ng/mL as quality control samples. For all hormone analyses, all serum samples analyzed were within the range of the respective standard curve. In general, all samples were analyzed in a single assay per hormone and quality control samples were in the expected range based on manufacturer specifications and previous experience. The intra-assay variability (based on coefficient of variation of each of the QA samples) was less than 15% for each assay.

5.4.2. TISSUE COLLECTION AND ORGAN WEIGHTS

The following organs from all males euthanized at scheduled termination were weighed (to the nearest 0.1 mg):

Adrenal glands[a]	Prostate (ventral and dorsolateral)
Epididymides[a]	Pituitary gland
Kidneys[a]	Seminal vesicles with coagulating gland (with accessory fluids)
Levator ani plus bulbocavernosus muscles	Testes[b]
Liver	Thyroid[c,d]

[a] = These paired organs were weighed together.
[b] = These paired organs were weighed separately.
[c] = Includes parathyroid glands; designated as "Thyroid Glands" on all tables.
[d] = Weighed after fixation in 10% neutral-buffered formalin.

Small tissues such as the adrenals and pituitary, as well as tissues that contain fluid were weighed immediately to prevent tissues from drying out prior to weighing.

To minimize systematic bias in the weighing procedures, organ harvesting and weighing procedures were divided as equally as possible among the prosecting and weighing technicians, such that all animals from a group were not processed by a single individual.

5.4.3. TISSUE FIXATION AND PROCESSING

The right testis and right epididymis were placed in Bouin's fixative for approximately 24 hours, after which they were rinsed and stored in 70% alcohol until histological processing. The thyroid, with attached trachea, was fixed in 10% neutral-buffered formalin for at least 24 hours. Then the thyroid was dissected from the trachea, blotted and weighed to the nearest 0.1 mg and placed in 70% ethanol until histological processing. The right testis, the right epididymis and the thyroid were then embedded in paraffin, sectioned and stained with hematoxylin and eosin (H&E) for subsequent

histological evaluations. Sections of 2-4 microns were made for the testis (transverse) and for the epididymis (longitudinal).

5.4.4. MICROSCOPIC EVALUATION

Testis, epididymis and thyroid histology were evaluated for pathologic abnormalities and potential treatment-related effects. In accordance with the USEPA OPPTS 870.3800 Guideline (1998), testicular histopathological examination included identification of retained spermatids, missing germ cell layers or types, multinucleated giant cells, or sloughing of spermatogenic cells into the lumen and examination of the intact epididymis included the caput, corpus and cauda epididymis via a longitudinal section in order to identify lesions such as sperm granulomas, leukocytic infiltration, aberrant cell types within the lumen or the absence of clear cells in the epithelium.

Three sections of the paired thyroid lobes from each animal were qualitatively evaluated for follicular epithelial height and colloid area as described by Capen and Martin (1989), using a 5 point grading scale (1=shortest/smallest; 5=tallest/largest) and any abnormalities/lesions noted. There was great variability in the microscopic appearance of the thyroid follicles in some animals, both within an individual section of thyroid and between sections of the same lobe. In general, when variability was noted, follicles toward the center of the thyroid lobes were given greater emphasis than those follicles at the periphery of the lobe, and sections of thyroid that were closer to the center of the lobe were given greater emphasis than those sections that were obtained closer to the periphery. Other microscopic changes were also recorded when present.

The pathologist reading the slides was presented only with the coded identity of the group to which a sample belonged. After the raw data were collected, the codes were translated to test substances and dosage groups for the purposes of the pathologist's interpretation and report. Microscopic examinations were conducted by Karen S. Regan, DVM, DACVP, DABT, Consulting Pathologist.

5.5. STATISTICAL METHODS

All statistical tests were performed using appropriate computing devices or programs. Analyses were conducted using two-tailed tests (except as noted otherwise) for minimum significance levels of 5% and 1%, comparing each test substance-treated group to the control group. Each mean was presented with the standard deviation (S.D., Appendices G, I and L only), standard error of the mean (S.E.) and the number of animals (N) used to calculate the mean. The coefficients of variation were calculated by the sponsor, and are presented with S.D. in Appendix H. The tables in Appendix H are intended to be an extension of the regular report tables, as such, the footnotes used in Appendix H correspond to those footnotes in the regular report tables. Due to the different rounding conventions inherent in the types of software used, the means, standard deviations, coefficients of variation and standard errors of the mean on the summary and individual tables may differ by ±1 in the last significant figure. Upon review of the individual data for biological plausibility, no data points were identified as potential outliers. Therefore, no statistical outlier tests were conducted.

All endpoint measures (PND 21 body weight, body weight gain from PND 23 to 53, age and body weight at preputial separation, body and organ weights at necropsy and serum hormones) were analyzed per test substance (control and 2 dosage levels). All endpoints were analyzed for heterogeneity of variance using Levene's test (Levene, 1960). For the PND 21 and 53 body weights, body weight gains from PND 23-53 and serum hormone values, if the data were homogeneous, a parametric one-way analysis of variance (ANOVA) was used to determine intergroup differences (Snedecor and Cochran, 1980). If the ANOVA revealed statistically significant ($p<0.05$) intergroup variance, Dunnett's test (Dunnett, 1964) was used to compare the test substance-treated groups to the control group. If the data were not homogeneous and normal, the data were analyzed by the Kruskal-Wallis nonparametric ANOVA test (Kruskal, 1952). If the results of this ANOVA were statistically significant, Dunn's test (Dunn, 1952) was applied to the data to compare all test substance-treated groups to the control group.

For organ weights and age and body weight at preputial separation, if the data were homogeneous using Levene's test, the data were analyzed using a parametric one-way ANOVA followed by Dunnett's test, as described above. In accordance with the statistical tree presented in Appendix F, if the data were not homogeneous using Levene's test, 1 of 5 methods of transformation were applied to the data in order to achieve homogeneity. The methods of transformation were applied to heterogeneous data in the following order: Log_{10} (x+1), x^2, $\sqrt{x}$, 1/x and rank sum. In addition, organ weights and age and body weight at preputial separation (using transformed data when necessary) were also analyzed by Analysis of Covariance (ANCOVA) using the body weight on PND 21 as the covariant. If the ANCOVA revealed statistically significant ($p<0.05$) intergroup variance, Dunnett's test (Dunnett, 1964) was used to compare the LSmean of each test substance-treated group to the control group (SAS Institute, 1999-2001).

5.6. DATA RETENTION

The Sponsor has title to all documentation records, raw data, specimens or other work product generated during the performance of the study. All work product generated by WIL Research Laboratories, LLC, including raw paper data and specimens, are retained in the Archives at WIL Research Laboratories, LLC, as specified in the study protocol.

Reserve samples of the test and vehicle control substances, pertinent electronic storage media and the original final report are retained in the Archives at WIL Research Laboratories, LLC in compliance with regulatory requirements.

6. RESULTS

6.1. CONTROL MALES

Fifteen males were assigned to the control group. This control group was used as a comparison to evaluate the effects of each test substance on pubertal development and thyroid function.

6.2. DE-71-TREATED MALES

6.2.1. IN-LIFE DATA

Table 1, Figure 1 and Appendices G, H and I (overall summary tables, coefficients of variation and individual tables, respectively)

There were no clinical observations in animals treated with 30 or 60 mg/kg/day DE-71 at the daily examinations or approximately 1 hour following dose administration. The mean body weight gain between PND 23 and 53 was slightly less in the 60 mg/kg/day DE-71 group compared to the control group mean (238.0 ± 6.72 g compared to 248.4 ± 5.76 g, respectively), but the difference was not statistically significant. There was also a slight decrease (4.31%, not statistically significant) in the mean final body weight on PND 53 in this group.

The mean absolute ages of attainment of balanopreputial separation were 43.9 ± 0.47, 44.7 ± 0.64 and 44.9 ± 0.51 days in the vehicle control, 30 and 60 mg/kg/day DE-71 groups, respectively. There were no occurrences of partial balanopreputial separation or persistent threads for any animal. The mean absolute body weights were 222.7 ± 4.61, 227.1 ± 5.08 and 222.8 ± 4.16 g at the time of preputial separation in the same respective groups (PND 21 body weight adjusted weights were also similar across groups). Based on the absolute mean data there was a slight delay (1 day) in the 60 mg/kg/day group for this developmental landmark in the absence of a corresponding increase in mean body weight at this age. However, the PND 21 body weight-adjusted age of preputial separation reduced the difference in mean age to 0.6 days between the control and 60 mg/kg/day group, reducing the likelihood that this was a true delay due to DE-71 in the

current study. By comparison, 30 and 60 mg/kg/day DE-71 in a similar study design have been previously shown to delay the mean age at preputial separation in Wistar rats by 1.7 and 2.1 days, respectively (Stoker, 2004).

6.2.2. NECROPSY, HISTOPATHOLOGICAL AND HORMONE DATA

Tables 2A, 2B, 3 and Appendices G, H, I and J (overall summary tables, coefficients of variation, individual tables and photographs of microscopic lesions, respectively)

In the 30 and 60 mg/kg/day DE-71 groups, mean absolute and PND 21 body weight-adjusted liver weights were significantly ($p<0.01$) increased in a dose-related manner compared to the control group. This weight change is consistent with the induction of hepatic enzymes by DE-71, as demonstrated previously (Zhou, 2001). There were no significant differences in mean or PND 21 body weight-adjusted organ weights for the kidneys, paired seminal vesicle with coagulating glands, prostate lobes, epididymides, testes, levator ani plus bulbocavernosus muscle, adrenal glands, pituitary or thyroid gland with DE-71 treatment compared to controls. However, in the 60 mg/kg/day group there were slight decreases in the mean absolute weights of the paired seminal vesicle with coagulating glands (9.3%), ventral prostate (12.5%) and levator ani plus bulbocavernosus muscle (10.9%) compared to the mean absolute weights in the control group. This slight decrease in paired seminal vesicle and ventral prostate weights is consistent with a previous study with 60 mg/kg/day DE-71, where statistically significant decreases in these two mean absolute organ weights were observed.

There was an increased mean thyroid follicular epithelial cell height (1 = shortest; 5 = tallest) and decreased mean colloid area (1 = least; 5 = most) in DE-71 treated animals compared to control animals (see following text table and plates 1 and 2 in Appendix J). The changes for both follicular epithelial cell height and colloid area were dose-related. Similar changes in these 2 parameters have been previously reported in pubertal rats dosed with 60 mg/kg/day DE-71 (Stoker, 2004). There were no other microscopic effects considered related to DE-71 in the thyroid and there were no

treatment-related macroscopic or microscopic findings in the testis or epididymis. Follicular degeneration, previously reported in pubertal animals treated with similar doses of DE-71 (Stoker, 2004), was not observed in the current study. Microscopic findings similar to those reported as degeneration in that study were observed in this study in animals from all groups, including the control group, and were interpreted as artifactual.

Diagnosis	**0**	**30 mg/kg/day**	**60 mg/kg/day**
Follicular Epithelial Height (total)	15	15	15
Grade 1	2	1	0
Grade 2	11	1	1
Grade 3	2	8	2
Grade 4	0	4	11
Grade 5	0	1	1
Mean	**2.0**	**3.2**	**3.8**
Colloid Area (total)	15	15	15
Grade 1	0	1	5
Grade 2	0	7	7
Grade 3	8	5	3
Grade 4	4	2	0
Grade 5	3	0	0
Mean	**3.7**	**2.5**	**1.9**

Although mean thyroid weights were not elevated in the 30 and 60 mg/kg/day DE-71 groups compared to the control group, mean serum T_4 (µg/dL) levels (1.50 ± 0.100 and 0.94 ± 0.057, respectively were markedly lower (statistically significant, $p<0.01$) than the control group (6.68 ± 0.346). Conversely, the mean serum TSH (ng/mL) levels were increased (26.1 ± 2.55 and 26.0 ± 3.59, respectively,) compared to the control group (15.8 ± 1.15); the differences were statistically significant ($p<0.01$ or $p<0.05$). These data are consistent with the statistically significant decrease in T_4 and increase in TSH in the male pubertal assay with 30 and 60 mg/kg/day DE-71 demonstrated previously (Stoker, 2004). There were no test article-related effects on serum testosterone. Mean serum testosterone levels (ng/dL) in the 30 and 60 mg/kg/day DE-71 groups

(322.8 ± 53.60 and 298.5 ± 45.66, respectively) were not significantly different from the control group (240.7 ± 38.93) and did not occur in a dose-related manner.

6.3. 2-CHLORONITROBENZENE-TREATED MALES

6.3.1. IN-LIFE DATA

Table 4, Figure 2 and Appendices G, H and I (overall summary tables, coefficients of variation and individual tables, respectively)

There were no clinical observations in animals treated with 25 or 100 mg/kg/day 2-chloronitrobenzene at the daily examinations or approximately 1 hour following dose administration. The mean body weight gain was decreased in animals dosed with 100 mg/kg/day for the first 2 days of dose administration (PND 23-24 and 24-25) compared to controls. This decreased mean body weight gain resulted in decreased mean body weights (5.3% to 6.4%) from PND 24 through 27. For the rest of the dose administration period the mean body weight gain was similar between the control and 100 mg/kg/day groups. By the scheduled day of euthanasia (PND 53), the final mean body weight in this group was 5.3% less than the control group (not statistically significant). Consistent with this slight decrease in the 100 mg/kg/day group final body weight, the PND 23 to 53 body weight gain was slightly less in this group relative to the control group (232.2 ± 4.77 g compared to 248.4 ± 5.76 g, respectively). The mean body weights and body weight gains in the 25 mg/kg/day group were similar to the control group for the duration of the study.

There was a 2 day delay in the mean absolute and PND 21 body weight-adjusted age that preputial separation was complete in the 100 mg/kg/day 2-chloronitrobenzene group compared to the control group (statistically significant, $p<0.05$ or $p<0.01$). In addition, there was a slightly increased (not statistically significant) mean absolute and PND 21 body weight-adjusted mean body weight on the day of preputial separation. There were no occurrences of partial balanopreputial separation or persistent threads for any animal.

6.3.2. NECROPSY, HISTOPATHOLOGICAL AND HORMONE DATA

Tables 5A, 5B, 6 and Appendices G, H, I and J (overall summary tables, coefficients of variation, individual tables and photographs of microscopic lesions, respectively)

Mean absolute and PND 21 body weight-adjusted liver weights were increased (statistically significant, $p<0.01$) in a dose-related manner in the 25 and 100 mg/kg/day 2-chloronitrobenzene groups compared to the control group. This increased liver weight is consistent with previous data demonstrating that 2-chloronitrobenzene induces liver necrosis, inflammation and hepatocytomegaly (IUCLID, 2003). There was also a statistically significant ($p<0.05$ or $p<0.01$) decrease in mean absolute and PND 21 body weight-adjusted weights of the paired seminal vesicle with coagulating glands, ventral prostate, levator ani plus bulbocavernosus muscle and pituitary in the 100 mg/kg/day group compared to the control group. There were no statistically significant differences in the mean absolute or PND 21 body weight-adjusted organ weights for kidney, dorsal/lateral prostate, epididymides, testes, adrenal glands or thyroid gland following 2-chloronitrobenzene treatment compared to the control group. However, in the 100 mg/kg/day group, there were slight decreases in the mean absolute weights of the dorsal/lateral prostate (13.5%) and the thyroid gland (24.4%) compared to the mean absolute weights in the control group. There was a slight increase (not statistically significant) in the mean serum TSH level in the 100 mg/kg/day group (19.1 ± 1.37 ng/mL) compared to the control group (15.8 ± 1.15 ng/mL). The slight decrease in mean thyroid gland weight and the slight increase in mean serum TSH level in the 100 mg/kg/day group were considered incidental since the thyroid histopathology and mean serum T_4 in the 100 mg/kg/day group were all similar to controls. The decrease in the mean dorsal/lateral prostate weight in the 100 mg/kg/day group is consistent with the decreases observed in the other androgen-dependent organs and with the 39% decrease in mean serum testosterone in this group compared to the control group. Notably, organ weight changes and/or microscopic changes in these organs were

not reported in rats and mice treated with 2-chloronitrobenzene in adult repeated-dose toxicity studies (IUCLID, 2003).

Despite the lack of organ weight differences in the testis compared to controls, there were test chemical-related changes in the testis histopathology in the 100 mg/kg/day 2-chloronitrobenzene group. Specifically, minimal to moderate degeneration of spermatids was observed in 6 of 15 animals. This lesion was characterized by the abnormal presence of degenerating round spermatids with pyknotic or ring nuclei in stages 9-11 of spermatogenesis, the presence of degenerating elongating spermatids, most often in stages 9-11, often with degenerating elongating spermatids in these same tubules. Affected round spermatids were sometimes sloughed into the tubular lumen or were present near the basement membrane of the tubule, suggesting phagocytosis of the abnormal spermatids by Sertoli cells (see Plate 5 compared to Plate 4 in Appendix J). In lesser-affected animals, the lesion may have involved only a portion of the tubular epithelium in stages 9-11, while the moderately affected animals, all of the tubular epithelium was affected, and the lesion was observed in more stages, typically 9-14. Decreased elongating spermatids, observed in two 100 mg/kg/day males, was characterized by partial loss of elongating spermatids in the seminiferous epithelium, primarily in stages 12-14. In these tubules, normal elongating spermatids were present in portions of the epithelium. Occasional elongating spermatids were sometimes observed near the tubular basement membrane (phagocytosis by Sertoli cells). This lesion was considered part of the spectrum of degeneration of spermatids previously described. Both affected animals had other testis lesions (either spermatid degeneration or retained spermatids). Spermatid retention was observed in four of fifteen 100 mg/kg/day group animals. This finding was characterized by the abnormal presence of step 19 spermatids along the tubular lumen of the seminiferous tubule in stages 9-11 (see Plate 6 compared to Plate 4 in Appendix J). Normally, step 19 spermatids are released at stage 8. Stage 19 spermatids were generally no longer observed by stage 12. The finding was minimal in all animals. These types of testis lesions have not been previously reported in adult

rodents administered 2-chloronitrobenzene. It may be that the pubertal animal is more sensitive to this test chemical. The testis lesions in the 100 mg/kg/day animals were generally subtle, making detection difficult. There were no epididymal lesions associated with 2-chloronitrobenzene treatment. Numbers of animals with hypospermia in these groups were similar to controls.

In the 25 mg/kg/day group, there were no effects on organ weights (except liver), serum hormones (testosterone, T_4 or TSH) or histopathology of the thyroid, testis or epididymis.

6.4. DIBUTYL PHTHALATE-TREATED MALES

6.4.1. IN-LIFE DATA

Table 7, Figure 3 and Appendices G, H and I (overall summary tables, coefficients of variation and individual tables, respectively)

In the 500 and 1000 mg/kg/day dibutyl phthalate groups, 3/15 and 13/15 animals, respectively, were observed with wet clear material around the mouth approximately 1 hour following dosing. These findings were observed sporadically between PND 25 and PND 50 and were considered test substance-related. There were no other notable clinical observations in these groups. In the 1000 mg/kg/day group, there were periodic decreases in mean body weight gain throughout the dose administration period, resulting in a statistically significant decrease ($p<0.01$) in mean body weight gain between PND 23 and 53 (229.8 ± 2.92 g) compared to the control mean body weight gain (248.4 ± 5.76 g). This decrease in the 1000 mg/kg/day group mean body weight gain was reflected as a 6.5% decrease (statistically significant, $p<0.01$) in absolute final body weight on PND 53 compared to the control group.

The mean absolute ages of attainment of balanopreputial separation were 43.9 ± 0.47, 45.3 ± 0.41 and 45.5 ± 0.64 days in the vehicle control, 500 and 1000 mg/kg/day dibutyl phthalate dose groups, respectively (no statistical significance across groups). There were no occurrences of partial balanopreputial separation or persistent threads for any animal. The values for the PND 21 body weight-adjusted age for attainment of the

developmental landmark were identical to the absolute age. The mean absolute and PND 21 body weight-adjusted body weights on the day that preputial separation completed was similar across groups. From this data set it is unclear whether or not the 1.4 or 1.6 day difference in the 2 dibutyl phthalate groups represents a true delay in preputial separation. There are previous data indicating that oral administration of 500 mg/kg/day dibutyl phthalate results in a 3.9-day delay in preputial separation when Long Evans rats are dosed starting on PND 21 (Gray, 1999) and a 1.9-day delay when Alpk:ApfSD rats are dosed starting on PND 22 (Ashby, 2000). The differences between the current study and the previous studies are likely attributable to the strain differences, but also the age at which daily dose administration was initiated.

6.4.2. NECROPSY, HISTOPATHOLOGICAL AND HORMONE DATA

Tables 8A, 8B, 9 and Appendices G, H, I and J (overall summary tables, coefficients of variation, individual tables and photographs of microscopic lesions, respectively)

Administration of 1000 mg/kg/day dibutyl phthalate to male rats from PND 23 to 53 resulted in statistically significant increases ($p<0.01$) in mean absolute and PND 21 body weight-adjusted liver weight, and significant decreases ($p<0.01$) in multiple reproductive mean absolute and adjusted organ weights, including seminal vesicles, levator ani plus bulbocavernosus muscle, dorsal/lateral prostate, epididymides and testes when compared to the control group. At 500 mg/kg/day, paired epididymides, testes and levator ani plus bulbocavernosus muscle mean absolute and adjusted weights were significantly decreased ($p<0.01$). Paired seminal vesicle with coagulating gland weights were also statistically significant at 500 mg/kg/day, but only when adjusted for PND 21 body weight (ANCOVA). Other mean organ weights that showed decreases relative to the control group, but were not statistically significant, were the 18.4% and 18.7% decreases in mean absolute ventral prostate weights at 500 and 1000 mg/kg/day, respectively and the 9.4% decrease in mean absolute dorsal/lateral prostate weight at 500 mg/kg/day. The testis and epididymis weight changes were consistent with the microscopic findings described below. Significant reductions in mean paired epididymis and paired seminal

vesicle weights, and increased mean liver weights, were reported at 500 mg/kg/day dibutyl phthalate in a previous male pubertal study (Ashby, 2000). The organ weight changes in the current study are all consistent with the reported ability of dibutyl phthalate to inhibit testosterone biosynthesis (Lehmann, 2004).

A dose-related increase in the incidence and severity of degeneration of the seminiferous tubules was observed in the testes of 500 and 1000 mg/kg/day dibutyl phthalate-treated animals (see Plate 7 compared to Plate 4 in Appendix J). All animals at 1000 mg/kg/day had moderately severe to severe degeneration, characterized by a complete or nearly complete loss of germinal epithelium lining the seminiferous tubules. At 500 mg/kg/day dibutyl phthalate, the testis lesion was present in 10/15 animals and ranged in severity from minimal to severe. In lesions of lesser severity, there were a lesser number of tubules with nearly complete germ cell loss.

Seven of fifteen animals in the 500 mg/kg/day dibutyl phthalate group had decreased elongated spermatids, characterized by a reduction or absence of elongated spermatids in stages 9-14. Six of the 7 animals with this lesion also showed minimal to moderately severe degeneration of the seminiferous tubules, as described above. Thus decreased elongated spermatids was thought to represent an early stage of the more severe change, degeneration, in which most or all of the germinal epithelium was lost. In the dibutyl phthalate-treated groups, degeneration and decreased elongated spermatids correlated with decreased mean testis weights and with the macroscopic observations of small and soft testes. Phthalate esters are Sertoli cell toxicants that cause germ cell detachment from Sertoli cells and sloughing of germ cells into the tubular lumen, and the testis lesions observed in this study are consistent with those previously described for phthalate esters (Creasy, 2002).

Minimal to mild interstitial cell hypertrophy was observed in 4/15 animals in the 500 mg/kg/day group and all 1000 mg/kg/day animals (see Plate 8 compared to Plate 4 in Appendix J). Hypertrophy was noted when the groups of interstitial cells appeared larger

than what would be expected in an untreated animal. Interstitial cell hypertrophy has not been previously reported in peripubertal dibutyl phthalate-treated animals, but has been observed when male rats were exposed to dibutyl phthalate in utero (Mylchreest, 2002). The size of the interstitial cells in animals in the current study may have been over-estimated because of the marked loss of seminiferous tubule epithelium that causes interstitial cells to be more apparent, and lack of untreated control comparison during the reading (blinded read). Thus, the significance of this finding is uncertain.

Hypospermia was a relative change based on the apparent density of mature sperm in the cauda epididymis. Fourteen of fifteen animals treated with 500 mg/kg/day dibutyl phthalate and all animals treated with 1000 mg/kg/day dibutyl phthalate showed increased severity of hypospermia when compared to controls (see Plate 10 compared to Plate 9 in Appendix J). Additionally, 13/15 animals treated with 500 mg/kg/day dibutyl phthalate and all animals in the 1000 mg/kg/day dibutyl phthalate group showed increased severity of cellular luminal debris within the epididymal ducts. Both of these changes are considered secondary to the testis lesions present in these animals. The hypospermia correlates with the decreased mean epididymis weights and the macroscopic observation of small epididymis in the dibutyl phthalate groups.

Mean total testosterone (ng/dL) was decreased in the 500 and 1000 mg/kg/day dibutyl phthalate groups (112.5 ± 23.53 and 95.1 ± 14.65) compared to the control group (240.7 ± 38.93); the differences in both treated groups were statistically significant ($p<0.01$). This dose-dependent decrease in serum testosterone with dibutyl phthalate is consistent with the reported ability of dibutyl phthalate to inhibit testosterone biosynthesis (Lehmann, 2004) and correlates with the testis changes observed microscopically.

Mean total T_4 was decreased in both the 500 and 1000 mg/kg/day dibutyl phthalate groups; the decreases were statistically significant ($p<0.05$ and $p<0.01$, respectively). Mean TSH, however, was significantly decreased in the 1000 mg/kg/day group and was

unchanged in the 500 mg/kg/day group compared to the control group. There was no correlating organ weight or histopathologic changes in the thyroids at either dosage. Decreased mean T_4 levels (but not TSH) have been reported in other male assays with dibutyl phthalate and the authors postulated that many chemicals that are not thyrotoxicants or endocrine modulators can transiently alter thyroid hormone homeostasis in rodents without long term morphological or functional effects on the thyroid (O'Connor, 2002). Without supportive changes in thyroid weights or histopathology, the relationship of the decreased T_4 and TSH to the administration of dibutyl phthalate and its significance is uncertain.

6.5. VINCLOZOLIN-TREATED MALES

6.5.1. IN-LIFE DATA

Table 10, Figure 4 and Appendices G, H and I (overall summary tables, coefficients of variation and individual tables, respectively)

The only clinical finding observed in the vinclozolin groups was single instances of 2 animals having wet clear material around the mouth at approximately 1 hour after dose administration on PND 52 (the day before scheduled euthanasia). There were no other clinical findings at the daily examinations or approximately 1 hour following dose administration with vinclozolin at 30 and 100 mg/kg/day. There was no test chemical effect on body weight gain (PND 23-53) or final body weight.

In the absence of any direct effect on body weight, vinclozolin at 30 and 100 mg/kg/day delayed mean absolute and PND 21 body weight-adjusted age of complete preputial separation by approximately 2 and 5 days (both statistically significant, $p<0.05$ and $p<0.01$, respectively). This is consistent with previous studies that reported a 2.4- and 5.4-day delay (George, 2003) or a 2.8- and 6.6-day delay (Rocca, 2003) in preputial separation compared to controls at the same dose levels under the same general pubertal study design. In addition, mean body weight at the age attainment of this developmental landmark was increased by 8.4% and 21.2% in the 30 and 100 mg/kg/day vinclozolin

groups, respectively, compared to the control group. This increase in mean body weight at the time of preputial completion is consistent with the 2 previous reports described above, in all 3 studies the increased mean body weight at both 30 and 100 mg/kg/day was statistically significant. There were no occurrences of partial balanopreputial separation or persistent threads for any animal.

6.5.2. NECROPSY, HISTOPATHOLOGICAL AND HORMONE DATA

Tables 11A, 11B, 12 and Appendices G, H and I (overall summary tables, coefficients of variation and individual tables, respectively)

Statistically significant and test article related changes in absolute organ weights were observed primarily in the 100 mg/kg/day vinclozolin group. Mean absolute and PND 21 body weight-adjusted levator ani plus bulbocavernosus muscle, paired seminal vesicles and epididymides weights were significantly decreased ($p<0.01$) with 100 mg/kg/day vinclozolin treatment. Mean weights of these organs were also decreased at 30 mg/kg/day, but only the paired epididymis weight changes were statistically significant ($p<0.05$) at this lower dose. There were no correlating histopathologic changes in the epididymides with vinclozolin in this study. The decreased mean absolute and adjusted levator ani plus bulbocavernosus muscle and seminal vesicles were also significantly decreased in previous male pubertal studies (George, 2003; Rocca, 2003). Other mean organ weights that showed decreases relative to the control group, but were not statistically significant, were the 10% and 19.8% decreases in mean absolute ventral prostate weights at 30 and 100 mg/kg/day, respectively, and the 9.2% decrease in mean absolute dorsal/lateral prostate weight at 100 mg/kg/day. The decrease in ventral and dorsal/lateral mean prostate weights at 100 mg/kg/day was consistent with statistically-significant decreases reported in a previous male pubertal study (Rocca, 2003) and the decreasing trend observed for the mean ventral prostate weight in the George (2003) study. Mean absolute liver and adrenal gland weights were significantly increased in the 100 mg/kg/day group ($p<0.01$). Increased adrenal gland weights have been reported previously in another pubertal rat study at 100 mg/kg/day vinclozolin (Rocca, 2003).

A statistically significant ($p<0.01$) decrease in mean total T_4 was observed in the 100 mg/kg/day vinclozolin group compared to the control group. There was a slight increase in TSH levels at 100 mg/kg/day vinclozolin, but it was not statistically significant. There were no correlating effects on thyroid weights or microscopic changes in the thyroid at either dosage. Decreased mean total T_4 levels have been reported in male pubertal and adult animals dosed with 100 mg/kg/day vinclozolin without corresponding changes in TSH or thyroid histopathology (George, 2003; O'Connor, 2002).

Mean testosterone levels (ng/dL) were 240.7 ±38.93, 327.7 ± 68.25 and 380.7 ±63.67 in the control, 30 and 100 mg/kg/day dose groups, respectively. The increases in the treated groups were not statistically significant, but were considered treatment-related. Androgen receptor antagonists typically produce increased testosterone levels in pubertal male rats (Lambright, 2000) but, at the dosages used in this study, vinclozolin has not been reported to cause histopathologic effects in the testis or epididymis (George, 2003; Kelce, 1997). Histopathologic effects were also absent in this study at both dosages of vinclozolin.

7. DISCUSSION

7.1. DE-71

Increased follicular epithelial cell height and decreased colloid area of the thyroid, together with the coordinate decrease in mean serum T_4 and increase in mean serum TSH in both DE-71 dose groups observed in the current study are consistent with a previous report in pubertal Wistar rats at similar dosages of DE-71 (Stoker, 2004). Mean absolute and PND 21 body weight-adjusted liver weights were also significantly increased in a dose-related manner in the DE-71 treated groups, possibly due to the reported induction of hepatic biotransformation enzymes by DE-71.

The slight delay in the absolute mean age of complete preputial separation observed in the 60 mg/kg/day group was not considered a true delay since the PND 21 body weight-adjusted difference between controls and DE-71 was only 0.6 days. This is contrary to the delayed onset of preputial separation of 1.7 (30 mg/kg/day) and 2.1 days (60 mg/kg/day) observed previously in Wistar rats (Stoker, 2004).

7.2. 2-CHLORONITROBENZENE

There was a significant delay (2 days) in the mean age of complete preputial separation in the 100 mg/kg/day 2-chloronitrobenzene group compared to control. There was a slightly increased (not statistically significant) mean body weight on the day of preputial separation. Mean liver weights were significantly increased compared to control group in both dose groups. Consistent with the observed 39% decrease in mean serum testosterone, the seminal vesicles, ventral prostate, levator ani plus bulbocavernosus muscle and pituitary in the 100 mg/kg/day dose group were significantly decreased compared to the control group. The dorsal/lateral prostate weight was also slightly decreased. Although testes weights were not affected by 2-chloronitrobenzene, there was some unexpected histopathology at the 100 mg/kg/day dose. Specifically, degeneration of round and elongated spermatids and decreased elongating spermatids were observed in

addition to retention of spermatids in late stage seminiferous tubules. These testicular lesions were generally subtle.

7.3. DIBUTYL PHTHALATE

Dibutyl phthalate significantly decreased mean weights of epididymides, both testes, seminal vesicles and levator ani plus bulbocavernosus muscles at 500 and 1000 mg/kg/day. At 1000 mg/kg/day, the mean dorsal/lateral prostate weight was significantly decreased, and there were also slight decreases in mean weights of dorsal/lateral prostate at 500 mg/kg/day and of ventral prostate at both dosage levels. The growth and development of the above-mentioned organs are dependent on androgens; therefore, the decreased weights of these organs are not surprising considering serum testosterone was significantly decreased by 53% and 60% in the 500 and 1000 mg/kg/day dibutyl phthalate groups, respectively, compared to the control group. In addition, mean liver weights were significantly increased in the high-dose group.

The organ weight effects on the testes and epididymides correspond convincingly with the histopathology observed at both dosage levels. Specifically, minimal to severe degeneration of the seminiferous tubules, characterized by loss of germinal epithelium, as well as reduction and absence of elongated spermatids and minimal to mild interstitial cell hypertrophy were observed. In the epididymis, there was an increased incidence and severity of hypospermia, as well as increased severity of cellular luminal debris within the epididymal ducts. These epididymal changes were considered secondary to the lesions in the testes, and are consistent with the decreased mean epididymis weights.

There was also a slight delay of 1.4 and 1.6 days in the mean age of preputial separation the animals dosed with 500 and 1000 mg/kg/day dibutyl phthalate from PND 23 to 53, respectively. It is unclear whether or not the 1.4 or 1.6 day difference in the 2 dose groups represents a true delay in preputial separation. Previous reports in the literature support a slight delay (1.9 days, Ashby, 2000 or 3.9 days, Gray, 1999) in preputial separation following 500 mg/kg/day dibutyl phthalate exposure to pubertal rats.

Although there was a significant decrease in body weight gain from PND 23 to 53 in the 1000 mg/kg/day dose group compared to the control group (229.8 ± 2.92 vs. 248.4 ± 5.76, respectively), this change was considered secondary to the above-described effects since most of those effects were observed in the 500 mg/kg/day group as well. This decreased mean body weight gain in the 1000 mg/kg/day group resulted in a statistically significant decrease (approximately 6.5%) in the mean final body weight compared to the control group.

The decrease in mean T_4 levels described in the current study have been also been reported in other male assays with dibutyl phthalate. It has been suggested that many chemicals that are not thyrotoxicants or endocrine modulators can transiently alter thyroid hormone homeostasis in rodents without long term thyroid effects (O'Connor, 2002). Without supportive changes in thyroid weights or histopathology, the relationship and significance of the decreased T_4 and TSH to the administration of dibutyl phthalate in this study is uncertain.

7.4. VINCLOZOLIN

Vinclozolin at 30 and 100 mg/kg/day significantly delayed the mean age of preputial separation by approximately 2 and 5 days, respectively. This is consistent with previous studies that reported a 2.4- and 5.4-day delay (George, 2003) or a 2.8- and 6.6-day delay (Rocca, 2003) compared to controls at the same dosage levels under the same general pubertal study design. Secondary to the older age of preputial separation in these animals, there was an increased mean body weight at the time of preputial separation compared to controls.

With 100 mg/kg/day vinclozolin, there were significantly decreased epididymides, levator ani plus bulbocavernosus muscle, and paired seminal vesicle weights; mean weights of these organs were also decreased at 30 mg/kg/day, but only the mean epididymides weight was significant at this lower dose. There were no correlating histopathologic changes in the epididymides with vinclozolin in this study. Other mean

organ weights that showed decreases relative to control, but were not statistically significant, were the 10% and 19.8% decrease in mean absolute ventral prostate weights (30 and 100 mg/kg/day groups, respectively) and the 9.2% decrease in mean absolute dorsal/lateral prostate weight (100 mg/kg/day group). This decrease in ventral and dorsal/lateral mean prostate weights at 100 mg/kg/day is consistent with significant decreases reported in a previous male pubertal study (Rocca, 2003) and a decreasing trend observed for the mean ventral prostate weight in the George study. The decreased levator ani plus bulbocavernosus muscle and seminal vesicles were also significantly decreased in previous male pubertal studies (George, 2003; Rocca, 2003).

Mean testosterone levels were increased in both treatment groups, although the changes were not statistically significant. Vinclozolin is an androgen receptor antagonist (Kelce, 1997) and this increase in testosterone is a known effect (Lambright, 2000). The changes in reproductive organ weights described above are consistent with this mechanism of action and were expected results. At the dosages used in this study, vinclozolin has not been reported to cause histopathologic effects in the testis or epididymis (George, 2003; Kelce, 1997). This is consistent with the lack of histopathological changes observed in this study. Mean liver and adrenal gland weights were also significantly increased in the 100 mg/kg/day group in the current study. Increased adrenal gland weights have been reported previously in other pubertal rat study at 100 mg/kg/day vinclozolin (Rocca, 2003).

8. CONCLUSIONS

In general, the results of this male pubertal study successfully identified the sensitive features for each the chemicals tested. Specifically, the thyroid toxicity induced by DE-71 was confirmed at both 30 and 60 mg/kg/day by changes in T_4 and TSH, and effects on thyroid histopathology. 2-chloronitrobenzene at 100 mg/kg/day was identified to have some unexpected antiandrogen-like effects characterized by a slight delay in preputial separation, testicular histopathology, slightly reduced testosterone, and decreased reproductive organ weights (not including the testis or epididymis). Dibutyl phthalate at 500 and 1000 mg/kg/day produced the expected effects of reduced serum testosterone, decreased reproductive organ weights (including testes and epididymides) and histopathology of the testis and epididymis (and possibly a delay in preputial separation at the high-dose). Vinclozolin produced effects consistent with previous male pubertal reports, specifically the marked delay in preputial separation (30 and 100 mg/kg/day), reduced reproductive organ weights (not including the testis) in the absence of any testicular or epididymal histopathology, and slightly elevated serum testosterone. Both dibutyl phthalate and vinclozolin produced alteration in T_4 and TSH, but the significance of these findings in the absence of thyroid histopathology is unknown. When considered collectively, the results of this study were considered acceptable for interlaboratory comparisons for the purposes of validating this male pubertal bioassay.

9. KEY STUDY PERSONNEL AND REPORT SUBMISSION

Report Submitted By:

13 DEC 2005

Christopher J. Bowman, PhD, DABT
Staff Toxicologist, Developmental and
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Study Director

Date

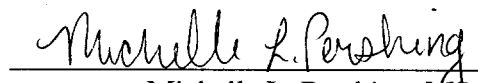
Pathologist of Record:

12-Dec 2005

Karen S. Regan, DVM, DACVP, DABT
Consulting Pathologist

Date

Report Prepared By:

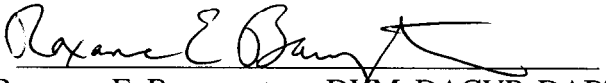


13 Dec 05

Michelle L. Pershing, MS
Senior Study Analyst

Date

Report Reviewed By:



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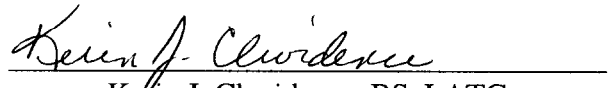
Date



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KEY STUDY PERSONNEL AND REPORT SUBMISSION (CONTINUED)

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FH: Donald G. Stump, PhD, DABT
Associate Director, Developmental and
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13 Dec. 2005
Date

Evelyn Tanchevski, BS
Group Supervisor, Study Analysis and Reports

13 Dec 2005
Date

Study Personnel:

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Theresa M. Rafeld	Group Supervisor, Formulations Laboratory
Daniel W. Sved, PhD	Director, Metabolism and Analytical Chemistry
Bennett J. Varsho, BS, DABT	Operations Manager, Developmental, Reproductive and Neurotoxicology
Robert A. Wally, BS, RAC	Acting Manager, Reporting and Regulatory Technical Services

10. QUALITY ASSURANCE UNIT STATEMENT

10.1. PHASES INSPECTED

Date(s) of Inspection(s)	Phase Inspected	Date(s) Findings Reported to Study Director	Date(s) Findings Reported to Management	Auditor(s)
05-Jan-2005	Protocol Review	05-Jan-2005	16-Feb-2005	J.Tooman
03-Feb-2005	Test Article Preparation	04-Feb-2005	25-Mar-2005	J.House / K.Dobbs
04-Feb-2005	Protocol Amendment I Review	04-Feb-2005	25-Mar-2005	L.Goodrich
08-Feb-2005	Body Weights / Clinical Observations	08-Feb-2005	25-Mar-2005	K.Dobbs
09-Feb-2005	Computerized Randomization Using MPUPJT - PND 21	09-Feb-2005	25-Mar-2005	E.Crawford
10-Feb-2005	Test Article Administration	10-Feb-2005	25-Mar-2005	K.Dobbs
23-Feb-2005	Animal Care/Equipment	23-Feb-2005	25-Mar-2005	S.Solomon
14-Mar-2005	Blood Collection	14-Mar-2005	23-Apr-2005	K.Dobbs
14-Mar-2005	Necropsy	14-Mar-2005	23-Apr-2005	K.Dobbs
06-Apr-2005	Trimming of Tissues	06-Apr-2005	28-May-2005	K.Dobbs
06-Jun-2005	Study Records (I-1)	06-Jun-2005	25-Jul-2005	L.Rush
06-Jun-2005, 10-Jun-2005	Study Records (I-2)	10-Jun-2005	25-Jul-2005	L.Rush
09-Jun-2005, 10-Jun-2005	Study Records (I-3)	10-Jun-2005	25-Jul-2005	L.Rush
10-Jun-2005	Study Records (I-4)	10-Jun-2005	25-Jul-2005	L.Rush
10-Jun-2005	Study Records (N-1)	10-Jun-2005	25-Jul-2005	L.Rush
27-Jun-2005	Study Records (H-1)	27-Jun-2005	25-Jul-2005	L.Rush
27-Jun-2005	Study Records (C-2)	22-Jul-2005	25-Aug-2005	L.Rush
28-Jun-2005	Study Records (P-1)	28-Jun-2005	25-Jul-2005	L.Rush
29-Jun-2005	Protocol Amendment III Review	29-Jun-2005	25-Jul-2005	P.Brant
15-Jul-2005	Hormone Analysis	15-Jul-2005	25-Aug-2005	E.Crawford
14-Jul-2005, 15-Jul-2005	Study Records (A-1 to A-3)	15-Jul-2005	25-Aug-2005	E.Crawford

Date(s) of Inspection(s)	Phase Inspected	Date(s) Findings Reported to Study Director	Date(s) Findings Reported to Management	Auditor(s)
20-Jul-2005	Study Records (C-1)	20-Jul-2005	25-Aug-2005	E.Crawford
18-Jul-2005, 22-Jul-2005	Study Records (Rx-1)	22-Jul-2005	25-Aug-2005	L.Rush
21-Aug-2005	Draft AC Appendix - DE-71	21-Aug-2005	22-Sep-2005	E.Crawford
21-Aug-2005	Draft AC Appendix - Peroxide in Corn Oil	21-Aug-2005	22-Sep-2005	E.Crawford
21-Aug-2005	Draft AC Appendix - Vinclozolin, Dibutyl Phthalate, 1-Chloro-2-Nitrobenzene	21-Aug-2005	22-Sep-2005	E.Crawford
31-Aug-2005, 01-Sep-2005, 02-Sep-2005	Draft Final Report	02-Sep-2005	17-Oct-2005	L.Rush

This study was inspected in accordance with the U.S. EPA Good Laboratory Practice Standards (40 CFR Parts 160 and 792), the standard operating procedures of WIL Research Laboratories, LLC and the Sponsor's protocol and protocol amendments with the following exceptions. The data located in Appendices A (Certificates of Analysis), B (Analyses of Test Substances) and H (Coefficients of Variation) were the responsibility of the Sponsor. The data located in Appendix D (Feed Lot Analyses) were the responsibility of the manufacturer and were collected according to cGMP standards. Quality Assurance findings, derived from the inspections during the conduct of the study and from the inspections of the raw data and draft report, are documented and have been reported to the study director. A status report is submitted to management monthly.

The raw data and draft report were audited by the WIL Quality Assurance Unit prior to submission to the Sponsor to assure that the Final Report accurately describes the conduct and the findings of the study. Quality control (QC) and quality assurance (QA) procedures followed those outlined in the Quality Assurance Project Plan (QAPP) that was prepared for this study (Appendix K).

The raw data, the retention sample(s), if applicable, and the final report will be stored in the Archives at WIL Research Laboratories, LLC or another location specified by the Sponsor.

10.2. APPROVAL

This study was inspected according to the criteria discussed in Section 10.1.

Report Audited By:

Elizabeth S. Crawford, BS
Compliance Specialist

Lori A. Rush, BS, LAT, RQAP-GLP
Sponsor Specialist, Quality Assurance

13 Dec 2005
Date

Report Released By:

Heather L. Osborn, BS, RQAP-GLP
Manager, Quality Assurance

13 Dec 2005
Date

11. REFERENCES

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12. DEVIATIONS FROM THE PROTOCOL AND QAPP

This study was conducted in accordance with the protocol and protocol amendments, except for the following.

- **Protocol Section 6.2** states that controls will be set to maintain an average daily relative humidity of 45 ± 5%. On 16 January 2005, the average relative humidity was 37.4%, on 17 January 2005 the average relative humidity was 39.9%, on 20 January 2005 the average relative humidity was 30.8%, on 21 January 2005 the average relative humidity was 37.6%, on 27 January 2005 the average relative humidity was 38.6% and on 3 February 2005 the average relative humidity was 37.7%.
- According to the QAPP (Appendix K), balances are required to be checked using standard check weights encompassing the weight range to be employed. This was not performed at the time of fixed thyroid weight collection on 14, 15 and 16 March 2005.

These deviations did not negatively impact the quality or integrity of the data nor the outcome of the study.