

SUPPORT

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The Goodyear Tire & Rubber Company

Akron, Ohio 44316-0001



8EHQ-96-13573

Certified Mail

January 10, 2001

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Attn: Section 8 (e) Coordinator
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U.S. Environmental Protection Agency
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Dear Ladies/Gentlemen:

Subject: Supplemental Information Regarding a TSCA Section 8(e) Notice

Reference: EPA Document Control Number: 8EHQ-96-13573

This submittal does not contain Confidential Business Information.

As promised in The Goodyear Tire & Rubber Company's previous submittal of data, enclosed is the following final report:

Two-Generation Reproductive Toxicity Evaluation of WINGSTAY 100 Administered in the Feed to CD® (Sprague-Dawley) Rats, Research Triangle Institute, December 8, 2000.

Also enclosed for your review is the following study:

Mechanistic Study of WINGSTAY 100, Research Triangle Institute, February 7, 2000.

The identity of the material is as follows:

Chemical Abstract Name: 1,4-Benzenediamine, N,N'-mixed Ph and Tolyl derivs.

Chemical Abstract Number: 68953-84-4*

The conclusions of the final study was that exposure to WINGSTAY 100 in the diet for two generations resulted in parental toxicity at 120, 400, and 1500 ppm, with overt



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toxicity at 400 and 1500 ppm. Including reduced body weights, weight gains, feed consumption and food efficiency, renal lesions (polycystic kidneys) in F0 (females only) and F1 parents and in F1 and F2 offspring; dystocia, delayed parturition, perinatal mortality, and increased pup body weights in surviving litters. At 120 ppm, polycystic kidneys were present in F1 and F2 weanlings and in F1 adults, prolonged gestation in F1 (but not F0) females, and increased pup weights in F1 and F2 newborn offspring. There was "no observable adverse effect level" (NOAEL) for adult, reproductive, or postnatal toxicity established in this study.

My address and telephone number are as follows:

The Goodyear Tire & Rubber Company
Department 100D
1144 East Market Street
Akron, Ohio 44316-0001
Telephone Number:(330) 796-2362

Sincerely,

A handwritten signature in black ink that reads "Michael W. Smith". The signature is written in a cursive style with a large, stylized initial "M".

Michael W Smith
Section Manager, Chemical Information
Systems & Regulatory Affairs

Enclosures (2)

FINAL REPORT
VOLUME I OF IV

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TITLE: Two-Generation Reproductive Toxicity Evaluation of Wingstay 100 Administered in the Feed to CD® (Sprague-Dawley) Rats

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The Goodyear Tire and Rubber Company

STUDY INITIATION DATE: May 21, 1996

IN-LIFE PERFORMANCE DATES: June 10, 1996-March 6, 1997

LABORATORY COMPLETION DATE: July 2, 1997

FINAL REPORT DATE: December 8, 2000

RTI IDENTIFICATION NUMBER: 65C-6429-400/200

Author:

Approved:

 12/8/00
 Rochelle W. Tyl, Ph.D., DABT Date
 Study Director
 Life Sciences and Toxicology
 Research Triangle Institute

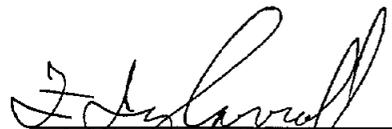
 12/8/00
 F. Ivy Carroll, Ph.D. Date
 Vice President
 Chemistry and Life Sciences
 Research Triangle Institute

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RTI Project No.: 65C-6429-400
RTI Protocol No.: RTI-569 (Definitive Study)

Two-Generation Reproductive Toxicity Evaluation of Wingstay 100
Administered in the Feed to CD® (Sprague-Dawley) Rats

Sponsor: The Goodyear Tire and Rubber Company
Research Division
142 Goodyear Boulevard
Akron, OH 44305-0001

* * * * *

ABSTRACT

Male and female CD® (Sprague-Dawley) weanling rats (the F0 generation) were administered Wingstay 100 (CAS No. 68953-84-4) in 2% corn oil in the feed at 0, 120, 400, and 1500 ppm, available *ad libitum*, 30 animals/sex/dose, for ten weeks. Body weights and feed consumption were recorded weekly, and clinical signs were recorded at least once daily. Vaginal cytology was evaluated for the last three weeks of the prebreed period. Animals were then randomly mated within treatment groups for a two-week mating period to produce the F1 generation, with exposure continued. F0 males were necropsied after the delivery period, with histologic evaluation of reproductive and target tissues for high dose and control males. F1 litters were culled to ten pups on postnatal day (pnd) 4 and weaned on pnd 21. At weaning, three weanlings/sex/litter were necropsied, and 30/sex/dose were selected as F1 parents of the F2 generation. All nonselected F1 weanlings were euthanized and discarded with the concurrence of the Sponsor. F0 females were then necropsied with histopathology of reproductive and target organs for high dose and control animals. Selected F1 weanlings, 30/sex/dose, were administered Wingstay 100 in the diet for a ten-week prebreed exposure period (with acquisition of vaginal patency in females and preputial separation in males assessed and vaginal cytology evaluated during the last three weeks). They were then mated for a two-week mating period as described above. At weaning of F2 litters, three weanlings/sex/ litter were necropsied; all nonselected F2 weanlings were euthanized and discarded with concurrence of the Sponsor. F1 males were necropsied after the delivery period, with histopathology as described above. After weaning of the F2 litters, parental F1 females were then necropsied with histopathology as described above.

Adult toxicity was present for F0 and F1 parental animals at 120, 400, and 1500 ppm (all Wingstay 100-exposed groups). At 400 and 1500 ppm, there was maternal mortality in F0 and F1 parental females associated with dystocia, delayed parturition and perinatal mortality of F1

and F2 offspring, reductions in body weight, weight gain, feed consumption, and food efficiency, consistent and persistent at 1500 ppm, occasional and sporadic at 400 ppm. At 120 ppm, there were few or no effects on these parameters. Treatment-related histologic lesions were limited to the kidney, specifically polycystic kidneys, with dose-related incidence and severity observed in F0 females at 1500 ppm (confounded by the fact that only F0 kidneys with gross lesions were examined histologically), and in F1 weanlings and adults and F2 weanlings at all dietary doses. There was no evidence of F0 or F1 male reproductive toxicity: no effects on acquisition of preputial separation in F1 males, no effects on mating or fertility indices, no histopathologic findings in male reproductive organs, and no effects on seminal parameters (epididymal sperm number, motility, morphology) or on calculated testicular daily sperm production. For F0 and F1 female parental animals, there were also no effects on mating or fertility indices, no effects on acquisition of vaginal patency in F1 females, no histopathologic findings in F0 or F1 female reproductive organs, no difference between high dose and control F0 and F1 females in paired ovarian follicle counts, and no major effects on estrous cyclicity (no changes in incidence of female cycling or in cycle length for both F0 and F1 females, and no changes in incidence of abnormal cycling in F0 females; but significant increases in the incidence of F1 females with abnormal cycles at 400 and 1500 ppm, and significant increases in the percentage of F1 females in metestrus at necropsy).

The peri-parturition findings were observed at all dietary doses. There were no effects of treatment on mating or pregnancy rates, no effects on implantation sites/litter or sex ratio of pups at birth (or during lactation). There were effects on parturition and perinatal survival as follows: postimplantation loss/litter was significantly increased at 400 and 1500 ppm for F1 litters and at 1500 ppm for F2 litters, the numbers of total pups and live pups per litter were significantly reduced at 400 and 1500 ppm for F1 and at 1500 ppm for F2 litters (significant for live pups, reduced but not statistically significantly, for total pups), the numbers of dead pups per litter were significantly increased and the live birth index was significantly reduced at 1500 ppm for F0 and F1 dams. Gestational length was significantly prolonged at 400 and 1500 ppm for F0 dams with F1 litters and at 120, 400 and 1500 ppm for F1 dams with F2 litters. Associated with the prolonged gestation was increased pup body weights per litter at 120 and 400 ppm on pnd 0 for F1 pups and at 120, 400 and 1500 ppm on pnd for F2 pups; F1 (but not F2) pups were also significantly heavier at 400 ppm on pnd 4, and F1 and F2 pups were significantly heavier at 400 ppm on pnd 21. Pup weights at 1500 ppm were increased, but not statistically significantly, on pnd 0 and 4, confounded by systemic toxicity at this dietary dose.

Based on the incidence of polycystic kidneys and prolonged gestation (and increased pup weights) at 120, 400, and 1500 ppm, there was no "no observable adverse effect level" (NOAEL) for adult, reproductive or postnatal toxicity established in this study.

OBJECTIVES

This study was performed to evaluate the potential of Wingstay 100 administered in the feed to CD® rats to produce alterations in parental fertility, maternal pregnancy and lactation, and growth and development of the offspring for two generations, one litter per generation.

MATERIALS AND METHODS

Test Material and Dietary Formulations

The test material, Wingstay 100 (diaryl-*p*-phenylenediamine reaction product; CAS No. 68953-84-4), was received from The Goodyear Tire and Rubber Company (142 Goodyear Boulevard, Akron, OH 44305) in two shipments. The test material was black-blue-brown solid flakes with an amine odor and was identified by the supplier as Lot No. 137170393 for both shipments (date of manufacture March 17, 1993). The first shipment was received at RTI on September 20, 1995, as one container and received the RTI Log No. 8349-04-01. The gross weight was 2276.3 g and it was stored at room temperature under controlled conditions. The second shipment was received at RTI on June 10, 1996, as one container, received RTI Log No. 8349-08-01, and stored at room temperature under controlled conditions. An archive sample of the test material (RTI Log No. 8349-04-01) was retained frozen at approximately -20°C. The corn oil (Mazola®, CPR International, CAS No. 8001-30-7) was purchased twice. The first purchase was ten one-gallon bottles, supplier lot no. 224A6, on May 22, 1996, and received the RTI Log No. 8349-24-01. The second purchase was nine one-gallon bottles, supplier lot no. 624A6, on September 26, 1996, and received the RTI Log No. 8349-34-01. Pooled samples from all bottles of each purchase were taken for peroxide analysis, utilizing potentiometric titration on a Corning Model 140 pH meter, and the balance of the corn oil stored frozen (at approximately -15° to -20°C). The peroxide level for Lot No. 224A6 was assayed at 0.885 (7.2% RSD) meq/kg, and the peroxide level for Lot No. 624A6 was assayed at 1.58 (5.7% RSD) meq/kg; both were acceptable for use (<2 meq/kg per RTI SOPs).

The purity of bulk Wingstay 100 used as the test material was assumed to be 100% for formulation purposes (diaryl-*p*-phenylenediamine reaction product), based on information from the supplier (see Appendix I). The identity, purity, and stability of the test material were the

responsibility of the Sponsor. The remaining bulk material in the original container (from the second shipment) and the empty container (from the first shipment) have been returned to the Sponsor.

The basic diet was ground NIH-07 Certified Rodent Chow (Zeigler Brothers, Inc., Gardners, PA). The lot/batch numbers of the NIH-07 feed were retained in the study records. The feed was stored under controlled conditions at approximately 65°F, and the period of use did not exceed six months from the milling date. The Study Director examined the analyses of each feed batch/lot number. The analyses were retained in the study records; all feed lots/batches were suitable for use. Dosed diet preparations were formulated as follows: all measurements of the test material were made by weight and were not corrected for purity of the active ingredient. Wingstay 100 was dissolved in corn oil (Mazola®, CAS No. 8001-30-7; 2% weight/weight), heated to approximately 90°C, cooled to 60-70°C, and then mixed with the feed as described below. A feed "sandwich" technique was used whereby for a 90 kg batch, the appropriate amount of Wingstay 100 (e.g., 135.0 g for 1500 ppm, 10.8 g for 120 ppm, etc.) in corn oil (1800 g) was mixed with 5 kg of premix. The container for the premix was "rinsed" with 3.2 kg of purge feed and 40 kg of clean feed, the premix plus purge feed, and finally 40 kg clean feed were mixed (i.e., the "sandwich"). Initially, 30 kg aliquots were added to a 2 cubic foot blender (Patterson-Kelly Liquids/Solids V-Shell Blender) and blended for 15 minutes with an intensifier bar. All three aliquots were then transferred to a 5 cubic foot blender (V-Shell Blender, Lowe Industries, Inc., Crestwood, IL) and blended for 15 minutes without the bar. The control diet was formulated in the same manner with 2% corn oil (weight/weight).

Standards for acceptable accuracy of mixing were: the mean of the analyzed samples were within $\pm 15\%$ of nominal, and the % RSD (Relative Standard Deviation) for triplicate samples did not exceed 10%. If one or more of these standards were not met, the dosed diets were not administered to the animals until the problem was resolved by analysis of the archived sample of the dosed diet and/or reformulation and reanalysis. Initially, dosed feed formulations at 120 and 5700 ppm, encompassing the range of dosed feed concentrations employed, were evaluated for homogeneity and stability at freezer and ambient temperatures. Homogeneity was determined by analysis of samples taken one each (and analyzed in triplicate) from the left, right, and bottom port of the V-shell blender for 120 and 1500 ppm. Both formulations were found to be homogeneous. Formulations were stable for at least 49 days in sealed amber bottles at freezer temperatures (-15 to -20°C); formulations were stable for at least nine days under conditions which simulated presentation to animals (at room temperature, in open containers, exposed to light). Dosed feed formulations were made approximately every five

weeks and stored frozen. Feed jars were changed weekly. Verification of dosage concentrations was performed prospectively on all formulations for the first four formulation dates, and then prospectively once per month for all doses per formulation date. For analyses of dosing formulations, triplicate 10 g aliquots of each dosage formulation were weighed into scintillation vials and then transferred to separate 250 ml amber glass screw-top bottles, and extracting solvent (50 ml toluene) was added to each bottle. The bottles were then sonicated for 20 minutes at 40°C and then shaken on a platform shaker for 40 minutes. After the feed had settled for 5 minutes, an aliquot (approximately 3 ml) of each sample was transferred to a separate 1-dram vial and centrifuged for 5 minutes. An aliquot of each supernatant was transferred to a GC vial (0.1 ml supernatant plus 0.9 ml blank feed extract for 1500 ppm, 1.0 ml supernatant for 400 and 120 ppm) and 0.5 ml of internal standard solution (0.0600 mg of 9-phenyl carbazole/ml toluene), and analyzed by gas chromatography as described below. Six standard samples spanning the range of formulations assayed were prepared and analyzed each time that samples were analyzed. Samples and standards were analyzed by Hewlett Packard 5890 Series II gas chromatograph with a nitrogen/phosphorus detector, using a J&W DB-5 (15 m X 0.25 mm ID) 0.25 micron film capillary column. (Details of analytical methods and results are presented in Appendix I.)

All dosed feed formulations were analyzed prior to use; the formulations contained 97.3 to 107% of target concentrations (except for one high dose formulation which was assayed at 112% of target and used with the concurrence of the Sponsor's representative). Vehicle control feed formulations contained no Wingstay 100, with an estimated detection limit of 9.10 ppm. Dietary dose levels were 0, 120, 400, and 1500 ppm, based on results from a reproductive toxicity range-finding study also performed in this laboratory (see Appendix IV). All RTI technical staff involved in the in-life portion of this study, except for the Study Director, Laboratory Supervisor and personnel involved in formulation and analyses of the dosing solutions, were not informed of the dose levels employed; *i.e.*, they were "blind" for dose.

Study animals were administered the control feed or dosed diets, *ad libitum*, seven days per week, 24 hours per day, throughout the study.

Animals and Husbandry

One hundred thirty (130) virgin female and 130 virgin male outbred albino CD® (Sprague-Dawley) rats (CrI:CD®[SD]BR; birth date April 29, 1996) were received from Charles River Breeding Laboratories, Raleigh, NC (area R10) on June 10, 1996, 42 days old (males 120-180 g, females 100-160 g) upon arrival. (The actual dates of all major phases of the study

are presented in Text Table A.) These animals were quarantined for approximately one week during which time they were weighed, examined by a veterinarian, and representative animals were subjected to fecal examination and serum viral antibody analysis.

Text Table A. Study Schedule - Wingstay 100

Event	Date(s)
Animals arrived at RTI-----	6/10/96
Quality control - blood for viral antibody titers -----	6/11/96
Eartag and initial weighing -----	6/11/96
Weighing and randomization -----	6/17/96
F0 prebreed exposure (10 weeks) -----	6/18 – 8/27/96
F0 female vaginal cytology-----	8/7 – 8/27/96
F0 mating (2 weeks)-----	8/27 – 9/10/96
F0 gestation (approx. 22 days)-----	8/28/96 (first gd 0) – 10/2/96 (last gd 22)
F0 male necropsy -----	10/7 – 10/10/96
F0 lactation (3 weeks)-----	9/19/96 (first pnd 0) - 10/24/96 (last pnd 21)
Selection and necropsy of F1 weanlings-----	10/10 – 10/24/96
F0 female necropsy -----	10/10 – 10/24/96
F1 prebreed exposure (10 weeks) -----	10/29/96 – 1/7/97
F1 female vaginal cytology-----	12/17/96 – 1/7/97
F1 mating (2 weeks)-----	1/7 – 1/21/97
F1 gestation (approximately 22 days) -----	1/8/97 (first gd 0) – 2/13/97 (last gd 22)
F1 male necropsy -----	2/17 – 2/20/97
F1 lactation (3 weeks)-----	1/30/97 (first pnd 0) – 3/6/97(last pnd 21)
Necropsy of F2 weanlings-----	2/20 – 3/6/97
F1 female necropsy -----	2/20 – 3/6/97

For serum viral antibody analysis, within one day after receipt, five rats per sex were randomly chosen from the shipment of animals, sacrificed and blood collected for assessment of viral antibody status. Heat-inactivated serum was sent to Microbiological Associates, Bethesda, MD, for their Level 1 Rat Antibody Screen. The viral screen consisted of evaluation for the presence of antibodies against the following: Toolan H-1 virus (H-1), Sendai virus,

Pneumonia virus of mice (PVM), Rat coronavirus/sialodacryoadenitis (RCV/SDA), Kilham rat virus (KRV), CAR Bacillus, and Mycoplasma pulmonis (*M. Pul.*). Results of the physical examination, serology, and parasitology were negative for signs of infectious disease; the animals were considered to be in good health and suitable for use in this study.

There were 240 animals (120 males and 120 females) assigned to the study at the initiation of the F0 prebreed exposure period. F0 animals were uniquely identified prior to initiation of the study by ear tag. The weight variation of the study animals at initiation did not exceed $\pm 20\%$ of the mean weight for each sex. F1 animals were uniquely identified by ear tag at the time of weaning. The method and numbers for identification were documented in the study records.

All weanling and adult animals were euthanized by CO₂ asphyxiation. This includes animals not used in the study, F0 and F1 adults and F1 and F2 weanlings, at scheduled sacrifice, those weanlings not selected for parents or necropsy, and/or any sacrificed moribund. All pups during the lactation period were euthanized by CO₂ asphyxiation and decapitation. This includes pups culled on pnd 4 and any pups sacrificed moribund. Animals received with the initial shipment, but not used in the study, were euthanized or removed from the study room prior to the start of the treatment period and were used for methods development and training of the RTI staff. Records were kept documenting the fate of all animals received for the study. At all times, the animals were maintained, handled, and treated in accordance with the NIH Guide (NIH, 1985).

The experiment was carried out under standard laboratory conditions. The animals were individually housed during the quarantine period and upon the initiation of the treatment period in solid-bottom polycarbonate cages (8"x19"x10.5") with stainless steel wire lids (Laboratory Products, Rochelle Park, NJ), with Ab-Sorb-Dri® cage litter (Laboratory Products, Rochelle Park, NJ). The bedding contained less than the amounts of contaminants specified by the supplier, based on analyses of Sani-Pure Food Laboratories (Saddle Brook, NJ). Study animals were housed two per cage (one male:one female from the same dose level) during the mating period. Females were caged separately and individually once they were successfully mated (or at the end of the mating period), and each female with her litter was housed individually during lactation. All animals were housed in the RTI Animal Research Facility for the duration of the study. All animal rooms were on a 12-hour automatic light cycle per day and were air-conditioned; temperature and relative humidity were continuously monitored, controlled, and recorded using an automatic system (Barber-Colman Network 8000 System, Loves Park, IL). The protocol-mandated temperature range was 68-75°F, and the relative

humidity range was 40-70% (NIH, 1985). The animals were housed in the following Animal Research Facility rooms:

- (1) Room 403: F0 males from June 10, 1996 (date of arrival at RTI) – June 17, 1996 (quarantine), June 18 - August 27, 1996 (F0 Prebreed), August 27 - September 10, 1996 (F0 mating), September 11 - October 10, 1996 (last day of F0 male necropsy);
- (2) Room 404: F0 females from June 10, 1996 (date of arrival at RTI) – June 17, 1996 (quarantine), June 18 - August 27, 1996 (F0 prebreed), August 27 – September 10, 1996 (F0 mating), August 28 – October 24, 1996 (F0 gestation and lactation);
- (3) Room 503: F1 males from October 9, 1996 – January 7, 1997 (except for December 9-16, 1996; see room 206) (F1 prebreed), January 7 - 21, 1997 (F1 mating), January 22 - February 21, 1997 (last day of F1 male necropsy);
- (4) Room 504: F1 females from October 9, 1996 – January 7, 1997 (except for December 9-16, 1996; see room 207) (F1 prebreed), January 7–21, 1997 (F1 mating), January 8 – March 5, 1997 (F1 gestation and lactation);
- (5) Room 206: F1 males on December 9-16, 1996 (during F1 prebreed, while floor repair was occurring in Room 503); and
- (6) Room 207: F1 females on December 9-16, 1996 (during F1 prebreed, while floor repair was occurring in Room 504).

Except for a few, brief, minor excursions in temperature and relative humidity, all animal rooms were in range throughout the study. The minor excursions did not affect the design, conduct or conclusions of this study (see Protocol Deviations, at the end of Volume I, just before the GLP Compliance Statement).

NIH-07 Certified Ground Rodent Chow (Zeigler Brothers, Inc., Gardners, PA; batch numbers documented in the study records) was available *ad libitum*. The analyses of each feed batch for nutrient levels and possible contaminants were performed by the supplier, examined by the Study Director, and maintained in the study records.

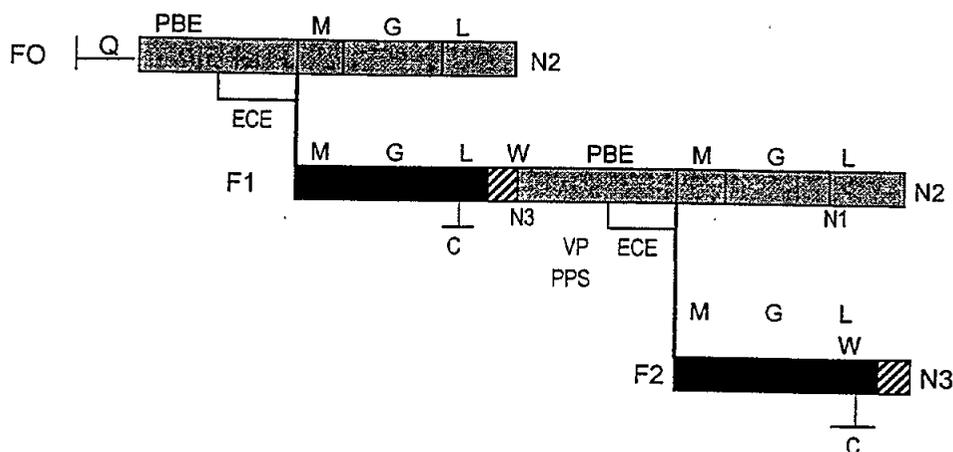
Water (tap water; source: City of Durham, Department of Water Resources, Durham, NC) was available *ad libitum* by polycarbonate water bottles with butyl rubber stoppers and stainless steel sipper tubes. Contaminant levels of the Durham City water were measured monthly by the supplier (and reported annually) and tap water analyzed at least annually by Balazs Analytical Laboratory, Sunnyvale, CA. The analyses of the tap water were examined by the Study Director and retained in the study records; the tap water was suitable for use.

Study Design

A graphic representation of the study design is presented in Text Figure A. The study began with 30 males/group and 30 females/group to yield at least 20 pregnant females/group at or near term. Exposure began for all F0 animals on Tuesday, June 18, 1996, when they were approximately seven weeks old. The route of administration was via dosed diets, as requested by the Sponsor. The target dietary concentrations were 0, 120, 400, and 1500 ppm. Animals were assigned to the different groups by means of randomization stratified by body weight, such that the body weights of all groups were homogeneous by statistical analysis at study initiation. The range of F0 male body weights at the start of the prebreed exposure period was 184.0 – 252.1 g. The range for F0 females was 170.9 – 205.1 g. All animals assigned to each dosage level were exposed to their respective dietary formulations *ad libitum*, 24 hours per day, seven days per week throughout the study.

TEXT FIGURE A

Wingstay 100 Reproductive Toxicity Study Design



KEY

- | | | | |
|---|---|-------|----------------------------------|
| Q = | Quarantine (one week) | PBE = | Pre-Breed Exposure (10 weeks) |
| M = | Mating (two weeks) | G = | Gestation (three weeks) |
| L = | Lactation (three weeks) | W = | Weaning (postnatal day 21) |
| VP = | Vaginal patency (evaluated in F1 females on postnatal day 22 to acquisition) | | |
| PPS = | Preputial separation (evaluated in F1 males on postnatal day 35 to acquisition) | | |
| N1 = | Necropsy of all paternal animals | N2 = | Necropsy of all maternal animals |
| N3 = | Necropsy of selected weanlings, three/sex/litter, if possible | | |
| ECE = | Estrous Cyclicity Evaluations (three weeks) | | |
| C = | Cull litters to 10 pups (with equal sex ratio) on postnatal day 4 | | |
|  | Direct dietary exposure | | |
|  | Possible indirect exposure from transplacental and/or translactational exposure | | |
|  | Both direct and possible indirect exposure (nursing pups also self-feeding) | | |

Observations for mortality were made twice daily (a.m. and p.m.), and the general condition of all animals was checked daily. Clinical examinations were conducted and recorded daily throughout the course of the study. This record included the time of onset and the degree and duration of symptoms. These cage-side observations included, but were not limited to, changes in: skin and fur, eyes, mucous membranes, respiratory system, circulatory system, autonomic and central nervous system, somatomotor activity, and behavior pattern.

The body weights of the male rats were determined and recorded initially and weekly through mating. The body weights of female rats were recorded in the same manner until confirmation of mating. During gestation, females were weighed on gestational days 0, 7, 14, and 20. Dams producing litters were weighed on lactational days (postnatal days) 0, 4, 7, 14, and 21. Body weight gains were computed.

Feed consumption measurements were recorded weekly for all F0 and F1 parental animals throughout the prebreed treatment periods. During pregnancy of F0 and F1 females, feed consumption was recorded for gestational days (gd) 0-7, 7-14, and 14-20. During lactation of F1 and F2 litters, maternal feed consumption was measured for lactational days 0-4, 4-7, 7-14, and 14-21, although maternal feed consumption after lactational day 14 was confounded by the contribution from the pups since pups were self-feeding by this time. Feed consumption was not measured during the cohabitation period, since two adult animals (breeding pair) were in the same cage. Feed consumption collection periods corresponded to the collection of the animals' body weight data; these data were employed to calculate their actual exposure to the test chemical as mg Wingstay 100/kg body weight/day. In addition, as mandated by the OPPTS draft guidelines (U.S. EPA, 1996), food efficiency was calculated per animal per interval as weight gain in grams for the interval divided by feed consumption in grams for the interval ($\times 100$ to allow reporting as a percentage).

For those brief interim periods, such as from the time of weaning until the formal prebreed started for F1 males and females and for males during mating (after "their" females were sperm positive), exposure to dosed feed continued, and F0 and F1 periodic body weights and daily clinical signs were recorded. These data are not presented in the final report since the times are brief and the numbers of animals in each group are variable, and the data are therefore not robust for statistical analysis. These data are retained in the study records.

Animals of the F0 generation were approximately seven weeks of age at the commencement of treatment. They were administered the dosed feed formulations *ad libitum* (24 hours per day, seven days per week) at their respective concentrations for at least ten weeks prior to mating (i.e., until they were approximately 17 weeks of age). For the last three

weeks of the prebreed exposure period, vaginal smears for estrous cyclicity and normality were taken for all F0 females. The slides from the prebreed period were evaluated for estrous cyclicity and normality.

The animals were then mated on the basis of one male to one female selected randomly within each dose group for a period of 14 days with no change in mating partners. Vaginal smears were taken daily during the 14-day mating period or until mating was confirmed. The slides taken during the mating period until mating was confirmed were retained, but were not evaluated for estrous cyclicity or normality unless it was necessary for interpretation of mating results. The observation of vaginal sperm or copulation plug was considered evidence of successful mating (Hafez, 1970). Females were examined daily during the cohabitation period for the presence of sperm or copulation plug in the vaginal tract. The day vaginal sperm (or plug) were observed was designated gestational day (gd) 0 (Hafez, 1970). Once vaginal sperm were observed, the male and female from that mating pair were individually housed. Any female which did not show evidence of successful mating after 14 days of cohabitation, was weighed weekly and feed consumption measured weekly until termination. If a female without a confirmed gd 0 date was, in fact, pregnant and delivered a litter, her lactational information was collected as described below. Beginning on gd 20, each female was observed twice daily (a.m. and p.m.) for evidence of littering. The dams were allowed to rear their young to day 21 postpartum. On day 21 postpartum, each litter was weaned. When each F1 litter reached day 21 postpartum, at least one male and one female pup per litter, if possible, were randomly selected to produce the F2 generation. These selected animals were then exposed to the dosed feed formulations at the same dietary concentration of Wingstay 100 as their parents. Each litter was represented at least once per sex, if possible, until a total of 30 per sex per treatment group were attained. Following this selection, three weanlings/sex/litter, if possible, were randomly selected for necropsy. All pups not selected as parents or for necropsy were examined externally, euthanized and discarded, with Sponsor concurrence.

Selected animals of the F1 generation were administered Wingstay 100 in the diet at their respective formulations *ad libitum* for at least ten weeks (with vaginal cytology taken for estrous cyclicity and normality during the last three weeks of the prebreed exposure period). During the F1 prebreed exposure period, each F1 female was observed beginning on pnd 22 for vaginal patency. The number of females with the vagina patent was recorded daily until all females had this response (usually by pnd 40 according to our historical data for this strain). In addition, during this approximate time period, each F1 male was observed for cleavage of the balanopreputial gland (preputial separation) beginning on pnd 35. The characteristic is present

when the prepuce can be completely retracted to expose the glans penis. The number of males with this separation was recorded daily until all males had this response (usually by pnd 50 according to our historical data for this strain). F1 offspring were approximately 13 to 15 weeks of age at the initiation of the mating period. They were mated as described above for the F0 animals, including, but not limited to, pairing one male:one female, and cohabitation for 14 days or until vaginal sperm were observed, whichever came first (with no change in pairing). Vaginal lavage continued throughout the mating period until mating was confirmed as described above for F0 females. There were no brother-sister matings. F2 litters were maintained as described above throughout lactation and were weaned on pnd 21. At weaning, three F2 weanlings/sex/litter were selected for necropsy and all remaining F2 weanlings were examined and euthanized as described above.

All pups (F1 and F2 litters) were sexed and examined as soon as possible after birth (date of birth designated pnd 0) to determine the number of viable and stillborn members of each litter. Thereafter, litters were evaluated for survival on 4, 7, and 14 days after birth and at weaning (pnd 21).

On day 4 after birth, the size of each litter was adjusted to ten pups by eliminating extra pups by random selection to yield, as nearly as possible, five males and five females per litter. Culled pups were sacrificed by decapitation and discarded. Survival indices were calculated at 0, 4, 7, and 14 days after birth and at weaning (pnd 21). All live pups were counted, sexed, weighed individually, and examined grossly at birth (pnd 0) and at 4, 7, and 14 days after birth and at weaning (pnd 21). The body weights and sexes were recorded on an individual basis, but the pups were not uniquely identified at this stage. All pups were examined for physical abnormalities at birth and throughout the preweaning period. All pups dying during lactation were necropsied, when possible, to investigate the cause of death.

At weaning (pnd 21), at least one female and one male (whenever possible) from each F1 litter for a total of 30/sex/dosage level were selected on a random basis to become parents of the next generation (F1 parents to produce F2 litters). All pups were available for selection (no pups were excluded from the selection process because of physical abnormalities or expectations of early demise). The parentage of each weanling was ascertained to avoid brother-sister matings. Three F1 and F2 weanlings/sex/litter, if possible, were randomly selected for necropsy. The F1 offspring not selected as parents or for necropsy, and F2 offspring not selected for necropsy were examined for gross external abnormalities, euthanized, and discarded after Sponsor concurrence. A gross internal examination was made on any pup appearing moribund or dying on test.

All random selections on offspring were performed as follows. At the time of selection, each pup was assigned a two-digit random number (two digits of five-digit numbers from random number tables). For adjustments of litter sizes on pnd 4, the pups with the lowest numbers per sex within each litter were culled (euthanized). For selections at weaning, the pups with the highest numbers per sex within each litter were selected as parents (F1 to produce the F2 generation) and then for necropsy (F1 pups), or selected for necropsy only (F2 pups).

All individual animal in-life data are presented in Appendix II.

Necropsy and Histology

All F0 and F1 parental animals in all groups (both generations) were subjected to a complete gross necropsy, with selected organs (see below) weighed. On the day of necropsy, prior to euthanasia, all F1 parental females were subjected to vaginal lavage to determine the stage of estrus at termination. In addition, three F1 and F2 weanlings per sex per litter were selected, if possible, and subjected to a gross (external and visceral) necropsy examination, with gross lesions and kidneys (left bisected longitudinally, right bisected transversely) retained in fixative, and brain, spleen, and thymus weighed. The tissues listed below were retained in buffered neutral 10% formalin. The gross necropsy included examination of the external surfaces; all orifices; cranial cavity; carcass; external and cut surfaces of the brain and spinal cord; the thoracic, abdominal, and pelvic cavities and their viscera; and cervical tissues and organs. Pituitaries from all F0 and F1 parental animals were retained in fixative for possible subsequent histopathologic examination. For F0 parental animals, only kidneys with gross lesions were retained; for F1 parental animals, all kidneys were retained (left bisected longitudinally, right bisected transversely) for possible subsequent histopathologic examination. Full histopathology of the organs listed below was performed for all high dose and control F0 and F1 animals selected for mating. Organs demonstrating treatment-related changes were also examined in the low- and mid-dose groups. Additionally, reproductive organs of the low- and mid-dose animals suspected of reduced fertility, e.g., those that failed to mate, conceive, sire, or deliver healthy offspring, or for which estrous cyclicity or sperm number, motility, or morphology were affected, were subjected to histopathological evaluation. Specific attention was focused on the examination of the parental reproductive organs as follows.

Testis: Besides gross lesions such as atrophy or tumors, testicular histopathological examination was conducted in order to identify treatment-related effects such as retained spermatids, missing germ cell layers or types, multinucleated giant cells, or sloughing of

spermatogenic cells into the lumen. Examination of the intact epididymis included the caput, corpus, and cauda, by evaluation of a longitudinal section, and was conducted in order to identify such lesions as sperm granulomas, leukocytic infiltration (inflammation), aberrant cell types within the lumen, or the absence of clear cells in the cauda epididymal epithelium (Russell et al., 1990).

Ovary: The postlactational ovary should contain primordial (small), growing, and antral follicles as well as the large corpora lutea of lactation. Histopathological examination should detect depletion of the primordial follicle population. Five ovarian sections were taken at least 100 μm apart from the inner third of each ovary. Examination included enumeration of the total number of primordial follicles from these ten sections for comparison with control ovaries. Examination should also confirm the presence or absence of small, growing and antral follicles and corpora lutea in comparison with control ovaries (Heindel et al., 1989).

Sacrifice of the parental males occurred after the completion of delivery of their litters, with concurrence of the Sponsor's Representative. At the time of sacrifice of F0 and F1 parental males, one testis (right) from each male was frozen at approximately -20°C for subsequent enumeration of testicular homogenization-resistant spermatid heads. (The left testis per male was retained in fixative for possible subsequent histopathologic examination; see above.) In addition, one cauda epididymis (the right one) was immediately removed, weighed, and seminal fluid from the cauda was assessed for sperm number, motility, and morphology. Sperm motility was assessed immediately after necropsy; number and morphology (at least 200 sperm per male, if possible) were evaluated at a later date using fixed sperm stained with Eosin Y. Epididymal sperm motility and number were determined using an HTM TOX-IVOS Automated Sperm Analysis System (Hamilton-Thorne Research, Beverly, MA). Epididymal sperm morphology was performed manually. (The left epididymis per male was retained in fixative for possible subsequent histopathologic examination; see below.) Sacrifice of the maternal animals occurred after F1 or F2 litters had been weaned. The gross lesions retained in fixative from the F1 and F2 weanling examinations, three/sex/litter, were subjected to histopathologic examination with emphasis on reproductive organs, if appropriate. In addition, some (initially one per sex per litter of the selected F1 weanlings) or all of the kidneys retained from the selected F1 and F2 weanlings were subjected to histopathologic examination. Histopathologic evaluation was conducted on the parental tissues from high dose and control groups, as specified below:

- ovaries with oviducts (2)
- vagina
- uterus with cervix
- prostate
- other tissues with gross lesions identified as being potentially treatment related (including kidneys)
- testis (1)
- epididymis (1)
- seminal vesicles (2)

Since the kidneys were identified as target organs, all of the kidneys retained in F0 animals (those with gross lesions) and in all kidneys in necropsied F1 weanlings and adults and in F2 weanlings from all groups were examined microscopically. A complete gross necropsy and histopathologic examination was conducted for any parental animals dying on test.

The fixed (buffered neutral 10% formalin) uteri from any females of the F0 or F1 generations failing to produce a litter were stained with potassium ferricyanide for confirmation of pregnancy status. This staining procedure did not interfere with subsequent histopathologic evaluation. The following organs were weighed from F0 and F1 parental animals: ovaries (2), uterus, testes (2), epididymides (2; total and caudae), seminal vesicles (2; with coagulating glands and their fluids), prostate, brain, liver, kidneys (2), adrenal glands (2), and spleen. Brain, spleen and thymus were weighed in selected F1 and F2 weanlings, three/sex/litter, subjected to necropsy. Organ weights were reported as absolute and relative to terminal body weight. Details of methods and results of histologic evaluation are presented in Appendix III.

Reproductive and Offspring Indices

The indices for reproductive performance and gestational and postnatal parameters which were calculated for this study are presented in Text Table B. The indices include those for F0 males and females to produce F1 litters and those for F1 males and females to produce F2 litters.

Text Table B. Reproductive and Offspring Indices

Females:	Mating index (%)	=	$\frac{\text{No. females sperm-positive}}{\text{No. females paired}} \times 100$
	Fertility index (%)	=	$\frac{\text{No. females pregnant}}{\text{No. females sperm-positive}} \times 100$
	Pregnancy index (%)	=	$\frac{\text{No. pregnant females}}{\text{No. males impregnating females}} \times 100$
Males:	Mating index (%)	=	$\frac{\text{No. males impregnating females}}{\text{No. males paired}} \times 100$
	Fertility index (%)	=	$\frac{\text{No. males siring litters}}{\text{No. males impregnating females}} \times 100$
	Pregnancy index (%)	=	$\frac{\text{No. pregnant females}}{\text{No. males impregnating females}} \times 100$
Offspring:	Gestational index (%)	=	$\frac{\text{Number of females with live litters}}{\text{Number of females pregnant}} \times 100$
	Live birth index (%)	=	$\frac{\text{Number of live pups at birth}}{\text{Total number of pups born}} \times 100$
	4-Day survival index (%)	=	$\frac{\text{Number of pups surviving 4 days (pre-cull)}}{\text{Total number of live pups at birth}} \times 100$
	7-Day survival index (%)	=	$\frac{\text{Number of pups surviving 7 days}}{\text{Total number of live pups at 4 days (post-cull)}} \times 100$
	14-Day survival index (%)	=	$\frac{\text{Number of pups surviving 14 days}}{\text{Total number of live pups at 7 days}} \times 100$
	21-Day survival index (%)	=	$\frac{\text{Number of pups surviving 21 days}}{\text{Total number of live pups at 14 days}} \times 100$
	Lactation index (%)	=	$\frac{\text{Number of pups surviving 21 days}}{\text{Total number of live pups at 4 days (post-cull)}} \times 100$

Calculation of Standard Reproductive Toxicity Parameters

Formulas for calculating standard reproductive toxicity parameters are presented in Text Table C.

Text Table C. Formulas for Calculating Standard Reproductive Toxicity Study Parameters^a

The following endpoints are calculated for each animal and then the mean is calculated using the animal values.

1. Body Weight Change.

body weight at end of measurement period - body weight at beginning of measurement period

2. Feed Consumption in grams per day.

$$\frac{((\text{feed weight at beginning of measurement period}) - (\text{feed weight at end of measurement period}))}{\text{number of days in measurement period}}$$

3. Feed Consumption in grams per day per kilogram body weight.

feed consumption in grams per day / average of all body weights taken during measurement period
in kilograms

4. Percent Feed Efficiency.

$$\frac{(\text{body weight change for measurement period} / ((\text{feed weight at beginning of measurement period}) - (\text{feed weight at end of measurement period}))) \times 100}$$

5. Test Material Intake in milligrams per day per kilogram body weight.

feed consumption in grams per day per kilogram body weight * (dose in ppm / 1000)

6. Relative Organ Weight.

$$(\text{organ weight} / \text{sacrifice body weight}) \times 100$$

7. Daily Spermatid Production per Testis

number of spermatid in testis / number of days for spermatid cycle (4.61 for rats)

8. Efficiency of Daily Spermatid Production

$$((\text{number of spermatid in testis}) / \text{testis weight in grams}) / \text{number of days for spermatid cycle}$$

9. Percent Abnormal Sperm

$$100 \times ((\text{no. blunt hook} + \text{no. banana} + \text{no. amorphous} + \text{no. pinhead} + \text{no. two heads and/or two tails} + \text{no. head only}) / (\text{no. normal} + \text{no. blunt hook} + \text{no. banana} + \text{no. amorphous} + \text{no. pinhead} + \text{no. two heads and/or two tails} + \text{no. head only}))$$

(continued)

Text Table C. Formulas for Calculating Standard Reproductive Toxicity Study Parameters^a (continued)

The following endpoints are calculated for each litter (dam) and then the mean is calculated using the litter (dam) values.

10. Percent postimplantation Loss per Dam and Arcsine Root Transformation:

$$100 \times \left(\frac{\text{no. implantation sites} - \text{no. live pups}}{\text{no. of implantation sites}} \right) \\ \text{arcsine (square root } \left(\frac{\text{no. implantation sites} - \text{no. live pups}}{\text{no. of implantation sites}} \right) \text{)}$$

11. Stillbirth Index per Dam and Arcsine Root Transformation:

$$100 \times \left(\frac{\text{no. dead pups delivered}}{\text{total number of pups delivered}} \right) \\ \text{arcsine (square root } \left(\frac{\text{no. dead pups delivered}}{\text{total number of pups delivered}} \right) \text{)}$$

12. Live Birth Index per Dam and Arcsine Root Transformation:

$$100 \times \left(\frac{\text{no. live pups delivered}}{\text{total number of pups delivered}} \right) \\ \text{arcsine (square root } \left(\frac{\text{no. live pups delivered}}{\text{total number of pups delivered}} \right) \text{)}$$

13. 4 Day Survival Index per Dam and Arcsine Root Transformation:

$$100 \times \left(\frac{\text{no. pups alive on postnatal day 4}}{\text{no. of pups alive on postnatal day 0}} \right) \\ \text{arcsine (square root } \left(\frac{\text{no. pups alive on postnatal day 4}}{\text{no. of pups alive on postnatal day 0}} \right) \text{)}$$

14. 7 Day Survival Index per Dam and Arcsine Root Transformation:

$$100 \times \left(\frac{\text{no. pups alive on postnatal day 7}}{\text{no. of pups alive on postnatal day 4 after culling}} \right) \\ \text{arcsine (square root } \left(\frac{\text{no. pups alive on postnatal day 7}}{\text{no. of pups alive on postnatal day 4 after culling}} \right) \text{)}$$

15. 14 Day Survival Index per Dam and Arcsine Root Transformation:

$$100 \times \left(\frac{\text{no. pups alive on postnatal day 14}}{\text{no. of pups alive on postnatal day 7}} \right) \\ \text{arcsine (square root } \left(\frac{\text{no. pups alive on postnatal day 14}}{\text{no. of pups alive on postnatal day 7}} \right) \text{)}$$

16. 21 Day Survival Index per Dam and Arcsine Root Transformation:

$$100 \times \left(\frac{\text{no. pups alive on postnatal day 21}}{\text{no. of pups alive on postnatal day 14}} \right) \\ \text{arcsine (square root } \left(\frac{\text{no. pups alive on postnatal day 21}}{\text{no. of pups alive on postnatal day 14}} \right) \text{)}$$

17. Lactational Index per Dam and Arcsine Root Transformation:

$$100 \times \left(\frac{\text{no. pups alive on postnatal day 21}}{\text{no. of pups alive on postnatal day 4 after culling}} \right) \\ \text{arcsine (square root } \left(\frac{\text{no. pups alive on postnatal day 21}}{\text{no. of pups alive on postnatal day 4 after culling}} \right) \text{)}$$

18. Percent Males per Litter and Arcsine Root Transformation:

$$100 \times \left(\frac{\text{no. males in litter for given postnatal day}}{\text{no. sexed in litter for given postnatal day}} \right) \\ \text{arcsine (square root } \left(\frac{\text{no. males in litter for given postnatal day}}{\text{no. sexed in litter for given postnatal day}} \right) \text{)}$$

(continued)

Text Table C. Formulas for Calculating Standard Reproductive Toxicity Study Parameters^a (concluded)

19. Average Fetal Body Weight per Litter:

sum of all individual pup weights in litter for given postnatal day / no. pups weighed in litter for given postnatal day

20. Average Male Fetal Body Weight per Litter:

sum of all individual male pup weights in litter for given postnatal day / no. male pups weighed in litter for given postnatal day

21. Average Female Fetal Body Weight per Litter:

sum of all individual female pup weights in litter for given postnatal day / no. female pups weighed in litter for given postnatal day

^a As required for FDA Good Laboratory Practices (subpart J, paragraph 58.185, no. 11), FIFRA Good Laboratory Practice Standards (subpart J, paragraph 160.185, no. 11) and TSCA Good Laboratory Practice Standards (subpart J, paragraph 792.185, no. 11).

Statistical Analyses

The unit of comparison was the male, the female, the pregnant female, or the litter, as appropriate. Quantitative continuous data (e.g., parental and pup body weights, organ weights, feed consumption, food efficiency, etc.) were compared among the three treatment groups and the one vehicle control group by the use of Bartlett's test for homogeneity of variances. If Bartlett's test indicated lack of homogeneity of variances (i.e., $p < 0.001$), then nonparametric statistical tests were employed for the continuous variables (see below; Winer, 1962). If Bartlett's test indicated homogeneous variances (i.e., $p > 0.001$), then parametric statistical tests were employed for the continuous variables as follows. Appropriate General Linear Models (GLM) procedures (SAS Institute Inc., 1989a,b, 1990a,b,c) for the proposed Analyses of Variance (ANOVA) are available at Research Triangle Institute (RTI). Prior to GLM analysis, an arcsine-square root transformation was performed on all litter-derived percentage data (Snedecor and Cochran, 1967) to allow use of parametric methods. For these litter-derived percentage data (e.g., periodic pup survival indices), the ANOVA was weighted according to litter size. GLM analysis was used to determine the significance of the dose-response relationship (Test for Linear Trend) and to determine whether significant dosage effects had occurred for selected measures (ANOVA). When a significant ($p < 0.05$) main effect for dosage occurs, Dunnett's Multiple Comparison Test (Dunnett, 1955; 1964) was used to compare each treatment group to the vehicle control group for that measure. A one-tailed test (i.e., Dunnett's Test) was used for all pairwise comparisons to the vehicle control group except that a two-tailed

test was used for parental and pup body weight parameters, feed consumption, food efficiency, and percent males per litter. Nonparametric tests, used for continuous data which did not have homogeneous variances, included the Kruskal-Wallis Test (Siegel, 1956) to determine if significant differences were present among the groups, followed by the Mann-Whitney U test for pairwise comparisons to the vehicle control group, if the Kruskal-Wallis test was significant. Jonckheere's test for k independent samples (Jonckheere, 1954) was used to identify significant dose-response trends for nonparametric continuous data. Frequency data such as reproductive indices (e.g., mating and fertility indices) were not transformed. All indices were analyzed by Chi-Square Test for Independence for differences among treatment groups (Snedecor and Cochran, 1967) and by the Cochran-Armitage Test for Linear Trend on Proportions (Cochran, 1954; Armitage, 1955; Agresti, 1990). When Chi-Square revealed significant ($p < 0.05$) differences among groups, then a Fisher's Exact Probability Test, with appropriate adjustments for multiple comparisons, was used for pairwise comparisons between each treatment group and the control group. For acquisition of developmental landmarks (e.g., vaginal patency and preputial separation), each treatment percent or mean was compared to the control percent or mean by Mann-Whitney U test (Siegel, 1956). For correlated data (e.g., body and organ weights at necropsy of the F1 and F2 weanlings, with more than one pup/sex/litter, SUDAAN® software (Shah et al., 1997) was used for analysis of overall significance, presence of trend, and pairwise comparisons to the control group values. For all statistical tests, the significance limit of 0.05 (one- or two-tailed) was used as the criterion for significance. A test for statistical outliers (SAS; 1990b) was performed on male and female body weights and feed consumption in g/day. If examination of pertinent study data did not provide a plausible, biologically-sound reason for inclusion of the data flagged as "outlier," the data were excluded from summarization and analysis and were designated as outliers. If body weight and feed consumption data for a given observational interval (e.g., study day [sd] 1-7 or 21-28 during the prebreed exposure period) were designated outliers or unrealistic, then summarized data encompassing this period (e.g., sd 0-70) also did not include this value.

Personnel

The evaluation of Wingstay 100 for reproductive toxicity in CD® (Sprague-Dawley) rats was conducted at Research Triangle Institute (RTI), Research Triangle Park, NC, under contract to the Goodyear Tire and Rubber Company, Akron, Ohio. Dr. A. Philip Leber, Goodyear Tire and Rubber Company, was the Sponsor's Representative. The RTI personnel indicated below contributed to the completion of this study.

Dr. R. W. Tyl served as Study Director. Reproductive toxicology personnel included Ms. M.C. Marr (Laboratory Supervisor), Ms. C.B. Myers (Reproductive Toxicity Study Supervisor and Data Specialist), Ms. F.S. Gerling, Ms. V.I. Wilson, Ms. N.M. Kuney, Ms. L.B. Pelletier, and Ms. M-S. Perry. Bulk chemical handling and dietary dosage formulations were provided by Mr. M.M. Veselica (Supervisor, RTI Materials Handling Facility), Mr. D.L. Hubbard, Mr. T.D. Burnette, and Mr. R.A. Price. Analyses of dosed feed formulations were performed by Ms. D.R. Brine, Analytical Chemistry Task Leader, and Mr. W.O. Poteat. Analyses of the test material were provided by the Sponsor (see Appendix I). Animal care was provided by Dr. D.B. Feldman, DVM, ACLAM, Veterinarian, and Mr. F.N. Ali, Manager of RTI Animal Research Facility personnel. Histologic preparations were made by Ms. T-Y. Chang (HT-ASCP) and Ms. P. Layman (HT-ASCP). Evaluation of histologic slides was by Dr. J.C. Seely, DVM, ACVP, PATHCO, Inc., Research Triangle Park, NC. Andrologic assessments were performed by Dr. P.A. Fail, Ms. S.W. Pearce, and Ms. S.A. Anderson. RTI Quality Assurance personnel were Ms. S. M. Taulbee, Manager (through September 30, 1998), Mr. D.L. Brodish, Manager (beginning October 1, 1998), Ms. C.D. Keller, Ms. P.D. Hall and Ms. M.E. Parker.

The final report was prepared by Dr. R.W. Tyl with assistance from C.B. Myers, M.C. Marr, and F.S. Gerling on data compilation and statistical analyses. The individual scientist reports were prepared and signed by the author(s).

The protocol and two amendments detailing the design and conduct of the study are presented in Appendix V (Volume IV). The protocol was signed by the Study Director on May 21, 1996.

Storage of Records

All original data sheets, records, biological specimens, blocks, and slides for the present study are stored in the RTI Archives, under the control of the RTI CLS Archivist, along with all biological samples collected during the course of the study which remain the responsibility of RTI. Work sheets and computer printouts which were generated in the statistical analysis of data are stored in the RTI Archives. Copies of this report are filed with the RTI Archives as well as with the Goodyear Tire and Rubber Company.

Compliance

All records, data, biological specimens, and reports will be maintained in storage for the time period specified by the appropriate testing guidelines or for as long as the quality of the preparation affords evaluation, whichever is less. The study was performed in compliance with

the U.S. EPA TSCA testing guidelines (U.S. EPA, 1985) and the U.S. EPA OPPTS Draft Testing Guidelines (U.S. EPA, 1996).

The toxicology laboratories at RTI are operated in compliance with Toxic Substances Control Act (TSCA) Good Laboratory Practice Standards (U.S. EPA, 1984; 1989a) and the RTI Animal Research Facility is fully accredited by the Association for Assessment and Accreditation of Laboratory Animal Care (AAALAC), International. This study was conducted in compliance with the TSCA GLP regulations and AAALAC accreditation standards.

RESULTS

Dosed Feed Formulations

Dosed feed formulations, encompassing the range of dosage concentrations employed in this study, were homogeneous and stable for at least 49 days in amber bottles under freezer conditions (-15 to -20°C) so formulations were used within the stability limits established and were stored frozen. All dietary formulations were analyzed at 97.3-107% of target dietary concentrations (except for one high dose formulation which assayed at 112% of target and was used with the Sponsor's concurrence) prior to use. No Wingstay 100 was detected in the vehicle control feed formulations, with an estimated detection limit of 9.10 ppm (Table 1 and Appendix I).

F0 Prebreed Exposure Period

Results are organized and reported chronologically. All individual in-life and necropsy data are presented in Appendix II; all individual histopathology data are presented in Appendix III.

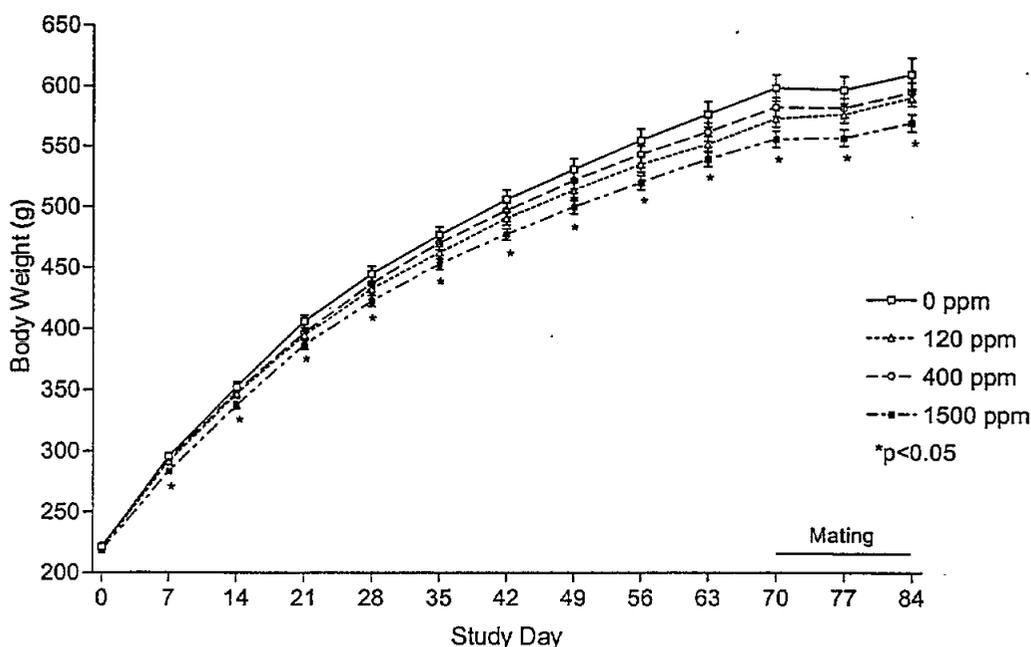
A summary of the fates of F0 males and females is presented in Table 2. One F0 male at 0 ppm (no. 207) was sacrificed moribund on sd 85; cause of his condition was determined to be leukemia. There were, therefore, 29, 30, 30, and 30 F0 males at scheduled sacrifice at 0, 120, 400, and 1500 ppm, respectively. One F0 female at 0 ppm (no. 204) was euthanized on sd 17; she had a probable broken left hind limb. No F0 females died at 120 ppm. At 400 ppm, one F0 female (no. 84) was found dead on gd 17 (sd 89), and two females died during early lactation (no. 150 on pnd 0, sd 96; and no. 156 on pnd 2, sd 97). At 1500 ppm, four females died or were euthanized during lactation (no. 44 on pnd 1, sd 95; no. 98 on pnd 0, sd 95; no. 202 on pnd 0, sd 96; and no. 220 on pnd 0, sd 95). Four females at 1500 ppm died during the holding period prior to scheduled sacrifice (no. 14 on sd 101, no. 58 on sd 100, no. 152 on sd

104 [euthanized moribund], and no. 164 on sd 99). There were therefore 29, 30, 27, and 22 F0 females at scheduled sacrifice at 0, 120, 400, and 1500 ppm, respectively.

F0 male body weights (Table 3 and Figure 1) were statistically equivalent among treatment groups at the start of the study (prebreed sd 0). Body weights, recorded weekly during the ten-week prebreed dosing period, exhibited a statistically significant reduction at 1500 ppm for all weeks evaluated, through the end of the prebreed dosing period (day 70) and through the two-week mating period (to day 84) (Table 3 and Figure 1). F0 male weekly body weight changes exhibited statistically significant reductions at 1500 ppm for weeks 1 (days 0-7), 6 (days 35-42), 8 (sd 49-56), 10 (sd 63-70) and for the entire prebreed period (sd 0-70), at 400 ppm and 120 ppm for week 3 (sd 14-21), and at 120 ppm for week 8 (sd 49-56).

Figure 1

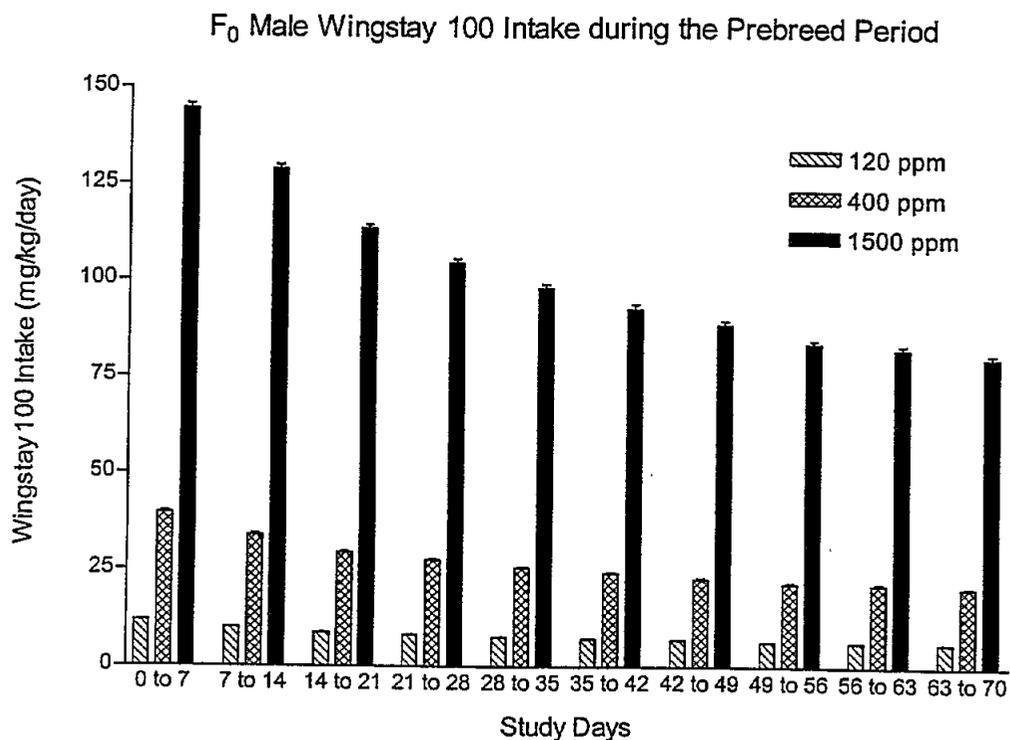
F₀ Male Body Weights during the Prebreed and Mating Periods



F0 male feed consumption, expressed as g/day, was significantly lower at 1500 ppm only for week 1 (days 0-7) during the ten-week prebreed dosing period. All other intervals and values at 120 and 400 ppm were equivalent. When the data were expressed as g/kg/day, the values at 1500 ppm were significantly increased for week 5 (sd 28-35), 7 (sd 42-49), 9 (sd 56-63) and 10 (sd 63-70). Percent food efficiency was significantly reduced at 1500 ppm for weeks 1, 6, 8, and 10, for the entire ten-week prebreed period (sd 0-70), and at 120 ppm for week 8. Wingstay 100 intake, expressed as mg/kg/day, exhibited the expected incremental

increases across dose groups, averaging approximately 8 mg/kg/day at 120 ppm, 26 mg/kg/day at 400 ppm and 98 mg/kg/day at 1500 ppm for the entire period (sd 0-70), increasing approximately 3.2x from 120 to 400 ppm and 3.8x from 400 to 1500 ppm. The Wingstay 100 intake values also exhibited the expected decreases within each dosed group over time. The test chemical intake ranged from 11.8, 39.6, and 144.4 mg/kg/day for the 120, 400, and 1500 ppm groups, respectively, during the first week of the prebreed period, to 6.2, 20.5, and 79.8 mg/kg/day for the last week of the prebreed period. These decreases within groups are due to the feed consumption in g/day remaining relatively constant and the body weights increasing markedly over time. Therefore, the feed consumption in g/kg/day (the basis for the test chemical intake calculations) decreased over time within each group (Table 4 and Figure 2).

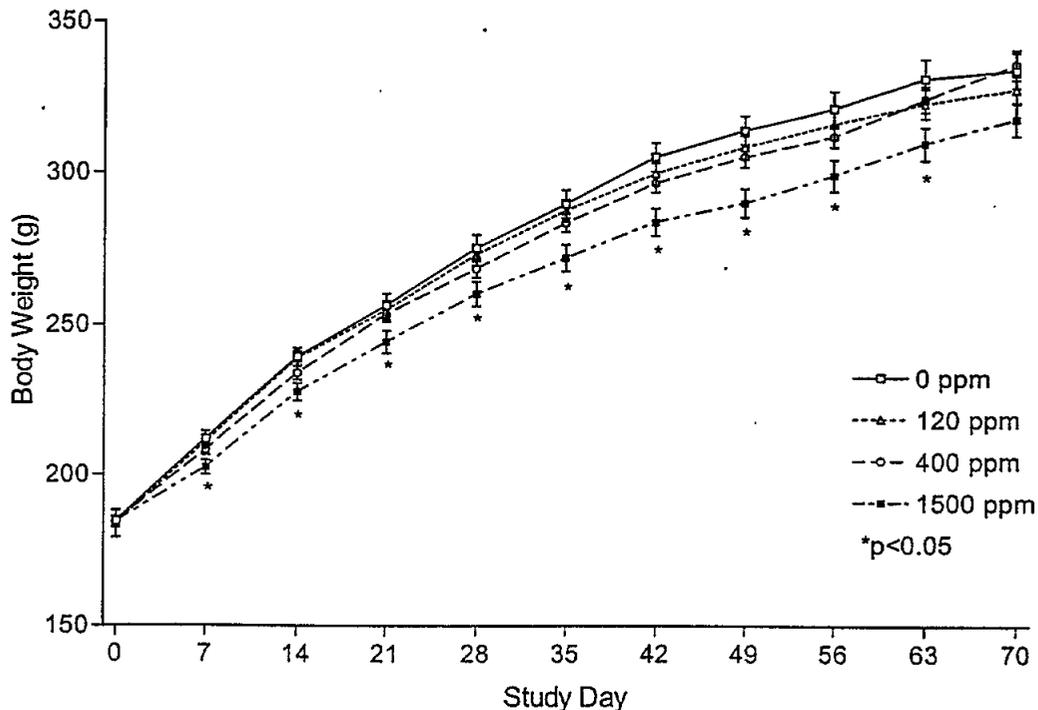
Figure 2



Clinical observations during the prebreed and mating periods for F0 males indicated no treatment-related clinical signs of toxicity, although sore(s) (various locations), a typical finding, did exhibit an apparent treatment- and dose-related incidence, with 1, 1, 4, and 7 animals at 0, 120, 400, and 1500 ppm with this observation. (Table 5).

F0 female body weights (Table 6 and Figure 3) were equivalent across all groups at the start of the study (prebreed, sd 0). Beginning at the end of week 1 (prebreed sd day 7) and continuing through nine of the ten-week prebreed exposure period, the mean weights at 1500 ppm were significantly lower. Weights at 120 and 400 ppm were not affected. Weekly body weights of females remaining sperm-negative during the two-week mating period, with diminishing numbers of females over time, were not statistically analyzed, although the mean values at 1500 ppm were clearly reduced. F0 female weekly body weight changes exhibited only one statistically significant reduction, at 1500 ppm for week 1 (sd 0-7), and only one statistically significant increase, at 400 ppm for week 10 (sd 63-70) (Table 6).

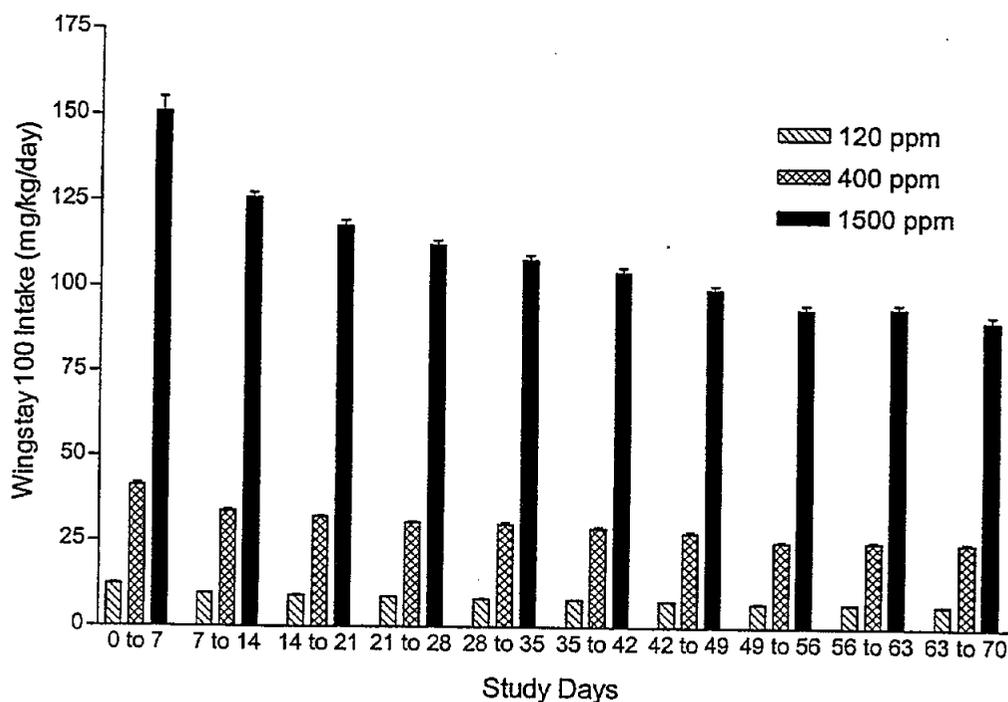
Figure 3

F₀ Female Body Weights during the Prebreed Period

F0 female feed consumption values (Table 7) during the ten-week prebreed dosing period, expressed as g/day, were unaffected across all groups except for a significant increase at 400 ppm for week 10 (sd 63-70). When the data were expressed on g/kg/day, maternal feed consumption values were significantly increased at 400 ppm for week 1 (sd 0-7), 5 (sd 28-35), 6 (sd 35-42), and for the entire prebreed period (sd 0-70), and at 400 and 1500 ppm for weeks 7 (sd 42-49), 8 (sd 49-56) and 10 (sd 63-70). There were no effects on feed consumption at

120 ppm. Percent food efficiency was significantly reduced at 1500 ppm for week 1 and for the ten-week prebreed exposure period, and at 400 ppm for week 4 and 10. Wingstay 100 intake (as mg/kg/day) exhibited the same incremental increases across dosed feed groups for the prebreed period (3.6x from 120 to 400 ppm and 3.7x from 400 to 1500 ppm) and the same decreases with increasing age (and weight) within dosed feed groups as did the F0 males. The test chemical intake within groups ranged from 12.2, 41.1, and 150.7 mg/kg/day at 120, 400, and 1500 ppm during the first week of the prebreed period, to 6.6, 24.6 and 90.1 mg/kg/day at 120, 400, and 1500 ppm during the last week of the prebreed period. Average intakes of test chemical during the ten-week prebreed were approximately 8.2, 29.2, and 107.5 mg/kg/day at 120, 400, and 1500 ppm, respectively (Table 7 and Figure 4).

Figure 4

F₀ Female Wingstay 100 Intake during the Prebreed Period

Clinical observations on F0 females during the prebreed dosing period are presented in Table 8. There were no treatment- or dose-related findings. Observations were essentially limited to occasional findings of thin fur, alopecia, rust-colored fur, and chromodacryorrhea in one to four females in all study groups (and one incident of a problem with a water bottle at

120 ppm and one female, no. 204, at 0 ppm euthanized and removed due to a fractured left hind leg).

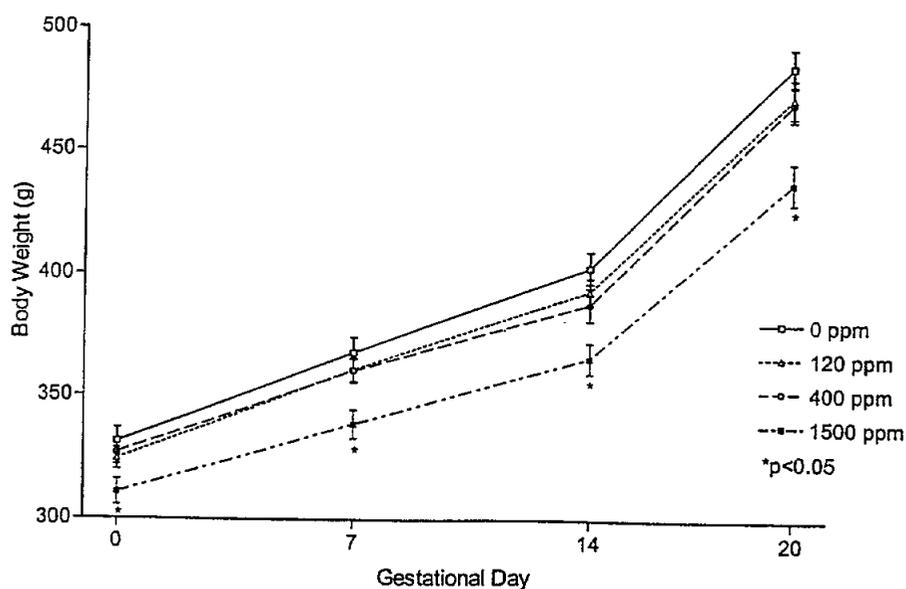
Vaginal cytology was evaluated for F0 females daily during the last three weeks of the ten-week prebreed exposure period. The results are presented in Table 9. Of the 29, 30, 30, and 30 females evaluated at 0, 120, 400, and 1500 ppm, only one each at 400 and 1500 ppm were not cycling. Of the females cycling, 5, 2, 2, and 4 had abnormal cycles at 0, 120, 400, and 1500 ppm. Cycle length was equivalent across all groups with a mean duration of 4.69, 4.50, 4.66, and 4.71 days at 0, 120, 400, and 1500 ppm, respectively. There were no statistically significant or toxicologically relevant differences among groups (Table 9).

Gestation - F0 Females for F1 Litters

Maternal gestational body weights (Table 10 and Figure 5) were statistically significantly reduced at 1500 ppm for all timepoints evaluated; gestational weight change (gd 0-20) was also significantly reduced at 1500 ppm (Table 10). Maternal gestational feed consumption, expressed as g/day, was significantly reduced at 1500 ppm for gd 7-14; when expressed as g/kg/day, feed consumption was significantly increased at 400 and 1500 ppm for gd 14-20. Percent food efficiency was significantly reduced at 1500 ppm for the gestation period (gd 0-20) (Table 11). Maternal gestational intake of Wingstay 100 was approximately 7.4, 25.3 and 95.7 mg/kg/day at 120, 400 and 1500 ppm, respectively (Table 11 and Figure 6).

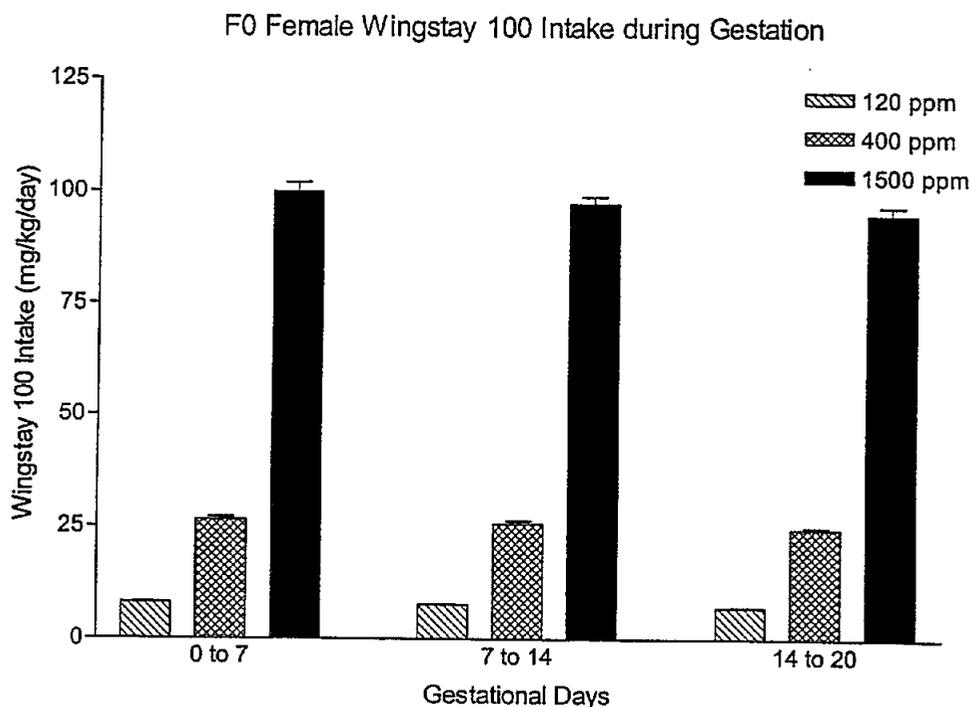
Figure 5

F₀ Female Body Weights during Gestation



Clinical observations of F0 dams during gestation are presented in Table 12, and included pale appearance, pup(s) stuck in vagina, and vaginal bleeding at 1500 ppm, and piloerection (a nonspecific indicator of stress) at 400 and 1500 ppm (Table 12).

Figure 6



Lactation - F0 Females For F1 Litters

Maternal lactational body weights (Table 13 and Figure 7) were statistically significantly increased at 1500 ppm for pnd 0, 4, and 7. Lactational weight change (pnd 0-21) was significantly increased at 1500 ppm. Maternal lactational feed consumption, expressed as g/day or g/kg/day, was statistically equivalent across all groups for all timepoints evaluated. Percent food efficiency was significantly increased at 120 and 1500 ppm for the lactation period (pnd 0-21). Maternal lactational intake of Wingstay 100 was approximately 19.4, 62.3, and 236.5 mg/kg/day at 120, 400, and 1500 ppm, respectively, confounded by pups self-feeding starting in the second week of postnatal life (Table 14 and Figure 8).

Figure 7

F₀ Female Body Weights during Lactation

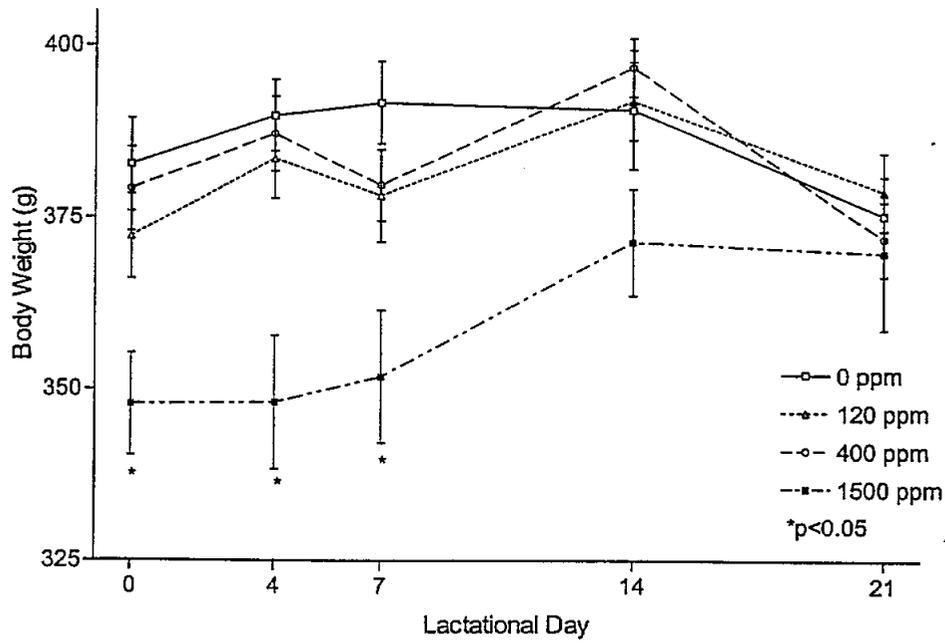
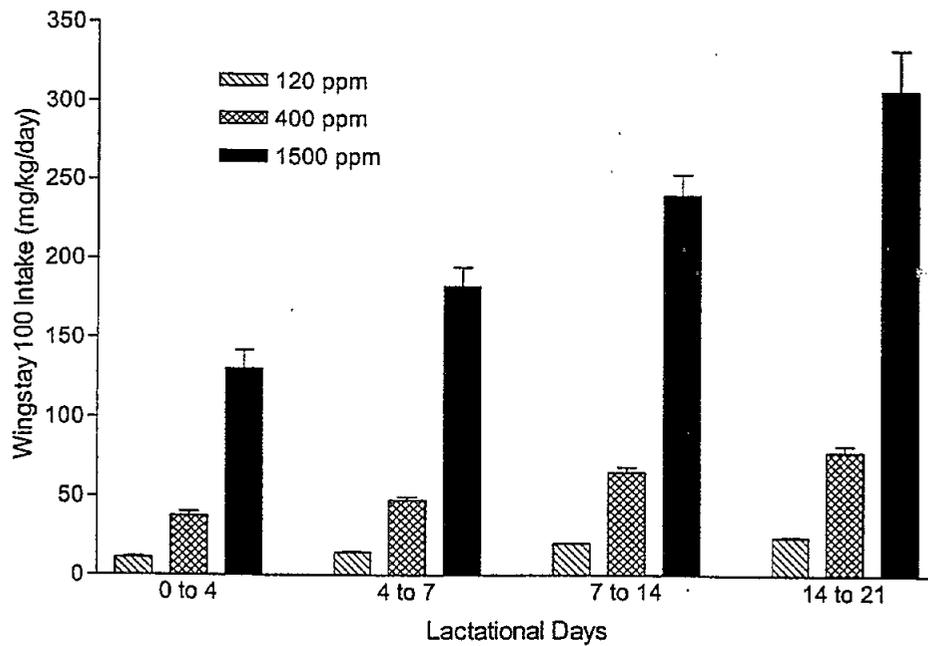


Figure 8

F₀ Female Wingstay 100 Intake during Lactation



Clinical observations of F0 dams during lactation (Table 15) indicated significant treatment- and dose-related findings at 400 and 1500 ppm. These observations included pale eyes, hunched posture, moribund (euthanized), rough coat and vaginal bleeding at 1500 ppm; chromodacryorrhea and found dead at 400 and 1500 ppm; and pale appearance and piloerection at 120, 400 and 1500 ppm; dystocia (difficult delivery) with pups retained in vagina at 120 and 1500 ppm (Table 15).

Reproductive and lactational indices for F0 animals producing F1 litters are presented in Table 16. F0 male and female mating, fertility, and pregnancy indices were equivalent across all groups. Gestational index was significantly reduced at 1500 ppm, and gestational length in days was significantly increased at 400 and 1500 ppm. The number of implantation sites per dam was equivalent across all groups, as was the number of total implantation sites per litter. However, the numbers of live pups per litter on pnd 0 was significantly reduced at 400 and 1500 ppm, and the number of dead pups per litter on pnd 0 was significantly increased at 1500 ppm. The numbers of litters on pnd 0 were 24, 26, 23, and 15 at 0, 120, 400, and 1500 ppm, respectively. Prenatal mortality index (postimplantation loss per litter) was significantly increased at 400 and 1500 ppm. Stillbirth index was significantly increased at 1500 ppm. Live birth index exhibited a significant decrease at 1500 ppm. Day 4, 7, 14, and 21 survival indices and lactation index were statistically equivalent across all groups, although the value at 1500 ppm for day 4 was clearly reduced (83.5%) relative to the control value (98.3%) with a very large error term (Table 16).

Sex ratio (% males per litter) on pnd 0, 4, 7, 14, and 21 was statistically equivalent across all groups. Litter sizes were significantly reduced at 400 and 1500 ppm for pnd 0 and 4 (precull) and 7 (postcull). Mean pup body weights per litter were significantly increased at 120 and 400 ppm (but not 1500 ppm) on pnd 0 for all pups and female pups (and at 120, 400, and 1500 ppm for male pups). Average pup body weights per litter (all pups and males and females separately) were significantly increased at 400 ppm on pnd 4. Female (but not male or total) pup body weights per litter were significantly increased at 400 ppm on pnd 7 and 14, and average pup and female (but not male) pup weight was significantly increased at 400 ppm on pnd 21 (Table 17).

Clinical observations of F1 pups during the lactation period (Table 18) indicated a treatment- and dose-related incidence of found dead (and missing and presumed dead) pups at 120, 400, and 1500 ppm. The total found dead (or missing and presumed dead) through pnd 14 (no deaths occurred subsequent to pnd 14) were 11, 10, 17, and 121 at 0, 120, 400, and 1500 ppm, respectively.

1500 ppm, respectively. Most observations indicated hypothermic pups and failure to nurse (no milk band) (Table 18).

Gross necropsy of F1 pups which died during the lactation and were available for examination (Table 19) included 4, 7, 8, and 114 dead pups at 0, 120, 400, and 1500 ppm, respectively. Gross necropsy indicated that most deaths occurred during the first four days of life, many with patent (open) ductus arteriosus and no air in lungs, indicating primary atelectasis (defective expansion of the pulmonary alveoli at birth), and no milk in stomach, although many pups died with closed ductus and air in lungs. There was an increased incidence of distended ureter/hydroureter and hydronephrosis at 1500 ppm relative to the incidence at low doses, but this was at least partially confounded by the increased number of perinatal deaths, and therefore more pups to examine, at 1500 ppm (Table 19).

Necropsy – F1 Weanlings

Necropsy body and organ weights of F1 weanling males and females, up to three per sex per litter, are presented in Table 20.

F1 weanling male body weight was significantly increased (109.2 % of control) at 400 ppm (and statistically but not significantly reduced at 1500 ppm, 96.4% of the control value). Absolute mean male thymus weight was significantly increased at 400 ppm (111.6% of control), and significantly decreased at 1500 ppm (88.8% of control). Absolute male spleen weight was significantly increased at 120 (120.0% of control) and 400 ppm (134.7% of control) and slightly but not significantly increased at 1500 ppm (107.9% of the control value). Absolute male brain weight was significantly increased at 400 ppm (105.3% of control) and significantly decreased at 1500 ppm (96.3% of control). Relative male thymus weight (as percentage of sacrifice weight) was significantly reduced at 1500 ppm (91.7% of control); relative male spleen weight was significantly increased at 120 ppm (113.7% of control) and 400 ppm (123.8% of control) and slightly, but not statistically significantly, increased (113.2% of control) at 1500 ppm. Relative male brain weights were equivalent across all groups (Table 20).

Necropsy body and organ weights of F1 weanling females are also presented in Table 20. F1 weanling body weight was significantly increased at 400 ppm (109.1% of control value). Absolute female thymus weight was also significantly increased at 400 ppm (112.7% of control). Absolute female spleen weight was significantly increased at 120 ppm (117.3% of control) and at 400 ppm (123.2% of control). Absolute female brain weight was significantly decreased at 1500 ppm (89.2% of control). Relative female thymus weights were equivalent across all groups. Relative female spleen weight was significantly increased at 120 ppm (112.7% of

control), 400 ppm (113.8% of control) and at 1500 ppm (116.6% of control). Relative female brain weight was significantly reduced at 400 ppm (88.8% of control) and at 1500 ppm (89.8% of control) (Table 20).

Summary of gross and microscopic findings for the F1 weanlings is presented in Table 21. All of the gross findings involved the urogenital system in both sexes with multiple clear cysts in both kidneys of varying sizes, observed in 25 females and 27 males at 1500 ppm, unilateral kidneys with white foci of female pups at 120 (three) and 400 ppm (one), but not in male pups. Hydronephrosis (right) was observed in 1-2 males at 120 and 400 ppm and enlarged renal pelvis (right) were observed in one male pup at 0 ppm. Microscopic findings were limited to the kidneys; treatment-related lesions included polycystic kidneys in females at 120 ppm (5, all minimal), 400 ppm (7 total; 6 minimal and 1 mild), and 1500 ppm (11 total, 2 minimal, 3 mild, 4 moderate and 2 marked), and in males at 120 ppm (1, minimal), 400 ppm (8 total, 7 minimal and 1 mild), and 1500 ppm (10 total, 1 minimal, 1 mild, 5 moderate and 3 marked). Renal tubule regeneration was also present in both sexes in all groups with an apparent dose-related incidence: 3, 6, 5, and 10 for females and 4, 3, 7, and 9 for males at 0, 120, 400, and 1500 ppm, respectively. Renal tubule dilation was observed in both sexes, with 4-7 per group per sex at 0, 120, and 400 ppm and only one of each sex at 1500 ppm (Table 21 and Appendix III).

F0 Parental Holding Period

F0 males were held after mating until the delivery of all F1 litters, sd 91-105. Male body weights were significantly reduced at 1500 ppm on sd 91, 98, and 105. Body weight change was unaffected across all groups for sd 84-91 and 91-98; for sd 98-105, body weight change was significantly decreased at 400 ppm (but not at 1500 ppm) (Table 22). F0 male feed consumption during this period was unaffected across groups when the data were expressed as g/day. When the data were expressed as g/kg body weight/day, feed consumption was significantly reduced at 1500 ppm for all three intervals, sd 84-91, 91-98, and 98-105. Percent food efficiency was significantly reduced only at 400 ppm and only for sd 98-105. Wingstay 100 intake was 5.6-5.8 mg/kg/day at 120 ppm, 18.7-19.1 mg/kg/day at 400 ppm, and 73.5-76.6 mg/kg/day at 1500 ppm (Table 23). Clinical observations in F0 males during this time (Table 24) indicated no treatment- or dose-related incidences or severities.

F0 females who were not pregnant, did not deliver live litters, or whose litters died during lactation were held through sd 112 until all dams were terminated at scheduled sacrifice. Their summarized body weights and weight changes during this period are presented in Table 25; the

data were not statistically analyzed due to small numbers of females per group which also changed over time. These mean values appeared approximately equivalent across groups (Table 25). Feed consumption in g/day and g/kg/day are also presented for these females in Table 26, with approximately equivalent values across all groups. Percent food efficiency was variable across groups within intervals and within groups across intervals with no consistent pattern (no statistical analyses were performed). Wingstay 100 intake was 5.9-6.3 mg/kg/day at 120 ppm, 19.4-21.5 mg/kg/day at 400 ppm, and 76.8-85.6 mg/kg/day at 1500 ppm (Table 26). Clinical observations for these females are presented in Table 27. Dams at 1500 ppm exhibited dehydration (one female) chromodacryorrhea (one female), pale eyes (one female) and ears (one female), pale appearance (two females), piloerection (four females), tremors (one female), found dead (three females), and euthanized moribund (one female) (Table 27).

Necropsy – F0 Parental Animals

Summary of F0 male terminal body weights, organ weights, and andrological assessments is presented in Table 28. Body weight at sacrifice was significantly reduced at 1500 ppm. There were no statistically significant effects of treatment on any absolute organ weights, including liver, paired kidney, paired adrenal gland, spleen, brain, paired testes, paired epididymides, prostate, and seminal vesicles with coagulating gland. Relative organ weights (organ weight as a percentage of sacrifice body weight) were unaffected for adrenal glands, spleen, brain, testes, epididymides, prostate, and seminal vesicles with coagulating gland. Relative liver weight was significantly increased (109.2% of control value) at 1500 ppm. Relative paired kidney weight was significantly increased at 400 ppm (106.0% of control value) and at 1500 ppm (110.7% of control value). There were no statistically significant differences among groups for any andrological parameters, including percent motile sperm, percent progressively motile sperm, epididymal sperm concentration ($10^6/g$), testicular spermatid head concentration ($10^6/g$), and percent abnormal sperm (Table 28).

Gross necropsy findings in F0 males included no apparent treatment- or dose-related findings in males who died on study (one male, no. 207, at 0 ppm) or who were sacrificed on schedule. Two kidneys at 120 ppm exhibited "pits" and one kidney at 1500 ppm exhibited a clear cyst on the surface (Table 29). Similarly, there were no treatment- or dose-related histopathologic findings in F0 male reproductive organs or in organs with gross lesions, including the retained kidneys (Table 29 and Appendix III).

Summary of F0 female terminal body and organ weights is presented in Table 30. Mean sacrifice body weights were statistically equivalent across groups (with a significant

downward trend, $p < 0.05$); the value at 1500 ppm was slightly, but not statistically significantly, reduced; 95.3% of the control value. Absolute paired adrenal glands, spleen, and brain weights were equivalent across groups. Absolute liver weight was significantly increased at 1500 ppm; 114.0% of the control value. Absolute paired kidney weights were significantly increased at 400 ppm (108.4% of control), 120 ppm (104.8% of control), and at 1500 ppm (106.8% of controls); the latter two means not statistically significantly different from the control group value. Absolute paired ovary weight was significantly reduced at 120 ppm (88.5% of control value) and increased, but not statistically significantly, at 400 ppm (129.5% of control) and at 1500 ppm (154.1% of control). Absolute uterine weight was significantly reduced at 400 ppm (79.6% of control) and at 1500 ppm (77.0% of controls). Relative adrenal, spleen, and brain weights were equivalent across all groups. Relative liver weight was significantly increased at 1500 ppm (119.8% of control). Relative paired kidney weight was significantly increased at 400 ppm (108.7% of control) and at 1500 ppm (112.0% of control). Relative paired ovary weight was significantly reduced at 120 ppm (88.0% of control) and increased, but not statistically significantly, at 400 ppm (130.9% of control) and at 1500 ppm (168.0% of control). Relative uterine weight was significantly reduced at 400 ppm (79.3% of control) and at 1500 ppm (80.4% of control). Paired ovarian follicle counts were equivalent between high dose and control females (Table 30).

Gross necropsy findings in F0 females who died on study (including one female with the probable broken left hind limb at 0 ppm which was euthanized, no females at 120 ppm, three females at 400 ppm, and eight females at 1500 ppm) included possible treatment-related effects on the adrenal glands, kidneys, liver, lungs, uterus, and vagina (retained/resorbing fetuses) at 400 and 1500 ppm. At scheduled sacrifice of surviving females, gross effects (probably treatment-related) were observed on the kidneys at 120 (one female) and 1500 ppm (three females) (Table 31).

Histopathologic findings which appeared treatment related included effects on the kidneys, specifically polycystic and cortical necrosis, and on the liver, specifically hematopoietic cell proliferation, and hepatocellular centrilobular necrosis, all at 1500 ppm (Table 31 and Appendix III).

F1 Prebreed Exposure Period

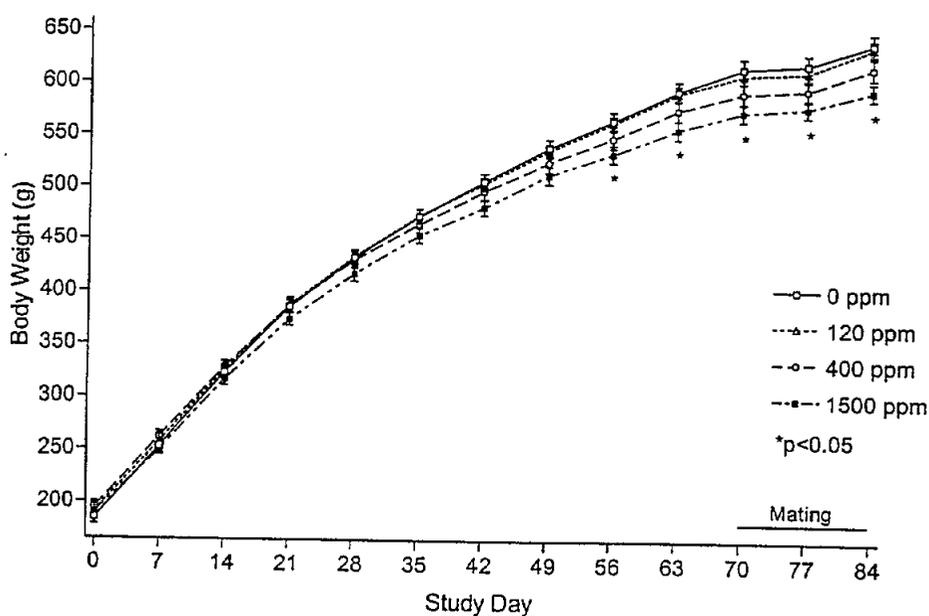
A summary of the fates of parental F1 males and females is presented in Table 32. No males died prior to scheduled sacrifice. One female each died at 0, 400, and 1500 ppm. The females at 400 and 1500 ppm died during lactation of their litters: female no. 528 at 400 ppm

died on pnd 3 (sd 100) and female no. 326 at 1500 ppm died on pnd 0 (sd 96). The female at 0 ppm (no. 390) died during the holding period after mating on sd 97.

The assessment of acquisition of reproductive developmental landmarks is presented in Table 33. The mean day of vaginal patency in F1 females ranged from 31.2 – 32.1 days with no differences among groups. The mean day of preputial separation in F1 males ranged from 41.7 – 42.2 days with no differences among groups (Table 33).

F1 male weekly body weights were equivalent across all groups for the first seven weeks (sd 0-49) and were statistically significantly reduced at 1500 ppm for the last three weeks (sd 56, 63 and 70) of the ten-week (70 day) prebreed dosing period (Table 34 and Figure 9). During the two-week mating period (days 70-84), weights were also significantly reduced at 1500 ppm for the entire period. Weekly F1 male body weight changes were significantly reduced at 1500 ppm for weeks 1 (sd 0-7), 6 (sd 35-42), 7 (sd 42-49), 9 (sd 56-63), and 10 (sd 63-70); at 400 ppm for weeks 5 (sd 28-35), 7 (sd 42-49) and 10 (sd 63-70); and at 120 ppm only for week 10 (sd 63-70). Weight changes for the entire 70-day prebreed period were significantly reduced at 400 and 1500 ppm. There were no differences among groups for weight changes during the two-week mating period, sd 70-77 and 77-84 (Table 34).

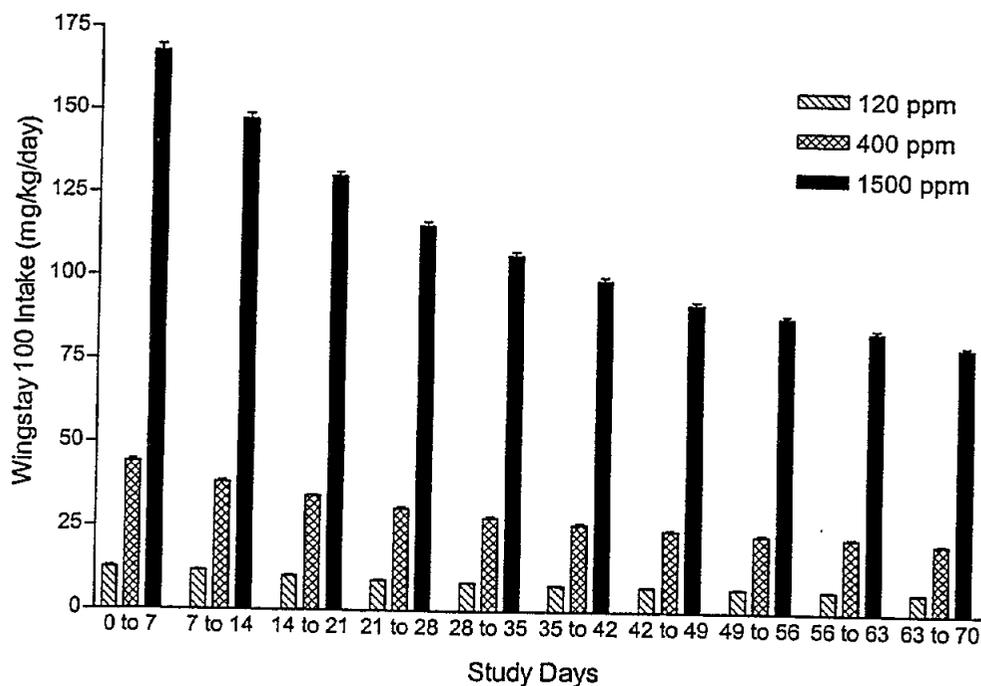
Figure 9

F₁ Male Body Weights during the Prebreed and Mating Periods

Feed consumption (Table 35), expressed as g/day, was significantly reduced at 400 and 1500 ppm for weeks 7 (sd 42-49) and 10 (sd 63-70). When feed consumption data were expressed as g/kg/day, feed consumption was significantly decreased at 120 ppm for week 1 (sd 0-7) and at 400 ppm for week 8 (sd 49-56) during the 12-week period. Percent food efficiency was significantly reduced at 1500 ppm for weeks 1, 6, 10, and for the entire ten-week prebreed period (sd 0-70); at 400 ppm for weeks 7 and 10; and at 120 ppm for week 10. It was significantly increased at 1500 ppm for week 7. Wingstay 100 intake (mg/kg/day) exhibited the expected incremental increases across dose groups, averaging approximately 8.1, 27.4, and 104.6 mg/kg/day at 120, 400, and 1500 ppm, respectively, for the ten-week prebreed. Test chemical intake also exhibited the expected decreases within each dosed group over time as the animals gained weight, with values from all three dose groups dropping approximately 50% over the ten-week period. The test chemical intake ranged from 12.5, 43.9, and 167.5 mg/kg/day for the first week, to 6.1, 20.6, and 79.4 mg/kg/day for the last week (sd 63-70) of the prebreed period at 120, 400, and 1500 ppm (Table 35 and Figure 10).

Figure 10

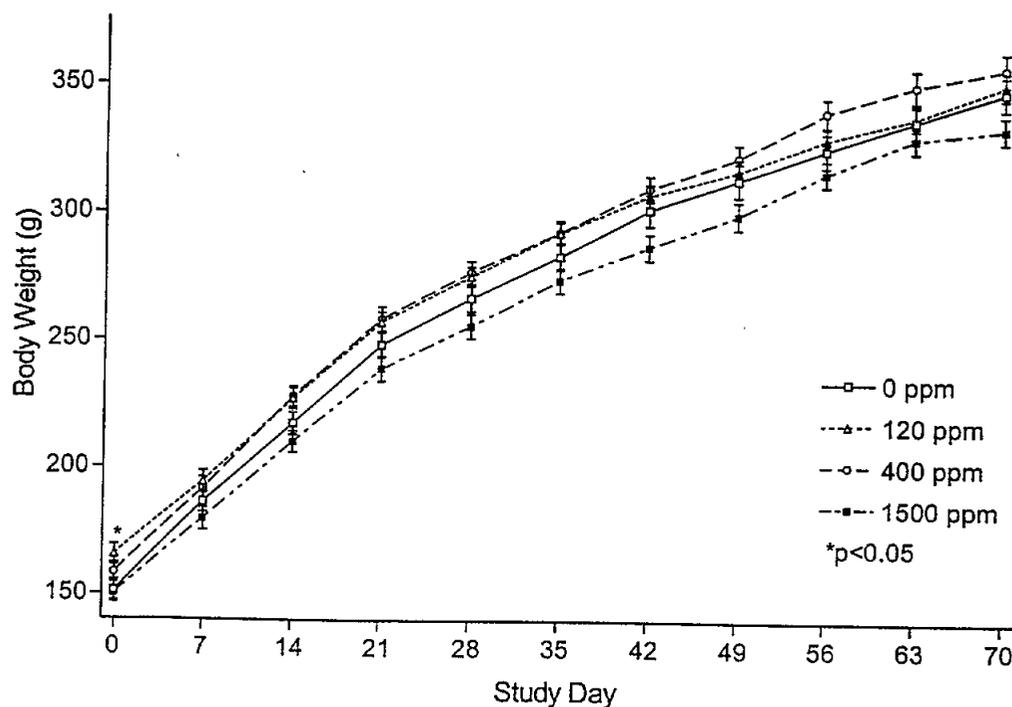
F₁ Male Wingstay 100 Intake during the Prebreed Period



Clinical observations of F1 males during the prebreed and mating periods were limited to occasional reporting of aggressive behavior, chromodacryorrhea, dehydration, diarrhea, sores (scabs), thin fur, alopecia, and vocalization in one to three males in groups, with no treatment- or dose-related incidence or severity (Table 36).

F1 female body weights and weight gains during the ten-week prebreed dosing period (Table 37) were essentially unaffected. The only statistically significant difference was an increase in mean body weight at 120 ppm on sd 0 (Table 37 and Figure 11). Statistical analyses of body weights and weight gains during the mating period (for females not yet found sperm-positive) were not performed due to the decreasing numbers of females included, as more females became sperm-positive and were weighed and recorded based on gestational days, with no apparent differences among groups (Table 37).

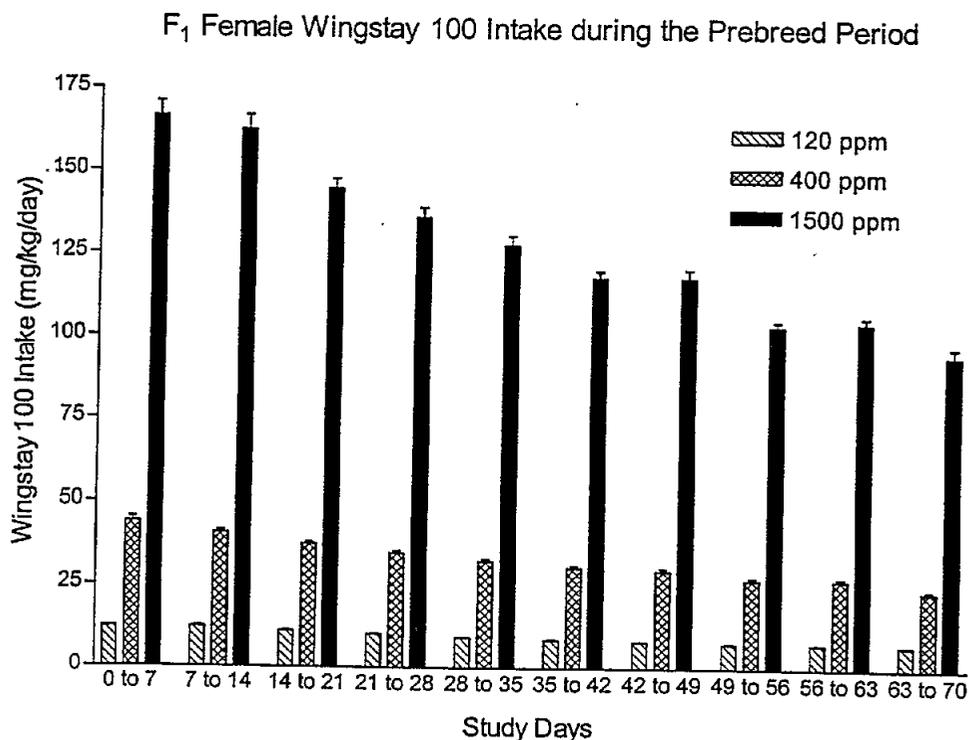
Figure 11

F₁ Female Body Weights during the Prebreed Period

F1 female feed consumption, expressed as g/day, was equivalent across all groups for all ten prebreed dosing weeks. When feed consumption data were expressed as g/kg/day, values for all groups were statistically equivalent for eight of ten weeks. Percent food efficiency was statistically equivalent across all groups for all intervals during the ten-week prebreed

exposure period. Wingstay 100 intake, calculated as mg/kg/day, exhibited the expected incremental increases across dosed groups, averaging approximately 9.2, 31.6, and 124.0 mg/kg/day at 120, 400, and 1500 ppm for the ten-week period. The intake values also exhibited the expected declines within groups over time as the F1 females gained weight over the ten-week period. Test chemical intake ranged from 12.2, 43.9, and 166.4 mg/kg/day during the first week, to 7.2, 23.6, and 94.5 mg/kg/day during the last week of the prebreed period (Table 38 and Figure 12).

Figure 12



Clinical observations of F₁ females during the prebreed dosing period included occasional findings of sores, alopecia, chromodacryorrhea, dehydration, enlarged eyes, piloerection, rough coat, stubby tail, and trimmed teeth. Blood in vaginal smear was observed in three females at 120 ppm. There were no treatment- or dose-related findings. (Table 39).

Results of the three-week vaginal cytology assessment are presented in Table 40. Thirty females per group were assessed. All but two females at 400 ppm were cycling. The percentage of females with abnormal cycles was significantly increased at 400 ppm (8 of 30; 28.6%) and at 1500 ppm (13 of 30; 43.3%), with no effects observed at 120 ppm (3 of 30; 10.0%) relative to the control value (2 of 30; 6.7%). Cycle length in days exhibited a significant

upward trend ($p < 0.05$) but no significant pairwise comparisons; however, the mean cycle length at 1500 ppm was 7.15 days versus the control value of 5.11 days (and 4.84 days at 120 ppm and 5.51 days at 400 ppm) (Table 40).

Gestation - F1 Females for F2 Litters

F1 maternal gestational body weights were statistically equivalent for all timepoints during gestation (gd 0, 7, 14 and 20), although the values at 1500 ppm were clearly reduced (Table 41 and Figure 13); maternal gestational weight change (gd 0-20) was significantly reduced at 400 and 1500 ppm (Table 41). Maternal feed consumption during gestation, expressed as g/day, was equivalent for all gestational timepoints and for the entire gestation period (gd 0-20). When the data were expressed as g/kg/day, there was a significant increase at 1500 ppm for gd 14-20. Percent food efficiency was significantly reduced at 400 and 1500 ppm for the gestational period (gd 0-20). Maternal intake of Wingstay 100 exhibited the expected incremental increases across dose groups, averaging approximately 7.7, 25.8, and 102.2 mg/kg/day at 120, 400, and 1500 ppm (Table 42 and Figure 14).

Figure 13

F1 Female Body Weights during Gestation

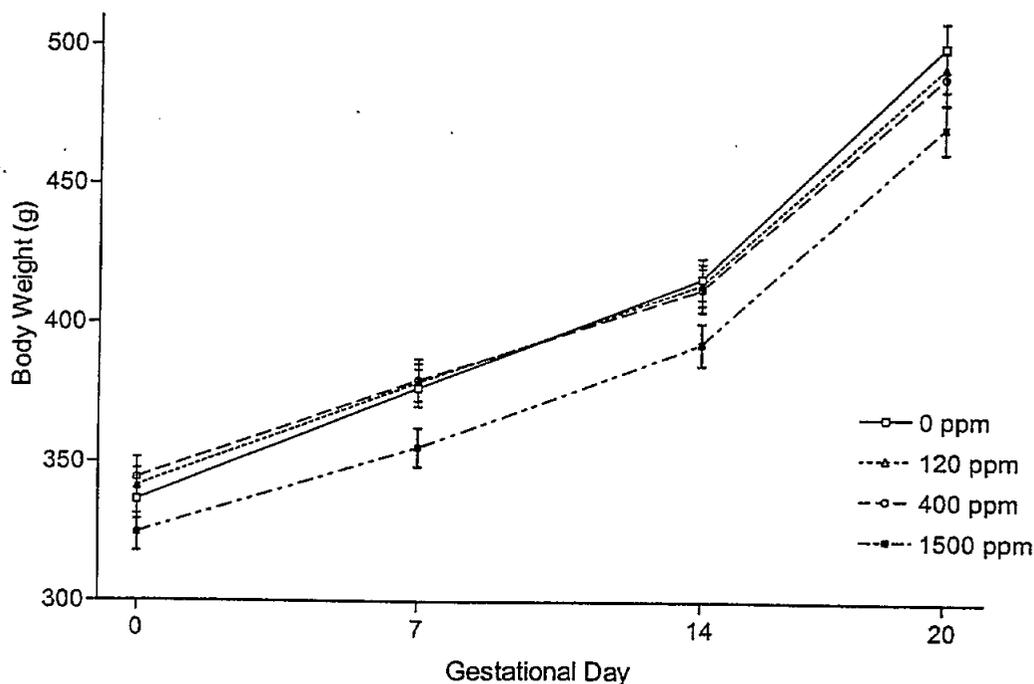
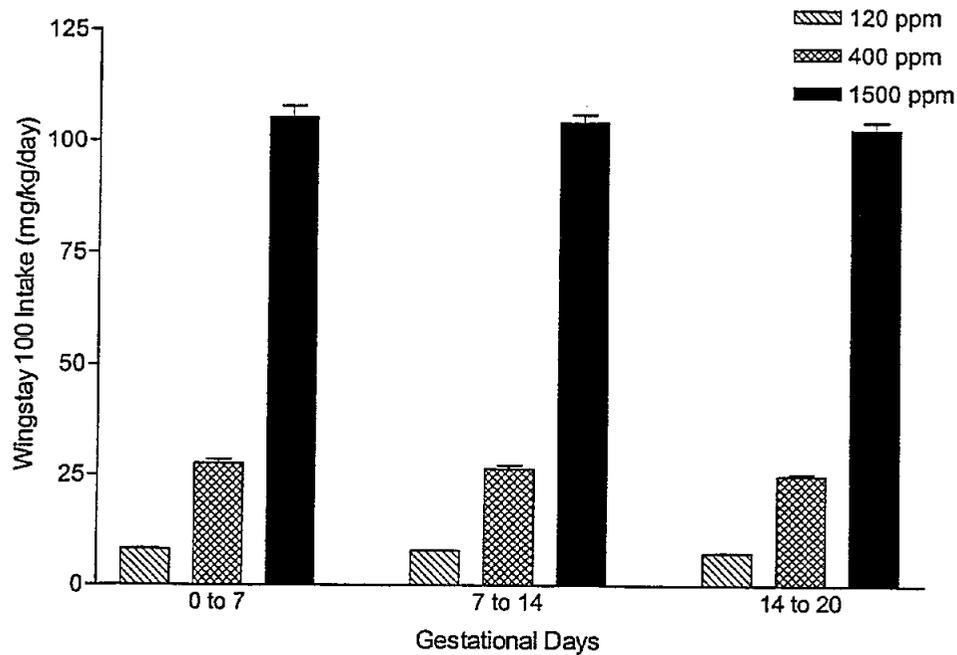


Figure 14
F₁ Female Wingstay 100 Intake during Gestation



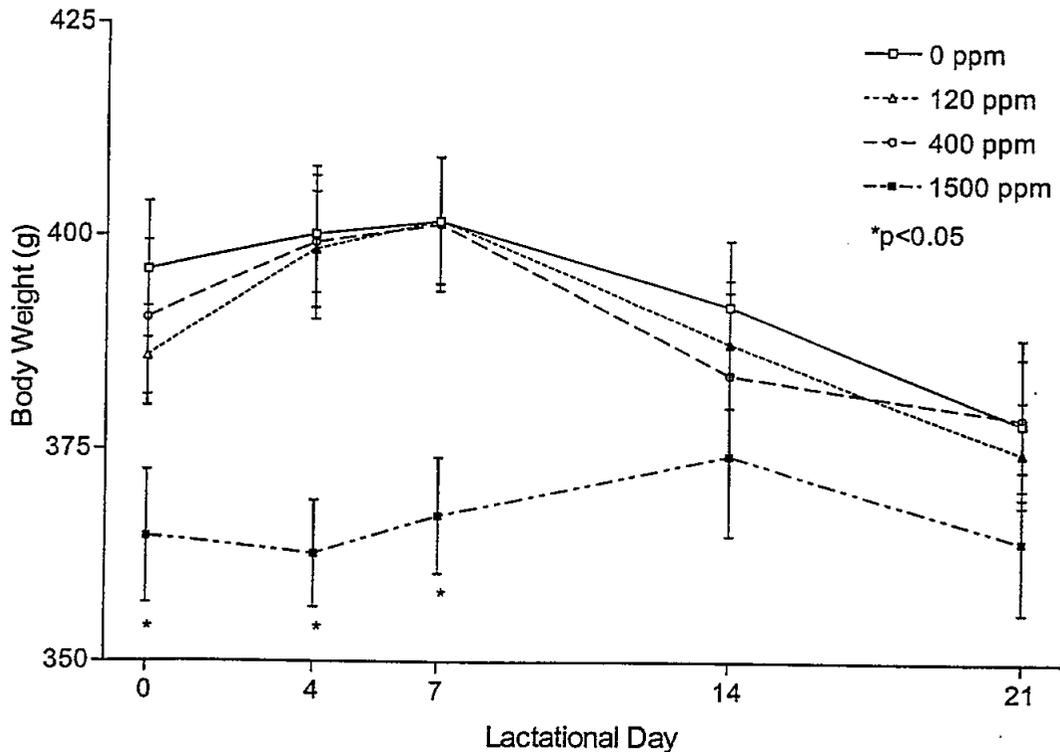
Clinical observations of F₁ females during gestation exhibited no treatment- or dose-related findings. Observations were limited to occasional findings of alopecia, pale appearance, piloerection, and sores in one to two animals in all groups (except the 400 ppm group) (Table 43).

Lactation - F₁ Females for F₂ Litters

The numbers of females that delivered litters (live and/or dead) on pnd 0 were 22, 23, 20, and 24. The numbers of live litters on pnd 0 were 22, 22, 20, and 21 at 0, 120, 400, and 1500, respectively. On pnd 4, the numbers of live litters were 21, 22, 19, and 19 at 0, 120, 400, and 1500 ppm, respectively. Maternal F₁ body weights during lactation exhibited significant reductions at 1500 ppm on pnd 0, 4, and 7, with no significant differences among groups for pnd 14 or 21 (Table 44 and Figure 15). Maternal lactational weight change (pnd 0-21) exhibited no significant differences among groups (Table 44).

Figure 15

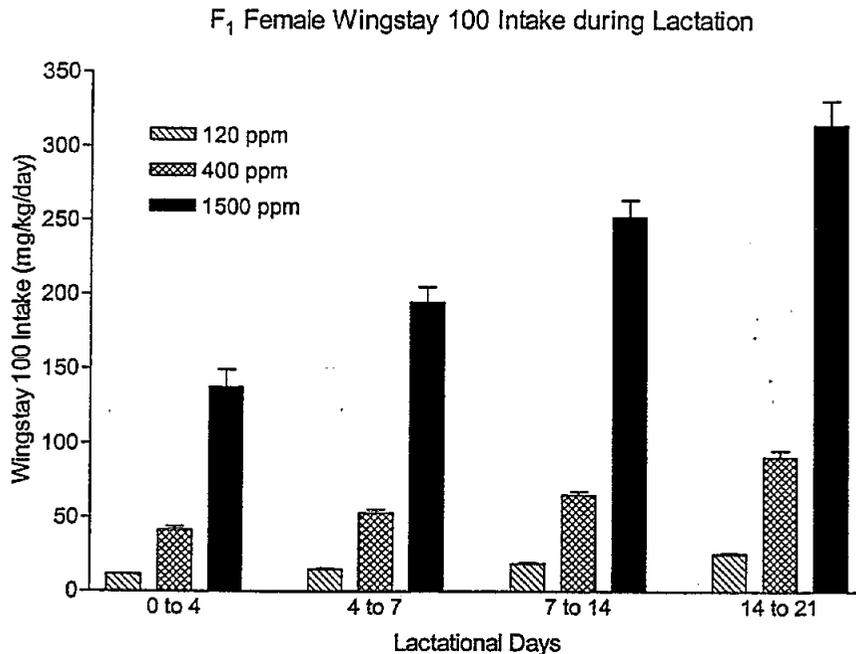
F₁ Female Body Weights during Lactation



Maternal F1 lactational feed consumption, expressed as g/day or g/kg/day, exhibited no significant differences among groups for any interval examined during lactation, pnd 0-21. Percent food efficiency was similarly statistically equivalent across all groups for the lactation period (pnd 0-21). Maternal intake of Wingstay 100, expressed as mg/kg/day, exhibited the expected incremental increases across dosed feed groups, with average intakes for the lactational period of 19.1, 67.3, and 248.5 mg/kg/day at 120, 400, and 1500 ppm, respectively, confounded by the pups self-feeding, beginning the second week of life (Table 45 and Figure 16).

Maternal F1 clinical observations during the lactation period indicated treatment- and dose-related clinical signs at 1500 ppm, including no milk production (also observed in one dam at 0 ppm), pale appearance (also observed in one dam each at 0 and 120 ppm), and piloerection (also observed in one dam each at 0 and 120 ppm). In addition, two dams were found dead, one each at 400 and 1500 ppm (Table 46).

Figure 16



Reproductive and lactational indices for F₁ parents and F₂ litters (Table 47) exhibited no treatment-related changes in mating index, fertility index (either sex), or pregnancy index. Mean gestational length in days was significantly increased at 120 ppm (22.8 days), 400 ppm (23.1 days), and 1500 ppm (23.2 days) relative to the control value (22.2 days). The number of implantation sites per litter were equivalent across all groups. Percent postimplantation loss per litter was significantly increased at 1500 ppm (32.63%) and clearly increased, but not statistically significantly, at 400 ppm (20.16%) and at 120 ppm (18.49%) relative to the control value (6.82%).

The total number of pups per litter on pnd 0 was statistically equivalent across groups (with a significant downward trend, $p < 0.05$). The number of live pups per litter on pnd 0 was significantly reduced, and the number of dead pups per litter on pnd 0 was significantly increased at 1500 ppm. Therefore, the stillbirth indexes significantly increased and the live birth index was significantly reduced on pnd 0 at 1500 ppm. Survival indices for pnd 4, 7, 14, 21, and the lactational index (pnd 4 postcull – pnd 21) were all equivalent across all groups (Table 47).

F₂ litter sex ratio (% male pups per litter) was equivalent across groups for all timepoints evaluated on pnd 0, 4, 7, 14, and 21. The number of pups per litter was significantly reduced only at 1500 ppm, on pnd 0 and 4 (prepull). Pup body weights per litter (all pups or males and

females separately) were significantly increased at 120, 400, and 1500 ppm for pnd 0, and equivalent across all groups for pnd 4 and 14. Pup body weight for males was only increased at 400 ppm on pnd 7. Pup body weights per litter on pnd 21 were significantly increased at 400 ppm (for all pups or separately by sex). Pup weights (for males only, but not for all pups or females) per litter were also significantly increased at 120 ppm on pnd 21 (Table 48).

Clinical observations of F2 pups during the lactation period (Table 49) indicated a treatment- and dose-related incidence of found dead, euthanized moribund, and missing and presumed dead pups at 120, 400, and 1500 ppm. The total found dead, euthanized, and moribund, or missing and presumed dead pups through pnd 14 (no deaths occurred subsequent to pnd 14) were 42, 32, 16, and 110 at 0, 120, 400, and 1500 ppm, respectively. Most observations in all groups indicated hypothermic pups, no milk in stomach, tail chewed off (in one female at 1500 ppm, presumably by dam; euthanized moribund), and incidental findings such as one female on pnd 14 with string-like tail and one male on pnd 21 with enlarged right eye, both at 1500 ppm (Table 49).

Gross necropsy of F2 pups, which died during the lactation period and were available for examination (Table 50), included 21, 16, 8, and 93 at 0, 120, 400, and 1500 ppm, respectively. Most deaths occurred in the first four days of life, many with patent (open) ductus arteriosus and no air in lungs, indicating primary atelectasis (defective expansion of pulmonary alveoli at birth), although many pups died with closed ductus and air in lungs. Many pups had autolyzed abdominal organs. Kidney lesions (including hydronephrosis) were observed in three pups at 0 ppm, one pup at 120 ppm, and two pups at 1500 ppm (Table 50).

Necropsy – F2 Weanlings

Necropsy body and organ weights of F2 weanling males and females, up to three per sex per litter, are presented in Table 51. F2 weanling male body weights were significantly increased at 120 and 400 ppm. Absolute mean male thymus weights were significantly increased at 120 ppm (113.6% of control values) and at 400 ppm (110.9% of controls). Absolute mean male spleen weight was also significantly increased at 120 ppm (115.1% of controls) and at 400 ppm (122.7% of controls). Absolute male brain weight was significantly reduced at 1500 ppm (93.0% of controls). Relative male thymus and spleen weights were equivalent across all groups. Male relative brain weight was significantly reduced at 120 ppm (93.8% of controls) and at 400 ppm (87.0% of controls).

Necropsy body and organ weights of F2 weanling females are also presented in Table 51. F2 weanling female body weight was significantly increased at 400 ppm. Absolute female thymus and spleen weights were equivalent across all groups. Absolute female brain weight was significantly reduced at 1500 ppm (95.0% of controls). Relative female thymus and spleen weights were equivalent across all groups. Relative female brain weight was significantly reduced at 400 ppm (91.4% of controls) (Table 51).

Summary of gross and microscopic findings for the F2 weanlings is presented in Table 52. All of the gross findings involved the kidneys (except for one male at 1500 ppm with an enlarged right eye and polycystic kidneys). The renal lesions presented as one or more clear cysts on the surface or within the kidney, one or more white foci on the surface, and hydronephrosis (dilation of the renal pelvis), predominantly at 1500 ppm (88 pups of 94 examined at 1500 ppm, seven of 111 examined at 400 ppm, five of 128 examined at 120 ppm, and seven of 120 examined at 0 ppm). Microscopic findings were also limited to the kidneys; treatment-related lesions included polycystic kidneys in females at 120 ppm (five total; all minimal), 400 ppm (eight total; seven minimal and one mild), and at 1500 ppm (15 total; five minimal, nine mild, none moderate, and one marked). In males, polycystic kidneys were observed at 120 ppm (three total; all minimal), at 400 ppm (six total; five minimal and one mild), and at 1500 ppm (15 total; five minimal, nine mild, and one moderate). Renal tubule dilatation in both sexes exhibited a dose-related decreasing incidence with many at 0 and 120 ppm, few at 400 ppm, and none at 1500 ppm. Renal tubule regeneration was present in both sexes, predominantly at 0 and 120 ppm, with far fewer at 400 and 1500 ppm (Table 52 and Appendix III).

F1 Parental Holding Period

F1 parental males were held from the end of mating until scheduled sacrifice after the delivery of the F2 litters (sd 91-105). F1 male body weights were significantly reduced at 1500 ppm for sd 84, 91, 98, and 105. Male weight change was equivalent across all groups for sd 84-91 and 91-98, and significantly reduced at 120 and 1500 ppm for sd 98-105 (Table 53). F1 male feed consumption, expressed as g/day, was equivalent across groups for sd 84-91 and sd 91-98, and significantly reduced at 1500 ppm for sd 91-98. F1 male feed consumption in g/kg/day was equivalent across groups for sd 84-91 and significantly reduced at 120 ppm for sd 91-98 and for sd 98-105. Percent food efficiency was significantly reduced at 1500 ppm for sd 98-105. Wingstay 100 intake in mg/kg/day ranged from 5.5 – 5.8 mg/kg/day at 120 ppm, 19.0 – 19.8 mg/kg/day at 400 ppm, and from 73.9 – 75.6 mg/kg/day at 1500 ppm (Table 54).

Clinical observations of the F1 males during this holding period involved only alopecia (various locations) and chromodacryorrhea in one male each at 0 ppm, and sore(s) (various locations) in one male at 1500 ppm (Table 55).

F1 females who were not pregnant, who did not deliver a live litter, or who lost their entire litter during lactation were also held until scheduled sacrifice through sd 112. Because of the small numbers per group, which changed over time, no statistical analyses were performed on body weight, weight gain, or feed consumption parameters. Body weights appeared equivalent across groups for sd 91 and 112 and slightly reduced at 1500 ppm for sd 98 and 105 (body weights at 400 ppm appeared slightly increased for sd 98, 105 and 112). F1 female body weight change during this period was variable (due to dam status and dam number), with no apparent reductions at 1500 ppm (Table 56).

F1 female feed consumption during this period in g/day and g/kg/day appeared approximately equivalent across all groups. Wingstay 100 intake ranged from 6.3 – 7.2 mg/kg/day at 120 ppm, 21.1 – 22.6 mg/kg/day at 400 ppm, and 77.6 – 97.7 mg/kg/day at 1500 ppm (Table 57). Percent food efficiency was approximately equivalent across all groups for sd 98-105 and 105-112. For sd 84-91, the parameter was extraordinarily high at 120 ppm; for sd 91-98, it was extraordinarily low at 120 ppm, both with very high standard errors.

F1 female clinical observations during this period included alopecia (various locations) in one female at 0 ppm, one female at 120 ppm, and one female at 1500 ppm; one female at 0 ppm found dead (on sd 97); one female with pale appearance at 1500 ppm; and piloerection in one female each at 120 and 1500 ppm (Table 58).

Necropsy – F1 Parental Animals

Summary of F1 male terminal body weights, organ weights, and andrological assessments is presented in Table 59. F1 male body weight at scheduled sacrifice was significantly reduced at 1500 ppm. Absolute liver, paired kidney, paired adrenal gland, and spleen weights were equivalent across all groups. F1 male absolute brain weight was significantly reduced at 1500 ppm (93.5% of control value). Absolute paired testes, paired epididymides, prostate, and seminal vesicle with coagulating gland weights were equivalent across all groups. Relative adrenal glands, spleen, brain, paired testes, paired epididymides, prostate, and seminal vesicles with coagulating gland weights were equivalent across all groups. Relative liver weight was significantly increased at 1500 ppm (108.8% of control value). Relative paired kidney weight was also significantly increased at 1500 ppm (110.1% of control). Percent motile sperm was statistically equivalent across all groups (with the value at

1500 ppm, 93.4% of the control value, not statistically significantly different). Percent progressively motile sperm was significantly reduced at 120 and 1500 ppm (but not at 400 ppm). Epididymal sperm concentration ($10^6/g$), testicular homogenization-resistant head count ($10^6/g$), and percent abnormal sperm were equivalent across all groups (Table 59).

Gross findings for F1 males at scheduled sacrifice (no F1 males died on study) were limited to the kidney (except for one male at 400 ppm with left epididymis and left testis reduced in size, and right testis enlarged). The renal findings included hydronephrosis in 6, 5, 1, and 3 males at 0, 120, 400, and 1500 ppm, respectively; polycystic kidneys in no males at 0 or 120 ppm, two males at 400 ppm, and 14 males at 1500 ppm. Irregular renal cortex was not present in males at 0 or 120 ppm, and was observed in one male at 400 ppm and three males at 1500 ppm; bilaterally enlarged kidneys were found in one male at 400 ppm.

Treatment-related microscopic findings in F1 males were limited to the kidneys, with polycystic kidneys observed in no males at 0 ppm and in 5, 10, and 21 males at 120, 400, and 1500 ppm, respectively. Renal tubule regeneration was also observed in 0, 1, 1, and 16 males at 0, 120, 400, and 1500 ppm, respectively (Table 60 and Appendix III).

Summary of F1 female terminal body weights and organ weights is presented in Table 61. Body weight at scheduled sacrifice (29, 30, 29, and 29 females at 0, 120, 400, and 1500 ppm) was equivalent across all groups. Absolute female liver, paired kidney, paired adrenal gland, spleen, paired ovary, and uterine weights were statistically equivalent across all groups. Absolute uterine weight was reduced at 400 ppm (82.9% of control value) and at 1500 ppm (79.6% of control value), not statistically significantly different. Absolute female brain weight was significantly reduced at 1500 ppm (94.4% of controls). Relative liver weight was statistically equivalent across all groups, but the value at 1500 ppm was 112.2% of the control value. Relative paired kidney, paired adrenal gland, spleen, brain, and uterine weights were equivalent across all groups (although the relative uterine weight at 400 ppm was 80.7% of the control value, and the relative uterine weight at 1500 ppm was 81.2% of the control value). Relative paired ovary weight was significantly reduced at 400 ppm (82.4% of controls); the value at 1500 ppm was 91.2% of controls, not statistically significantly different (Table 61).

Gross findings for F1 females who died on study (unscheduled deaths; one each at 0, 400, and 1500 ppm) are presented in Table 62. The one female at 0 ppm (no. 390; died on sd 97) exhibited red lesion on adrenal gland, small foci on pale heart ventricle, bilateral kidneys with cortex pale white and irregular, all liver lobes dark red and slightly swollen, all lung lobes with multiple small hemorrhages, and enlarged spleen. The one female at 400 ppm (no. 528; died on pnd 3, sd 100) exhibited all liver lobes pale white, all lung lobes congested with irregular

patchy consolidation, and one fetus retained in the vagina. The one female at 1500 ppm (no. 326; died on pnd 0, sd 96) exhibited urinary bladder with two tan calculi and wall thickened, bilateral kidneys irregular and pale, all liver lobes pale yellow, all lung lobes with irregular patchy consolidation, spleen reduced in size, five fetuses retained in left horn of uterus, five fetuses and numerous blood clots in right horn of uterus, and one placenta retained in vagina.

Gross findings for F1 females at scheduled sacrifice (29, 30, 29, and 29 at 0, 120, 400, and 1500 ppm, respectively) were predominantly in the kidney at 1500 ppm with one or more clear cysts and white foci, one to three females per finding. Additional observations included necrosis in abdominal fat in two females at 0 ppm, hydronephrosis in one female each at 0 ppm and 1500 ppm, adrenal gland with clear cyst at 1500 ppm, a mass on head in one female at 120 ppm, short tail in one female at 120 ppm, uterus distended with clear fluid in one female at 400 ppm, and right horn of uterus with five resorption sites in one female at 0 ppm (Table 62).

F1 maternal microscopic findings from 30 females each at 0 and 1500 ppm, and 12 females at 120 ppm and 13 females at 400 ppm (with gross lesions and/or unsuccessful reproduction) are also presented in Table 62. The only treatment-related findings were in the kidneys. Renal tubule dilatation in the renal papilla was observed in 0, 0, 1, and 4 females at 0, 120, 400, and 1500 ppm (renal tubule dilatation in the cortex and medulla did not exhibit a dose-related pattern of incidence). Chronic inflammation was observed in 0, 2, 4, and 9 females at 0, 120, 400, and 1500 ppm. Nephropathy was observed in 2, 4, 5, and 8 females at 0, 120, 400, and 1500 ppm. Polycystic kidneys were observed in 0, 2, 1, and 18 females at 0, 120, 400, and 1500 ppm. Renal tubule regeneration was observed in 1, 0, 1, and 14 females at 0, 120, 400, and 1500 ppm, respectively. Paired ovarian follicle count was equivalent between the high dose and control females. The stage of estrus at necropsy was also assessed. The percentage of females in metestrus was statistically significantly increased at 120, 400, and 1500 ppm, due to nonsignificant reductions in the percentages of females in diestrus at these dose levels, and of females in estrus at 400 and 1500 ppm. No other findings appeared treatment or dose related (Table 62 and Appendix III).

DISCUSSION

The present study evaluated exposure of CD® (Sprague-Dawley) rats to Wingstay 100 administered in the diet at 0, 120, 400, and 1500 ppm for two generations, one litter per generation. Adult toxicity was overt for F0 and F1 parental animals at 400 and 1500 ppm. At 1500 ppm, there were consistent and persistent reductions in body weights, weight gains, feed consumption, and food efficiency in both sexes and both generations. At 400 ppm, there were occasional and sporadic reductions in body weights, weight gains, feed consumption, and food efficiency in both sexes and both generations. At 120 ppm, there were few or no effects on these parameters. Parental liver weights exhibited a relatively consistent pattern of no effects on absolute weight (except for F0 females in which the absolute weight was statistically significantly increased at 1500 ppm) and clear increases in relative weight (statistically significant for F0 males and females and F1 males, and increased, but not statistically significantly in F1 females). In the absence of any remarkable pathology, it is most likely that the increased relative liver weights are due to decreased terminal body weights and to the induction of metabolizing enzymes in the liver and consequent increase in mass (Conney, 1967). Treatment-related histopathologic lesions were limited to the kidney, specifically polycystic kidneys, and were observed in F0 females at 1500 ppm (three of nine females with identified gross lesions), but not F0 males at 1500 ppm (no gross lesions, therefore no kidneys retained), and in F1 parental males and females at 120, 400 and 1500 ppm (Text Table D). The lesion was first identified in the F1 (and later F2) weanlings in all Wingstay 100-exposed groups as a more severe and acute lesion which partially resolved and exhibited a more chronic appearance in the F1 adults at the same dietary doses. The status of the F0 kidneys is not known, the cause of the lesions is not known, and the necessary and sufficient exposure duration is not known.

Text Table D. Summary of Incidence and Severity of Polycystic Kidneys in F0 Adults, F1 Weanlings and Adults, and F2 Weanlings^a

	WINGSTAY 100 (ppm)							
	MALES				FEMALES			
	0	120	400	1500	0	120	400	1500
F0 Adults^b								
No. Adults Examined	0	0	0	1	0	0	2	9
Polycystic Kidneys	0	0	0	0	0	0	0	3 ^c
minimal	0	0	0	0	0	0	0	2
moderate	0	0	0	0	0	0	0	1
F1 Weanlings								
No. Pups Examined	23	25	20	11	22	26	18	11
Polycystic Kidneys	0	1	8	10	0	5	7	11
minimal	0	1	7	1	0	5	6	2
mild	0	0	1	1	0	0	1	3
moderate	0	0	0	5	0	0	0	4
marked	0	0	0	3	0	0	0	2
F1 Adults								
No. Adults Examined	30	30	30	30	30	30	30	30
Polycystic Kidneys	0	5	10	21	0	2	1	18
minimal	0	5	10	15	0	2	1	11
mild	0	0	0	6	0	0	0	6
moderate	0	0	0	0	0	0	0	1
marked	0	0	0	0	0	0	0	0
F2 Weanlings								
No. Adults Examined	60	64	19	16	60	64	19	15
Polycystic Kidneys	0	3	6	15	0	5	8	15
minimal	0	3	5	5	0	5	7	5
mild	0	0	1	9	0	0	1	9
moderate	0	0	0	1	0	0	0	0
marked	0	0	0	0	0	0	0	1

^a Summarized from Summary Tables 21 (F1 weanlings), 29 (F0 adult males), 31 (F0 adult females), 52 (F2 weanlings), 60 (F1 adult males), and 62 (F1 adult females), and Appendix III, Histopathology Report. Only findings related to polycystic kidneys are reported here.

^b For F0 adults, only kidneys with gross lesions were examined histologically in any group.

^c The number of animals exhibiting the specified lesion.

There was no evidence of male reproductive toxicity at any doses in terms of no effects on acquisition of preputial separation in F1 males, on F0 or F1 male mating or fertility indices (summarized in Text Table E), and no histologic findings in F0 or F1 male reproductive organs.

Text Table E. Summary of Reproductive Parameters^a

Parameter	F0				F1			
	Wingstay 100, ppm				Wingstay 100, ppm			
	0	120	400	1500	0	120	400	1500
No. mating pairs	29	30	30	30	30	30	30	30
No. sperm positive	26	30	26	27	26	26	24	26
No. pregnant	24	27	24	25	22	23	22	24
No. pregnant with no live litters	0	1	1	10	0	1	2	2
No. with live litters - pnd 0	24	26	23	15**	22	22	20	21
- pnd 4	24	26	21	14	21	22	19	19
No. dams died								
- during gestation	0	0	1	0	0	0	0	0
- during lactation	0	0	2	4	0	0	1	1
- holding period	0	0	0	4	1	0	0	0
No. litters died - pnd 0-21	0	0	1	1	1	0	1	4
Gestational length, days	22.2	22.4	22.8**	23.5**	22.2	22.8**	23.1**	23.2**
No. implantation sites/litter	16.92	15.85	15.63	14.54	16.50	16.35	15.14	15.04
% Postimplantation loss/litter	10.67	14.92	26.06**	52.34**	6.82	18.49	20.16	32.63*
No. total pups/litter - pnd 0	15.7	14.9	12.3**	12.1**	15.7	14.5	15.2	13.3
No. live pups/litter - pnd 0	15.6	14.1	11.9*	7.6**	15.6	13.7	13.4	10.8**
No. dead pups/litter - pnd 0	0.1	0.3	0.4	4.1**	0.1	0.7	0.4	2.5**
Live Birth Index (%)	99.2	98.0	97.0	57.5**	99.2	91.9	97.2	77.8**
Sex ratio (% males) - pnd 0	50.4	54.1	55.2	44.1	44.4	47.1	46.2	48.6
Pup body weights (g) - pnd 0	6.38	6.79**	6.93**	6.63	6.32	6.89**	6.99**	6.63*
- pnd 4	10.97	11.40	12.32**	11.24	10.46	11.27	11.72	10.59
- pnd 7	18.24	18.62	19.47	17.61	17.08	18.33	19.16	17.08
- pnd 14	35.76	37.56	38.42	34.44	34.86	35.50	37.06	33.90
- pnd 21	55.13	59.62	60.78*	55.93	53.69	57.22	60.44**	53.62

^a Data taken from Tables 2 (F0) and 32 (F1) for animal fates, and Tables 16, 17 (F0), and 47, 48 (F1) for F0 mating to produce F1 litters and F1 mating to produce F2 litters.
* = p<0.05 versus control group value
** = p<0.01 versus control group value

There were no effects on seminal parameters in F0 males (in F1 males, the percentage of progressively motile sperm was significantly reduced at 120 and 1500 ppm, but not at 400 ppm, in the absence of any effects on sperm number, morphology, or on percent motile sperm), and no effects in F0 or F1 males on calculated daily sperm production (Text Table F). For parental females, there were also no effects on acquisition of vaginal patency, on mating or fertility, no histologic findings in reproductive organs, and no major effects on estrous cyclicity (no change in number of females cycling or in cycle length in F0 or F1 females, no changes in incidence of abnormal cycles for F0 females, but significant increases in F1 females with abnormal cycles at 400 and 1500 ppm). In addition, F1 females at necropsy exhibited a significant increase in the percentage of metestrus at 120, 400, and 1500 ppm (due to nonsignificant decreases in the percentage in diestrus at these doses and in the percentage in estrus at 400 and 1500 ppm). The stage of estrus at demise was not determined for F0 females. Since neither the F0 nor F1 females had any problem with mating or fertility at any dietary dose, it does not appear that these effects on estrous cyclicity had any consequences in this study. Both F0 and F1 females at 400 and 1500 ppm exhibited dystocia (difficulty during delivery), prolonged gestation (also significantly longer at 120 ppm for F1 females), and perinatal mortality of their F1 and F2 litters (Text Table E). F0 and F1 dams died during the peri-parturitional period at 400 and 1500 ppm (and at 1500 ppm for F0 dams only during the postmating holding period). This was associated, clearly temporarily and most likely causally, with the difficulty in delivery and prolonged gestational period (and the finding of retained pups in the reproductive tract at demise). Based on the data summarized in Text Table E, the effects on the parental females were not due to alterations in mating, fertility, pregnancy, or implantation (number of implantation sites/litter were equivalent across groups for both generations). Clinical observations indicative of maternal distress were not observed until the time of anticipated parturition. All parameters during the pregnancy (i.e., weight gain, feed consumption, and food efficiency) showed slight (but statistically significant) decreases at 1500 ppm but no effects at 400 or 120 ppm. What was affected was perinatal survival (increased postimplantation loss at 400 and 1500 ppm for F0 dams and at 1500 ppm for F1 dams), decreased number of total and live pups/litter at 400 and 1500 ppm for F0 dams and at 1500 ppm for F1 dams (significant for live pups, not significant for total pups), increased number of dead pups/litter at 1500 ppm for F0 and F1 dams, and reduced live birth index for F0 and F1 dams at 1500 ppm). Gestational length was also prolonged at 400 and 1500 ppm for F0 dams with F1 litters, and at 120, 400, and 1500 ppm for F1 dams with F2 litters. A likely corollary to prolonged gestation was the finding of increased pup body weights per litter, statistically significant for F0 and F1 dams at

120 and 400 ppm on pnd 0 (and increased but not statistically significantly for F1, and statistically significant for F2 litters at 1500 ppm, probably confounded by systemic toxicity); *i.e.*, the pups remained *in utero* for a longer period than the control pups did, with functional placentae and therefore continued nutrition and continued growth for at least part of the extended period. Note that there were increased perinatal deaths, including stillbirths, at these affected doses, so dams were delivering dead pups; *i.e.*, *in utero* term deaths did occur. The perinatal deaths also mean that dams at these doses were nursing fewer pups per litter which confounds the interpretation of larger pups per litter, especially on pnd 0 and 4 (pre-cull). The increased weight of the pups persisted through the end of lactation, pnd 21, at 120 and 400 ppm for both generations, with statistical significance achieved on pnd 4 at 400 ppm for F1 pups, and at 400 ppm on pnd 21 for both F1 and F2 pups (Text Table E).

TEXT TABLE F

Calculation of Daily Sperm Production and Efficiency of Daily Sperm Production (DSP)

Parameter	F0				F1			
	Wingstay 100, ppm				Wingstay 100, ppm			
	0	120	400	1500	0	120	400	1500
Paired testes wt., g ^a	3.8586	3.7453	3.7564	3.7157	4.0301	3.9388	3.9128	3.8328
Single testis wt., g ^b	1.9293	1.8727	1.8782	1.8579	2.0151	1.9694	1.9564	1.9164
SHC, 10 ⁶ /g testis ^a	115.03	117.22	116.41	123.49	124.85	133.17	125.52	128.55
SHC, 10 ⁶ /testis ^b	221.93	219.51	218.64	229.43	251.58	262.26	245.57	246.35
DSP ^c	48.141	47.616	47.427	49.768	54.57	56.96	53.27	53.44
Efficiency of DSP ^d	24.952	25.427	25.252	26.787	27.082	28.887	27.228	27.885

SHC = Testicular homogenization-resistant spermatid head counts

DSP = Daily Sperm Production

^a Summary data taken from Summary Tables 28 (for F0 males) and 59 (for F1 males)

^b Data calculated from summary data (paired testes weight/2)

^c Daily sperm production in 10⁶/g testis, calculated according to Sharpe *et al.* (1996) and Robb *et al.* (1978) as follows:

$$DSP = \frac{SHC \times 10^6 / \text{testis}}{4.61 \text{ days}}$$

where 4.61 is the number of days in the spermatogenic cycle in rats when the spermatids are stages VI-VIII (step 18 and 19 spermatids)

^d Efficiency of daily sperm production is calculated as in footnote "c", except that the numerator is SHC x 10⁶/gram testis (Sharpe *et al.*, 1996; Robb *et al.*, 1978).

Interestingly, the same situation has been reported for N,N'-diphenyl-*p*-phenylene-diamine (DPPD), a feed grade antioxidant (Oser and Oser, 1956) which comprises approximately 20% of Wingstay 100 (information provided by the Sponsor's Representative). In that study, the rats were exposed to DPPD in the diet at 0.025, 0.10, 0.40, and 1.60% for a two-week prebreed, mating, gestation, and lactation. The feed antioxidant delayed parturition from a mean of 22.1 days (range 21-23 days) in control to a mean of 24.7 days (range 22-27 days) at 1.60%, only partial litters delivered with dams dying during parturition at 0.40 and 1.60%, increased maternal mortality during and after parturition, increased numbers of stillborn pups at 0.10-1.60%, and increased pup birth weight at all dosed diets (5.9-6.6 g versus 5.5g in controls). The authors indicated that the pups appeared "larger than normal." The nonpregnant females exhibited no distress, and dams who were able to deliver all of their pups (alive and/or dead) survived and thrived. They also reported no effect on fertility and no increased resorptions.

Marois (1998) also reported that daily injections (sc?) of 20, 30, or 40 mg of DPPD, beginning on gd 17 or 14, delayed parturition. He also reported that injection (sc?) of 200 µg of prostaglandin F_{2α} initiated parturition.

Diphenylamine (DPA), which makes up 4-6% of Wingstay 100, is known to cause polycystic kidneys in rats (e.g., Evan and Gardner, 1976; Evan et al., 1978; Crocker et al., 1972, 1983; Alvarez et al., 1987; Merta and Zima, 1994; Resnick et al., 1976).

Therefore, a likely explanation for the dystocia/prolonged gestation and polycystic kidneys observed in this study and confirmed in a subsequent mechanistic study (Tyl, 2000) is the presence in Wingstay 100 of DPPD (at approximately 20%), known to cause dystocia and delayed parturition and of DPA (at approximately 4-6%), known to cause polycystic kidneys.

CONCLUSIONS

Exposure to Wingstay 100 in the diet for two generations in CD® (Sprague-Dawley) rats resulted in parental toxicity at 120, 400, and 1500 ppm, with overt toxicity at 400 and 1500 ppm, including reduced body weights, weight gains, feed consumption and food efficiency, renal lesions (polycystic kidneys) in F0 (females only) and F1 parents and in F1 and F2 offspring; dystocia, delayed parturition (prolonged pregnancy), perinatal mortality, and increased pup body weights in surviving litters. At 120 ppm, polycystic kidneys were present in F1 and F2 weanlings and in F1 adults, prolonged gestation in F1 (but not F0) females, and increased pup weights in F1 and F2 newborn offspring. There was no "no observable adverse effect level" (NOAEL) for adult, reproductive, or postnatal toxicity established in this study.

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PROTOCOL DEVIATIONS

A. Temperature and Relative Humidity Excursions:

The protocol mandates temperature range of 68-75°F and relative humidity of 40-70% for the animal rooms. Excursions outside these ranges for the RTI Animal Research Facility animal rooms used in this study are as follows:

1. Room 403, F0 Males (see text for occupancy dates)

Temperature			Relative Humidity		
Date	Duration	°F	Date	Duration	% RH
10/4/96	1 hour	78.2°	6/21/96	1 hour	71.5%
			7/3/96	1 hour	79.6%
			9/6/96	2 hours	Up to 77.2%
			(Hurricane Fran)		
			10/4/96	1 hour	73.0%

2. Room 404, F0 Females (see text for occupancy dates)

Temperature			Relative Humidity		
Date	Duration	°F	Date	Duration	% RH
10/4/96	1 hour	76.6°	6/21/96	1 hour	72.4%
			7/3/96	1 hour	80.3%

3. Room 503, F1 Males (see text for occupancy dates)

Temperature			Relative Humidity		
Date	Duration	°F	Date	Duration	% RH
No Excursions			10/28/96	1 hour	83.5%
			2/21/97	1 hour	82.4%

4. Room 504, F1 Females (see text for occupancy dates)

Temperature			Relative Humidity		
Date	Duration	°F	Date	Duration	% RH
2/21/97	1 hour	79.7°	10/20/96	1 hour	70.2%
			10/28/96	1 hour	80.2%
			12/2/96	1 hour	72.1%
			12/3/96	1 hour	73.5%
			12/5/96	1 hour	31.6%
			12/16/96	2 hours	Up to 71.3%
			12/19/96	2 hours	Up to 70.3%
			12/25/96	1 hour	73.2%
			1/1/97	1 hour	72.0%
			1/7/97	1 hour	71.0%
			1/8/97	1 hour	70.7%
			1/9/97	2 hours	Up to 78.5%
			2/21/97	1 hour	73.4%

5. Room 206, F1 Males (12/9 – 12/16/96)
No temperature or relative humidity excursions.
6. Room 207, F1 Females (12/9 – 12/16/96)
No temperature or relative humidity excursions.

The NIH Guide for the Care and Use of Laboratory Animals (U.S. Dept. HHS, PHS, NIH Publication No. 86-23, Revised 1985) recommends, for rats, a temperature range of 64.4 – 78.8°F and a relative humidity range of 40-70%. The brief excursions in temperature outside the protocol-mandated range did not exceed the NIH recommended temperature range, except for one hour at 79.7°F. The brief one-two hour excursions in relative humidity, up to 83.5% (for one hour) and down to 31.6% (for one hour), did exceed the NIH recommended range, but they were few, brief, and minor.

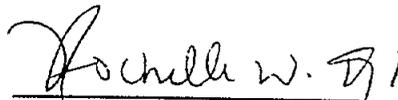
These few, brief and minor excursions in temperature and relative humidity did not affect the design, conduct, or conclusions of this study, in the Study Director's professional judgment.

B. Missing Room Log Sheets

The room log sheets for August 24, 1996 through October 10, 1996, were inadvertently misplaced. This is the only place in the raw data where a.m. and p.m. mortality checks were recorded. This deviation was documented in the study records. Data for this period on animal body weights, feed consumption, daily clinical observations, etc., were collected and are in the study records. The loss of these sheets had no impact on the design, conduct, or conclusions of the study.

C. F1 Male Preputial Separation

Four F1 males at 0 ppm and seven males at 120 ppm were inadvertently not checked for preputial separation on pnd 35. One male pup at 0 ppm was also not checked on pnd 36. Since these pups were negative for preputial separation on the subsequent day, this inadvertent protocol deviation had no impact on the study.

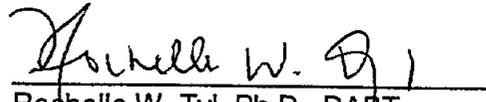

Rochelle W. Tyl, Ph.D, DABT
Study Director

12/8/00
Date

GOOD LABORATORY PRACTICES COMPLIANCE STATEMENT

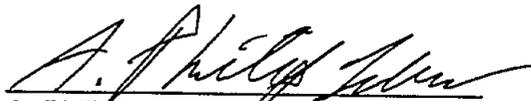
This study was performed in compliance with the Good Laboratory Practices (GLP) Standards promulgated by the U.S. Environmental Protection Agency Toxic Substances Control Act Good Laboratory Practice Standards, Final Rule, 40CFR Part 792 (*Federal Register* 54 [158], 34034-34050, August 17, 1989).

Prepared by:



Rochelle W. Tyl, Ph.D., DABT
Study Director/Research Director
Center for Life Sciences and Toxicology
Research Triangle Institute

12/8/00
Date



A. Philip Leber, Ph.D.
Project Coordinator
Goodyear Tire and Rubber Company
Sponsor's Representative

Dec 7, 2000
Date



Quality Assurance Statement

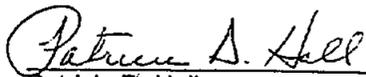
Study Title: Two-Generation Reproductive Evaluation of Wingstay 100 Administered in the Feed to CD® (Sprague-Dawley) Rats
Sponsor: The Goodyear Tire and Rubber Company
Study Code: Rt96-GY1B
Protocol Number: RTI-569

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

Inspections and Audits	Inspection and Audit Date(s)	Date Inspection/Audit Report Sent to Study Director and Management
Protocol Review	04/08 & 04/11/96	04/11/96, 05/23/96
Analysis for Peroxides	05/24/96	05/24/96
Quarantine	06/14/96	06/14/96, 06/24/96
F0 Dosing, Body and Food Weights	06/18/96	06/18/96, 06/24/96
Dose Analysis Data Audit	08/05/96	08/05/96
Dose Analysis Data Audit	08/05/96	08/05/96
Dose Analysis	08/20/96	08/20/96, 10/01/96
Vaginal Cytology	08/26/96	08/26/96, 10/01/96
Dose Formulation	09/23/96	09/23/96, 10/01/96
F0 Male Necropsy	10/07/96	10/08/96, 11/06/96
Sperm Analyses	10/07/96	10/08/96, 11/06/96
F0 Female and F1 Weanling Necropsy	10/11/96	10/11/96, 11/06/96
Dose Analysis Data Audit	11/14/96	11/14/96
Dose Analysis Data Audit	11/14/96	11/14/96
Data Audit	12/13/96	12/13/96
Dose Analysis Data Audit	12/24/96	12/24/96
F1 Dosing, Body and Food Weights	12/31/96	12/31/96, 02/06/97
Dose Analysis Data Audit	12/31/96	12/31/96
Dose Analysis Data Audit	01/10/97	01/10/97
Dose Formulation and Analysis Data Audit	06/11- 19/97	06/19/97

continued

Inspections and Audits	Inspection and Audit Date(s)	Date Inspection/Audit Report Sent to Study Director and Management
Dose Formulation	01/20/97	01/20/97, 02/06/97
Weaning, Body and Feed Weights	01/30/97	01/31/97, 02/06/97
Male Necropsy	02/17/97	02/17/97, 03/04/97
F1 Female and PND 21 Necropsy	02/20/97	02/20/97, 03/04/97
Dose Analysis Data Audit	06/19/97	06/19/97
Data Audit	10/10 – 11/20/97	11/20/97
Report Audit	10/01 – 11/30/97	11/30/97



Patricia D. Hall
Quality Assurance Specialist

12/8/00
Date

Approval:



David L. Brodish
Quality Assurance Manager

12/8/00
Date