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March 6, 2002

Re: 8EHQ-0201-14864

Dear Sir or Madam:

Enclosed as per our February 20, 2001 submission is a copy of the final report for the toxicology study entitled, "Two-Generation Reproductive Toxicity Evaluation of Butyl Benzyl Phthalate Administered in the Feed to CD® (Sprague-Dawley) Rats". The study was conducted at Research Triangle Institute under the direction of Dr. Rochelle W. Tyl on behalf of the European Council for Plasticizers and Intermediates (ECPI), of which Ferro Corporation is a member. Ferro Corporation reported to EPA in 2001 that Butyl Benzyl Phthalate (BBP) in this study decreased the litter-based anogenital distance at birth in the F1 male pups of the mid- and high-dose test groups, 3750 and 11,250 ppm dietary BBP, respectively. At the time of that report, the F1 breeding phase of the investigation had just ended.

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The enclosed report of the study, now final, states that dietary exposure of CD® (SD) rats to BBP for two generations, one litter per generation, at 0, 750, 3750, and 11,250 ppm, resulted in:

F0 and F1 parental systemic toxicity at 11,250 ppm;

F1 (but not F0) parental reproductive toxicity at 11,250 ppm;

F1 and F2 offspring toxicity at 3750 and 11,250 ppm

- -- Reduced body weights in F1 and F2 pups during lactation at 11,250 ppm
- -- Shortened anogenital distance in F1 and F2 males at 3750 and 11,250 ppm
- -- Delayed acquisition of puberty in F1 males and females at 11,250 ppm
- -- Retention of nipples and areolae in preweanling F1 and F2 males at 11,250 ppm, and male reproductive system malformations at 11,250 ppm.

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The effects observed at the high-dose (11,250 ppm) were anticipated based on published reports of BBP effects (Gray, L.E., Jr., J. Ostby, M. Price, D.N.R. Veeramachaneni, and L. Parks., "Perinatal exposure to the phthalates DEHP, BBP and DINP, but not DEP, DMP or DOTP alters sexual differentiation of the male rat." *Toxicol. Sci.* **58(2)**, 350-365, 2000). In the present study the only effect observed in either generation of the mid-dose animals was decreased anogenital distance at birth. However, this observation, which can suggest the possibility of reproductive impairment, did not manifest as a decrement in any parameter of maturation or reproductive performance in either generation.

Accordingly, the Study Director has concluded that, in rats, under the conditions of this study:

the F0 and F1 parental systemic and reproductive **NOAEL** is 3750 ppm (equivalent to approximately 250 mg/kg/day);
the F1 and F2 offspring reproductive toxicity **NOAEL** also is 3750 ppm (equivalent to approximately 250 mg/kg/day);
the F1 and F2 offspring reproductive toxicity **NOEL** (no observable effect level) in males is 750 ppm (equivalent to approximately 50 mg/kg/day), based on the significantly shortened anogenital distance in F1 and F2 male pups at birth at 3750 (and at 11,250) ppm, with no effects on reproductive development, structures, or functions at 3750 mg/kg/day.

Since the only effect seen in the F1 or F2 offspring at the 3750 ppm (mid-dose level) was shortened AG distance, and this effect did not manifest as any reproductive decrement in either generation, the AG distance change at 3750 ppm is not considered as an adverse effect. Moreover, adverse effects on offspring or on reproductive performance were only seen at doses systemically toxic to the parental generation (11,250 ppm), i.e., at parentally toxic doses.

The present two-generation study of dietary BBP was performed in compliance with U.S. EPA Toxic Substances Control Act (TSCA), Good Laboratory Practice Standards, and the U.S. EPA OPPTS Reproduction and Fertility Effects Testing Guidelines with the following enhancements:

1. Anogenital distance (AGD) and body weight were recorded for all live F1 and F2 offspring at birth on postnatal day (pnd) 0 (the OPPTS guidelines have AGD "triggered" in F2 offspring if developmental and/or reproductive effects are observed in F1 offspring).
2. F1 and F2 litters were standardized to ten (with as even a sex ratio as possible) on pnd 4 to remove the confounder of litter size on offspring survival and growth during lactation. All culled pups on pnd 4 were subjected to an external and visceral examination, with special attention on the male reproductive organs.
3. All F1 and F2 male preweanling pups were examined on pnd 11-13 for the presence of retained nipples and/or areolae.

4. At weaning on pnd 21, in addition to the three pups/sex/litter necropsied, all nonselected pups were necropsied, with special attention on the male reproductive organs.
5. Epididymal sperm number, motility, and morphology; enumeration of testicular homogenization-resistant spermatid heads for calculation of daily sperm production (DSP); and efficiency of DSP were assessed in all F0 and F1 adult males at scheduled necropsy.
6. Additional histopathology was performed on F0 and F1 adult males in all groups if they exhibited gross lesions, if they did not sire live litters (also F0 and F1 females if they did not produce live litters), and if there was evidence of potential treatment-related histopathologic findings in any organs at the high dose.

Because of the comprehensive nature of this study and the quality with which it was performed, Ferro Corporation is pleased to submit this report to EPA. If there are questions about the submission, feel free to contact me at: 216-750-6696.

Sincerely,

Alan J Olson

Alan Olson, P.E.
Director of Technology
Ferro Polymer Additives

cc: D. Cadogan, ECPI, Brussels

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VOLUME I OF III

FINAL REPORT

TITLE:	Two-Generation Reproductive Toxicity Evaluation of Butyl Benzyl Phthalate Administered in the Feed to CD® (Sprague-Dawley) Rats
AUTHORS:	Rochelle W. Tyl, Ph.D., DABT Christina B. Myers, M.S. Melissa C. Marr, B.A., RLATG
PERFORMING LABORATORY:	Reproductive and Developmental Toxicology Laboratory Center for Life Sciences and Toxicology Chemistry and Life Sciences RTI P. O. Box 12194 Research Triangle Park, NC 27709-2194
SPONSOR:	The European Council for Plasticizers and Intermediates (ECPI) Avenue E. Van Nieuwenhuyse 4, Box 1 B-1160 Brussels, Belgium
SPONSOR'S REPRESENTATIVE:	David Cadogan, Ph.D. Director ECPI
STUDY MONITOR:	John H. Butala, DABT Consultant to ECPI (through Ferro, a member company)
STUDY INITIATION DATE:	May 4, 2000
IN-LIFE PERFORMANCE DATES:	July 10, 2000 - March 29, 2001
LABORATORY COMPLETION DATE:	October 22, 2001
FINAL REPORT DATE:	January 31, 2002
RTI IDENTIFICATION NUMBER:	65C.07269.200

Author:

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

Approved:

Alvin M. Cruze 1/31/02

Alvin M. Cruze, Ph.D. Date
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RTI Project No.: 65C-07269-200
RTI Protocol No.: RTI-761

FINAL REPORT

Two-Generation Reproductive Toxicity Evaluation of Butyl Benzyl Phthalate Administered in the Feed to CD® (Sprague-Dawley) Rats

Sponsor: The European Council for Plasticizers and Intermediates (ECPI)
Avenue E. Van Neiuwenhuyse 4, Box 1
B-1160 Brussels, Belgium

ABSTRACT

The purpose of this study was to evaluate the potential of butyl benzyl phthalate (BBP), 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. This study was performed in compliance with U.S. EPA Toxic Substances Control Act (TSCA), Good Laboratory Practice Standards (U.S. EPA, 1989), and the U.S. EPA OPPTS Testing Guidelines (U.S. EPA, 1998).

Male and female CD® (Sprague-Dawley) rats (the F0 generation) were administered BBP (1,2-Benzenedicarboxylic acid, butyl phenylmethyl ester (9CI); CAS No. 85-68-7) in the feed at 0, 750, 3750, and 11,250 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 continuing. F0 males were necropsied after the mating period, with histologic evaluation of reproductive and other organs and andrological assessments (reproductive organ weights, epididymal sperm number, motility and morphology, testicular homogenization-resistant spermatid head counts, daily sperm production, and efficiency of daily sperm production). On the day of birth (postnatal day [pnd] 0), anogenital distance was measured and body weights recorded for all live F1 pups in all litters. F1 litters were standardized to ten pups on pnd 4 (with all culled pups examined externally and viscerally after euthanasia). On pnd 11-13, all F1 male pups were examined for retained nipples/aerolae on the ventrum. At weaning on pnd 21, up to three weanlings/sex/litter were necropsied, and 30/sex/dose were selected as F1 parents of the F2 generation. Any remaining F1 male pups not

selected as parents or for necropsy, which exhibited retained nipples, were also necropsied. F0 females were then necropsied with organ weights, stage of estrus at necropsy, enumeration of ovarian primordial follicles, and histopathology of reproductive and other selected organs. Selected F1 weanlings, 30/sex/dose, were administered BBP in the diet for a ten-week prebreed exposure period, with acquisition of vaginal patency in females and preputial separation in males assessed. Vaginal cytology for estrous cyclicity in F1 selected females was evaluated during the last three weeks of the prebreed exposure period, and they were mated for a two-week period, as described above. F1 males were necropsied after their mating period, with histopathology and andrological assessments (as described above). During the F2 lactation, pup anogenital distance was measured on pnd 0, and F2 male offspring were examined for retention of nipples and/or areolae on pnd 11-13. At weaning of the F2 litters, parental F1 females were necropsied with histopathology, as described above, and F2 weanlings, up to three/sex/ litter, were necropsied.

Dietary exposure of CD® (SD) rats to BBP for two generations, one litter per generation, at 0, 750, 3750, and 11,250 ppm, resulted in:

- F0 and F1 parental systemic toxicity at 11,250 ppm
  - -- Reduced body weights and weight gains
  - -- Increased absolute and relative liver weights
  - -- Histopathologic lesions in livers
- F1 (but not F0) parental reproductive toxicity at 11,250 ppm
  - -- Reduced mating and fertility indices in F1 parents to make F2 offspring
  - -- Reduced number of implantations and total and live pups per litter on pnd 0 for F2 offspring
  - -- Reduced F1 male epididymal sperm motility, progressive motility, sperm concentrations
  - -- Increased incidence of F1 adult male reproductive tract malformations (from perinatal exposure)
  - -- Increased gross and histopathologic lesions in F1 adult male testes and epididymides (from perinatal exposure)
- F1 and F2 offspring toxicity at 3750 and 11,250 ppm
  - -- Reduced body weights in F1 and F2 pups during lactation at 11,250 ppm
  - -- Shortened anogenital distance in F1 and F2 males at 3750 and 11,250 ppm
  - -- Delayed acquisition of puberty in F1 males and females at 11,250 ppm
  - -- Retention of nipples and areolae in preweanling F1 and F2 males at 11,250 ppm

-- Male reproductive system malformations at 11,250 ppm

Therefore, in rats, under the conditions of this study:

- The F0 and F1 parental systemic and reproductive NOAEL is 3750 ppm (equivalent to approximately 250 mg/kg/day)
- The F1 and F2 offspring reproductive toxicity NOAEL also is 3750 ppm (equivalent to approximately 250 mg/kg/day)
- The F1 and F2 offspring reproductive toxicity NOEL (no observable effect level) in males is 750 ppm (equivalent to approximately 50 mg/kg/day), based on the significantly shortened anogenital distance in F1 and F2 male pups at birth at 3750 (and at 11,250) ppm, with no effects on reproductive development, structures, or functions at 3750 mg/kg/day

OBJECTIVES

This study was performed to evaluate the potential of BBP 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, butyl benzyl phthalate (BBP; 1,2-Benzenedicarboxylic acid, butyl phenylmethyl ester (9CI); CAS No. 85-68-7), product name Santicizer® 160 plasticizer, was received from Solutia, Bridgeport, NJ, on February 2, 2000. The test material was a clear liquid and was identified by the supplier as Lot No. DL1120N, with a purity of 98.5%, as specified in the Certificate of Analysis (see Appendix V, attachment to protocol). All measurements of the test material, for diet formulation purposes, were made by weight and not corrected for the purity of BBP. The basic diet was ground Purina Certified Rodent Chow® (No. 5002, PMI Feeds, Inc., St. Louis, MO).

For the 750 and 3750 ppm doses, the appropriate amount of chemical was weighed into a portion of blank premix feed in a stainless steel bucket. The chemical/feed mixture was stirred thoroughly with a stainless steel spoon. The remainder of the premix feed (total 5 kg) was added and mixed. The 11,250 ppm dose was prepared by using two 5 kg premixes mixed with clean feed and blended in a V-shell blender for 15 minutes. This eliminated any lumps at this concentration. The premix for the 750 and 3750 ppm doses was then added to a V-shell blender using a sandwich technique (e.g., for an 80 kg formulation, 35 kg of blank (clean) feed was added first, then the 5 kg premix, then the premix feed container was purged by 5 kg of blank feed, and last a 35 kg portion of blank feed was added). The mix was then blended for 20 minutes. For the 11,250 ppm concentration, for an 80 kg formulation (for example), 20 kg of clean feed was added to a V-shell blender, followed by the 10 kg premix, then the premix feed container was purged with 15 kg of feed, and last a 35 kg portion of clean feed was added. This mix was also blended for 20 minutes. Analytical and archive samples were collected (and frozen) from the middle of the feed as it was discharged into a polyethylene bag.

For analysis, stock solutions were prepared by weighing specified weights of BBP into scintillation vials, dissolving the compound in hexane, transferring the solution quantitatively into 50-ml volumetric flasks, diluting to volume with hexane, and mixing. The stock solutions were 24.0 and 20.0 mg/ml. The internal standard solution was 2.0 mg/ml of diethylphthalate in hexane. Vehicle standards were prepared from aliquots of blank feed spiked with one of two stock solutions of hexane in 250-ml bottles. An aliquot of hexane was added to each standard bottle, and the bottles were capped and sonicated for 15 minutes without heat, then shaken on a platform shaker at low setting for 60 minutes. The feed was allowed to settle for at least 5 minutes. Approximately 18-20 ml of each extract was transferred to a separate scintillation vial and centrifuged for 5 minutes. An aliquot from each sample was transferred to a separate GC autosampler vial containing 0.1 ml of the internal standard solution. The vials were capped, and the samples were mixed prior to gas chromatographic analysis as follows. The samples were analyzed on a Hewlett-Packard 5890 Series II gas chromatograph with an HP 07673 autosampler. The column was a capillary, J&W DB-1701 (15 m x 0.22 mm ID), 0.25 μm film. The injection solvent was hexane. The injection volume was 1 μl, and the detector was flame ionization detector (FID) (see Appendix I for details on methods and results of analyses). Formulation samples were processed and analyzed in the same manner.

Standards for acceptable accuracy of mixing were: the mean of the analyzed samples was within ± 10% of the nominal concentration, and the percent 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.

Homogeneity and stability of the test material in the animal diets, at the concentrations to be used in this study, were determined by RTI as follows. Prior to the performance of this study, aliquots of treated diets, encompassing the range of dietary concentrations that were to be used in the study (700 ppm and 12,000 ppm), were used to assess the homogeneity and stability of the prepared diets. Homogeneity (three samples each from the left, right, and center of the V-shell blender) was established for the high and low dietary concentrations. Stability analyses were also performed on dosed feed samples at 700 and 12,000 ppm at room temperature in open containers (to simulate cageside exposures) for five and 12 days, and on dosed feed samples stored frozen (approximately -20°C) for 0, 20, 40, and 54 days. Cageside stability was confirmed for 12 days, and

frozen stability was confirmed for 54 days at 700 and 12,000 ppm. The dosed feed was formulated, based on the stability data, approximately every four to six weeks and stored frozen, and feed in the feed jars was changed at least every seven days. Analysis of dietary concentrations of test material was performed on all doses and control for the first four formulation dates and every other month thereafter. These analyses were performed prior to administration of the test diets to the animals.

All dosed feed formulations used in the study had analytical values of 90.9 to 107% of target concentrations. Vehicle control feed formulations contained no BBP, with an estimated detection limit of 35 ppm. Dietary dose levels were 0, 750, 3750, and 11,250 ppm. 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 dosed feed, were not aware of the dose levels (groups were identified by color and Rx codes). 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-five (134) virgin female and 135 virgin male outbred albino CD® (Sprague-Dawley) rats (Crl:CD®[SD] IGS BR) were received from Charles River Breeding Laboratories, Raleigh, NC (males from area R04, females from area R12) on July 10, 2000, 40-45 days old, with weights of males 151-175 g and females 126-150 g upon arrival (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. For serum viral antibody analysis, within one day after receipt, five rats per sex were arbitrarily chosen from the shipment of animals, sacrificed, and blood collected for assessment of viral antibody status. Heat-inactivated serum was sent to BioReliance Corporation (previously Microbiological Associates), Rockville, 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/sialoacryoadenitis (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.

Text Table A. Study Schedule

Event	Dates[a]
Animals arrived at RTI	07/10/00
Quality control – blood for viral antibody titers	07/11/00
Eartag and initial weighing	07/11/00
Weighing and randomization	07/18/00
F0 prebreed exposure (10 weeks)	07/19/00 – 09/27/00
F0 female vaginal cytology (3 weeks)	09/07/00 – 09/27/00
F0 mating (2 weeks)	09/27/00 – 10/11/00
F0 gestation (approximately 22 days)	09/28/00 – 10/31/00
F0 male necropsy	11/06/00 – 11/09/00
F0 lactation (3 weeks)	10/19/00 – 11/22/00
Selection and necropsy of F1 weanlings	11/09/00 – 11/22/00
F0 female necropsy	11/09/00 – 11/22/00
F1 prebreed exposure (10 weeks)	11/22/00 – 01/31/01
F1 female vaginal patency	11/10/00 - 12/06/00
F1 male preputial separation	11/23/00 - 03/02/00
F1 female vaginal cytology (3 weeks)	01/11/01 – 01/31/01
F1 mating (2 weeks)	01/31/01 – 02/14/01
F1 gestation (approximately 22 days)	02/01/01 – 03/07/01
F1 male necropsy	03/12/01 – 03/15/01
F1 lactation (3 weeks)	02/22/01 – 03/29/01
F2 anogenital distance (pnd 0)	02/22/01 - 03/08/01
Necropsy of F2 weanlings	03/15/01 – 03/29/01
F1 female necropsy	03/15/01 – 03/29/01

[a] A range of dates is provided, when appropriate, since study females became pregnant on different days during the two-week mating period.

Immediately after the formal quarantine period, after selection of quality control animals and study animals, four rats/sex were randomly selected and designated as sentinels. They were housed in the study room(s), singly in polycarbonate solid-bottom cages with bedding, and provided feed and water *ad libitum* (as described below for study animals). They were examined once daily by cageside observation for morbidity or mortality at the same time as clinical observations or morbidity/mortality checks for the study animals. No sentinels exhibited any clinical signs, morbidity, or mortality. At the time of the last necropsies of F0 and F1 parental females, two sentinel animals/sex/necropsy were terminated per time, blood samples collected, and serum samples prepared. All sentinel serum samples were submitted to BioReliance Corporation, Rockville, MD, for serological evaluation (see above). Analysis of serum (as described above) from sentinels sacrificed during the necropsy of the F0 and F1 parental animals was also negative.

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 ± 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 CO_2 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 animals sacrificed moribund. Any pups sacrificed moribund during the lactation period were euthanized by CO_2 asphyxiation or decapitation. F1 and F2 pups culled on pnd 4 were sacrificed by decapitation. Animals received with the initial shipment, but not used in the study, were euthanized on July 19, 2000. Records were kept documenting the fate of all animals received for the study.

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 with stainless steel wire lids (Laboratory Products, Rochelle Park, NJ), with Sani-Chips® cage litter (P.J. Murphy Forest Products Corp., Montville, NJ). Study animals were housed two per cage (one male:one female from the same dose level) during the mating periods. Females were caged separately and individually once they were successfully

mated (or at the end of the mating period). Females were housed with their litters throughout the lactation periods. All animals were housed in the RTI Animal Research Facility for the duration of the study. All animal rooms were on a 12-hour light cycle per day and were controlled for temperature and relative humidity. Temperature and relative humidity in the animal rooms were continuously monitored, controlled, and recorded using an automatic system (Barber-Colman Network 8000 System, Ver. 4.4.1, Loves Park, IL). The protocol-mandated temperature range was 64-79°F (18-26°C), and the relative humidity range was 30-70% (NRC, 1996). The animals were housed in the following Animal Research Facility rooms:

F0 Animals

- Room 303: F0 males from July 10, 2000 (date of arrival at RTI) through November 9, 2000 (the last day males were removed for necropsy)
- Room 305: F0 females from July 10, 2000 (date of arrival at RTI) through November 22, 2000 (last day of F0 female necropsy), with the exception of the mating period (September 27 to October 11, 2000) when F0 females were housed in Room 303 with F0 males until sperm positive

F1 Animals

- Room 503: F1 males from November 9, 2000 (first F1 male weanlings transferred) through March 15, 2001 (last day of F1 male necropsy)
- Room 504: F1 females from November 9, 2000 (first F1 female weanlings transferred) through March 29, 2001 (last day of F1 female necropsy), with the exception of the mating period (January 31 to February 14, 2001) when F1 females were housed in Room 503 with F1 males until sperm positive

Except for a few brief and minor excursions outside the target range in relative humidity, all animal rooms were in range throughout the study. There were no excursions outside the target range for temperature in any animal room throughout this study. The brief and minor excursions in relative humidity did not affect the design, conduct, or conclusions of this study (see Protocol Deviations after the references in this volume).

Purina Certified Ground Rodent Chow (No. 5002, PMI Feeds, Inc., St. Louis, MO; 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.

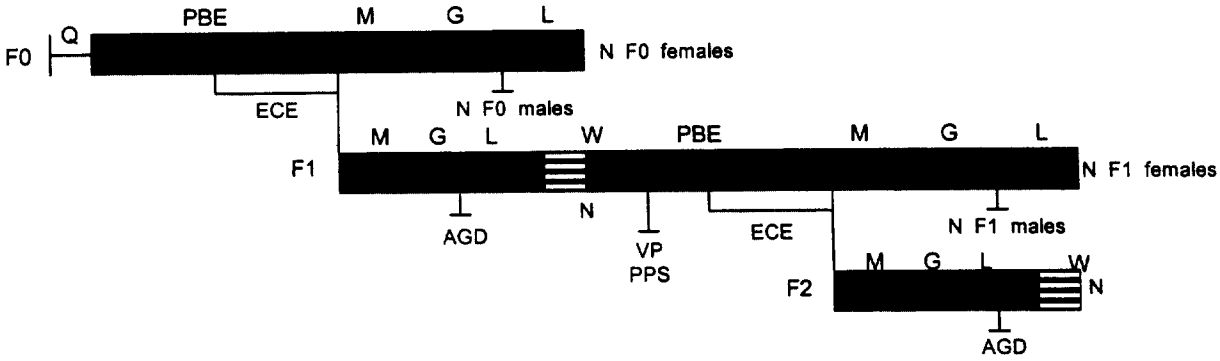
Water (tap water; source: City of Durham, Department of Water Resources, Durham, NC) was available *ad libitum* by plastic water bottles with butyl rubber stoppers and stainless steel sipper tubes. Contaminant levels of the Durham City water were measured at regular intervals by the supplier per EPA specifications. There were no known contaminants that may have affected the outcome of this study.

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 July 19, 2000, when they were approximately seven weeks old. The top dietary concentration (11,250 ppm) was selected to result in target intake of BBP of 750 mg/kg/day. Gray et al. (2000) reported that oral gavage administration of 750 mg/kg/day of BBP to maternal rats on gd 14 to pnd 3 resulted in retained nipples in preweanling male offspring and male reproductive system malformations in greater than 70% of the F1 male offspring at adulthood. Since BBP was administered in the feed in the present study, it was anticipated that the effects observed at 750 mg/kg/day might be reduced in incidence and severity but would still be present. The low dietary concentration (750 ppm) was selected to result in a target intake of BBP of 50 mg/kg/day. Mylchreest et al. (1998a,b, 1999) administered dibutyl phthlate (DBP) by oral gavage to maternal rats during gestation and lactation, and reported that 50 mg/kg/day DBP was the NOAEL. Both DBP and BBP share an intestinal metabolite, monobutyl phthalate (BBP is also cleaved to monobenzyl phthalate), which is the presumed proximal toxicant (Ema, 1992, 1993, 1994; Mylchreest et al., 1998a,b, 1999; Foster et al., 1999). The intermediate dietary concentration (3750 ppm) was selected to provide dose-response information and to result in a target intake of BBP of 250 mg/kg/day, five times the low dose intake and one-third the high dose intake. The target concentrations in the diet were therefore 0, 750, 3750, and 11,250 ppm. Animals were assigned to the different groups by means of randomization stratified by body weight, such that the body weights of all groups by sex were homogeneous by statistical analysis at study initiation. The range of F0 male body weights at the start of the prebreed exposure period was 221.5 – 264.0 g. The range for F0 females was 157.3 – 195.2 g. All animals assigned to each dose level were exposed to their

respective dietary formulations *ad libitum*, 24 hours per day, seven days per week, throughout the study.

Text Figure A
Butyl Benzyl Phthalate Reproductive Toxicity Study Design



KEY:

Q = Quarantine
PBE =Prebreed exposure
M = Mating
G = Gestation
L = Lactation
W = Weaning

ECE = Estrous cyclicity evaluations
VP = Acquisition of vaginal patency
PPS = Acquisition of preputial separation
AGD = Measurement of anogenital distance in F1 and F2 pups on pnd 0
N = Necropsy

Direct dietary exposure
Possible indirect exposure from transplacental and/or translactational exposure
Both direct and possible indirect exposure (nursing pups also self-feeding)

Parental F0 and F1 Animals

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 degree and duration of symptoms. These cage-side observations included, but were not limited to, changes in skin and fur, eyes, mucous membranes, respiratory and circulatory system, autonomic and central nervous system, somatomotor activity, and behavior pattern.

The body weights of the F0 and F1 male rats were determined and recorded initially and weekly through mating. The body weights of F0 and F1 female rats were recorded in the same manner until confirmation of mating. During gestation, F0 and F1 females were weighed on gd

0, 7, 14, and 20. Dams producing litters were weighed on pnd 0, 4, 7, 14, and 21, and 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 gd 0-7, 7-14, and 14-20. During lactation of F1 and F2 litters, maternal feed consumption was measured for pnd 0-4, 4-7, 7-14, and 14-21, although maternal feed consumption after pnd 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, but consumption was visually monitored and feed changed as necessary. 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 BBP/kg body weight/day. In addition, as mandated by the OPPTS guidelines (U.S. EPA, 1998), 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 (x 100 to allow reporting as a percentage). Feed was changed at least weekly throughout the study.

For those brief interim periods, such as from the end of mating to sacrifice for parental males and from the end of lactation to sacrifice for parental females, exposure to dosed feed continued and periodic body weights and daily clinical signs were recorded. These data are presented in this report for completeness. Since the times are brief and the number of females in each group were variable, the female data were not robust for statistical analysis. The male data were subjected to statistical analyses. 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 presence, normalcy, and duration of estrous cycles.

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 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. On the day of birth (pnd 0), anogenital distance was measured and body weight recorded for all live F1 pups in all litters. F1 litters were standardized to ten pups on pnd 4 (with all culled pups examined externally and viscerally after euthanasia). On pnd 11-13, all F1 male pups were examined for retained nipples on the ventrum. 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 then began exposure to the dosed feed formulations at the same dietary concentration of BBP 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 as well as any unselected male pups that had been positive for retained nipples. All F1 pups not selected as parents or for necropsy were examined externally, euthanized, and discarded.

Selected animals of the F1 generation were administered BBP in the diet at their respective formulations *ad libitum* for at least ten weeks. 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. The body weight on the date of acquisition was recorded for each F1 female. 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. The body weight on the date of acquisition was recorded for each F1 male.

The F1 animals were approximately 13 to 15 weeks of age at the initiation of the mating period. Estrous cyclicity was evaluated for the last three weeks of the prebreed exposure period and for the two-week mating period for F1 females (as described above for F0 females). The F1 animals 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 or a vaginal copulation plug was observed, whichever came first (with no change in pairing). Brother-sister matings were avoided. In addition, three F2 weanlings per sex per litter were selected and subjected to a gross (external and visceral) necropsy examination. At necropsy of F0 and F1 parental animals and selected F1 and F2 weanlings, gross lesions were retained in fixative, and selected organs were weighed and/or retained in fixative for possible subsequent histopathologic evaluation.

F1 and F2 Offspring Animals

All F1 and F2 pups were sexed and examined as soon as possible after birth 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). Individual anogenital distance and body weight were recorded on pnd 0 for all F1 and F2 offspring.

On day 4 after birth, the size of each litter was adjusted by eliminating extra pups by random selection to yield, as nearly as possible, five males and five females per litter. Culled pups were examined externally and viscerally after euthanasia. 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), 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. On pnd 11-13, all F1 and F2 male pups were examined for retained nipples and areolae. Any male with one or more retained nipples was marked on the tail with an indelible ink code.

At weaning, 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. At necropsy of selected F1 and F2 weanlings, any male unscheduled for retention or necropsy with one or more retained nipples were also necropsied (with the ventrum shaved to identify any retained nipples). The F1 offspring not selected as parents or for necropsy and the F2 offspring not selected for necropsy were examined for gross external abnormalities, euthanized, and discarded. A gross internal examination was made on any pup appearing moribund or dying on study.

All random selections of offspring were performed as follows. At the time of selection, each pup was assigned a two-digit random number (from two-digit random number tables). For standardization of litter sizes on pnd 4, the pups with the lowest numbers per sex within each litter were culled (euthanized and examined). For selection at weaning, the pups with the highest numbers per sex within each litter were selected as parents (F1 to produce the F2 generation), and up to three pups/sex with the highest or lowest random numbers (depending on the technician performing the selections) were selected for necropsy (F1 and 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 were subjected to a complete gross necropsy, with selected organs (see p. 26) weighed. On the day of necropsy, prior to euthanasia, all F0 and 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, as well as all male pups which were positive for nipple retention and subjected to a gross (external and visceral) necropsy examination. Gross lesions were retained in fixative, and brain, spleen, and thymus of both sexes, and testes, epididymides (males), and ovaries, uterus with cervix and vagina (females) weighed. The adult and weanling males, identified on pnd 11-13 as having retained nipples, were shaved on the ventrum and re-examined for retained nipples. 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. Full histopathology of the organs listed below was performed initially for ten high dose and ten control F0 and F1 animals selected for mating. Since treatment-related reproductive organ malformations were observed in the F1 high dose males (as anticipated), reproductive organs from the remaining F1 high dose and control males, as well as from all F1 males at the mid dose (3750 ppm), were also evaluated histopathologically. Also, since apparent treatment-related histopathologic lesions were observed in livers of F0 and F1 males and F1 females at the top dose (11,250 ppm), ten additional control livers per sex (for a total of 20/sex/generation), and 20 livers/sex/generation at 750 and 3750 ppm were also examined histopathologically. Additionally, reproductive organs of the animals from all groups 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 any 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 detects depletion of the primordial follicle population, if present. Ten ovarian sections were taken at least 100 μm apart from the inner third of each ovary from ten control and ten high dose F0 and F1 females. Examination included enumeration of the total number of primordial follicles (consisting of early oocytes with a prominent nucleolus through oocytes with a single follicular

epithelial cell layer) from these 20 sections for comparison with control ovaries. This examination also confirms 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 F0 and F1 parental males occurred after the completion of delivery of their litters. At the time of sacrifice of F0 and F1 parental males, the right testis from each male was frozen at approximately -20°C for subsequent enumeration of testicular homogenization-resistant spermatid heads and calculation of daily sperm production and efficiency of daily sperm production (Robb et al., 1978; Sharpe et al., 1995). (The left testis per male was fixed in Bouin's solution for 24 hours and then transferred to 70% ethanol for embedment in glycol methacrylate (GMA) plastic, and subsequent histopathologic examination; see above.) In addition, the right cauda epididymis 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 were evaluated at a later date using fixed sperm (200 per male if possible) stained with Eosin Y. Epididymal sperm motility and number were determined using an HTM TOX-IVOS (Version 10.8 S)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 subsequent histopathologic examination; see below.) Sacrifice of the F0 and F1 maternal animals occurred after F1 and F2 litters had been weaned. Any gross lesions, and weighed organs were retained in fixative from the F1 and F2 weanling examinations, three/sex/litter as well as any unselected male pups with retained nipples, for possible subsequent histopathologic examination with emphasis on reproductive organs, if necessary and appropriate.

Histopathology

Histopathologic evaluation was conducted on the F0 and F1 parental tissues initially from ten randomly-selected animals per sex from the high dose and control groups, as specified below:

- ovaries with oviducts (2)
- vagina
- uterus with cervix
- prostate
- testis (1)
- epididymides with contents (2; one intact and one minus the cauda)
- seminal vesicles with coagulating glands and fluids

- thyroid gland
- liver (final numbers: 20/sex in F0 and F1 control groups, 20/sex/group in F0 and F1 low and mid dose groups)
- other tissues with gross lesions identified as being potentially treatment related

In addition, gross lesions and reproductive tissues from males and/or females, which were unsuccessful breeders or exhibited other evidence of possible reduced fertility, were subjected to histopathologic examination, as required by the testing guidelines. The protocol specified that any of the above organs or tissues showing gross alterations were to be evaluated microscopically in the remaining animals from the high dose and control groups, for which ten/sex were initially examined, and in the other dose groups, as necessary.

A complete gross necropsy (with no organs weighed) and histopathologic examination were conducted for two F0 males dying on study (one each at 750 ppm and at 11,250 ppm) and for two F0 females dying on study (one each at 0 ppm and at 750 ppm). For F1 animals, one male at 750 ppm died on study, one male was euthanized moribund and one male died at 3750 ppm, and two males were euthanized moribund at 11,250 ppm. A complete gross necropsy and histopathologic examination were conducted on these males. No unscheduled deaths occurred in the F1 females.

The fixed (buffered neutral 10% formalin) uteri from any females of the F0 and 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 (and retained in fixative) from all surviving F0 and F1 parental animals at scheduled sacrifice:

ovaries (2)	spleen
uterus	prostate
pituitary (fixed)	testes (2)
adrenal glands (2)	brain
liver	kidneys (2)
thyroid gland	pancreas

seminal vesicles (2; with coagulating glands and their fluids)
epididymides with contents and fluids (2; total and caudal weight for either one or both)

The following organs were weighed from selected F1 and F2 weanlings, up to three/sex/ litter, subjected to sacrifice and necropsy:

brain	spleen	thymus	uterus with cervix and vagina
testes (2)	epididymides (2)	ovaries (2)	

Organ weights were reported as absolute and relative to terminal body weight. The pathology report, with individual and summarized data, is presented in Appendix III.

Reproductive and Offspring Indices

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

Calculation of Standard Reproductive Toxicity Parameters

The calculations used to summarize standard reproductive toxicity parameters for this study are presented in Text Table C.

Text Table B

Reproductive and Offspring Indices

Females:	Mating index (%)	=	$\frac{\text{No. females sperm positive}}{\text{No. females paired}} \times 100$
	Fertility index (%)	=	$\frac{\text{No. females pregnant}}{\text{No. females sperm positive}} \times 100$
	Gestational index (%)	=	$\frac{\text{No. females with live litters}}{\text{No. females pregnant}} \times 100$
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:	Live birth index (%)	=	$\frac{\text{No. live pups at birth}}{\text{Total no. pups born}} \times 100$
	4-Day survival index (%)	=	$\frac{\text{No. pups surviving 4 days (precull)}}{\text{Total no. live pups at birth}} \times 100$
	7-Day survival index (%)	=	$\frac{\text{No. pups surviving 7 days}}{\text{Total no. live pups at 4 days (postcull)}} \times 100$
	14-Day survival index (%)	=	$\frac{\text{No. pups surviving 14 days}}{\text{Total no. live pups at 7 days}} \times 100$
	21-Day survival index (%)	=	$\frac{\text{No. pups surviving 21 days}}{\text{Total no. live pups at 14 days}} \times 100$
	Lactation index (%)	=	$\frac{\text{No. pups surviving 21 days}}{\text{Total no. live pups at 4 days (postcull)}} \times 100$

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

   ((feed weight at beginning of measurement period) - (feed weight at end of measurement period)) / 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 Food Efficiency

   (body weight change for measurement period / ((feed weight at beginning of measurement period) - (feed weight at end of measurement period))) x 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

   (organ weight / sacrifice body weight) x 100

7. Daily Spermatid Production per Testis

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

8. Efficiency of Daily Spermatid Production

   ((number of spermatids in testis / testis weight in grams) / number of days for spermatid cycle)

9. Percent Abnormal Sperm

   100 x ((no. blunt hook + no. banana + no. amorphous + no. pinhead + no. two heads and/or two tails + no. head only) / (no. normal + no. blunt hook + no. banana + no. amorphous + no. pinhead + no. two heads and/or two tails + no. head only))

(continued)

Text Table C (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 x ((no. implantation sites - no. live pups) / no. of implantation sites)

    arcsine (square root ((no. implantation sites - no. live pups) / no. of implantation sites))

11. Stillbirth Index per Dam and Arcsine Root Transformation

    100 x (no. dead pups delivered / total number of pups delivered)

    arcsine (square root (no. dead pups delivered / total number of pups delivered))

12. Live Birth Index per Dam and Arcsine Root Transformation

    100 x (no. live pups delivered / total number of pups delivered)

    arcsine (square root (no. live pups delivered / total number of pups delivered))

13. Four-Day Survival Index per Dam and Arcsine Root Transformation

    100 x (no. pups alive on pnd 4 / no. of pups alive on pnd 0)

    arcsine (square root (no. pups alive on pnd 4 / no. of pups alive on pnd 0))

14. Seven-Day Survival Index per Dam and Arcsine Root Transformation

    100 x (no. pups alive on pnd 7 / no. of pups alive on pnd 4 after culling)

    arcsine (square root (no. pups alive on pnd 7 / no. of pups alive on pnd 4 after culling))

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

    100 x (no. pups alive on pnd 14 / no. of pups alive on pnd 7)

    arcsine (square root (no. pups alive on pnd 14 / no. of pups alive on pnd 7))

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

    100 x (no. pups alive on pnd 21 / no. of pups alive on pnd 14)

    arcsine (square root (no. pups alive on pnd 21 / no. of pups alive on pnd 14))

17. Lactational Index per Dam and Arcsine Root Transformation

    100 x (no. pups alive on pnd 21 / no. of pups alive on pnd 4 after culling)

    arcsine (square root (no. pups alive on pnd 21 / no. of pups alive on pnd 4 after culling))

(continued)

Text Table C (continued)

18. Percent Males per Litter and Arcsine Root Transformation

    100 x (no. males in litter for given pnd / no. sexed in litter for given pnd)

    arcsine (square root (no. males in litter for given pnd / no. sexed in litter for given pnd))

19. Average Fetal Body Weight per Litter

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

20. Average Male Fetal Body Weight per Litter

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

21. Average Female Fetal Body Weight per Litter

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

[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. Treatment groups were compared to the concurrent control group using either parametric ANOVA under the standard assumptions or robust regression method (Zeger and Liang, 1986; Royall, 1986; Huber, 1967) that do not assume homogeneity of variance or normality. The homogeneity of variance assumption was examined via Levene's Test (Levene, 1960), which is much more robust to the underlying distribution of the data than the traditional Bartlett's Test. If Levene's Test indicated lack of homogeneity of variance ($p<0.05$), robust regression methods were used to test all treatment effects. The robust regression methods use variance estimators that make no assumptions regarding homogeneity of variance or normality of the data. They were used to test for overall treatment group differences, followed by

individual tests for exposed vs. control group comparisons (via Wald Chi-Square Tests), if the overall treatment effect was significant. The presence of linear trends was analyzed by GLM procedures for homogenous data or by robust regression methods for nonhomogenous data (SAS Institute Inc., 1989a,b, 1990a,b,c, 1996, 1997). Standard ANOVA methods, as well as Levene's Test, are available in the GLM procedure of SAS® Release 6.12 (SAS Institute Inc., 1997), and the robust regression methods are available in the REGRESS procedure of SUDAAN® Release 7.5.3 (Shah et al., 1997). Both packages are GLP compliant.

If Levene's Test did not reject the hypothesis of homogeneous variances, standard ANOVA techniques were applied for comparing the treatment groups. The GLM procedure in SAS® 6.12 was used to evaluate the overall effect of treatment and, when a significant treatment effect was present, to compare each exposed group to control via Dunnett's Test (Dunnett, 1955, 1964). For the litter-derived percentage data (e.g., periodic pup survival indices), the data were subjected to arcsin of the square root transformation prior to analysis. The transferred data were analyzed using ANOVA was weighted according to litter size. 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 and organ weight parameters, feed consumption, percent males per litter, and anogenital distance.

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. Acquisition of developmental landmarks (e.g., vaginal patency and preputial separation), as well as F2 newborn anogenital distance, were analyzed by Analysis of Covariance (ANCOVA; in addition to ANOVA analysis) using body weight at acquisition or measurement as the covariate. For correlated data (e.g., body and organ weights at necropsy of 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.

A test for statistical outliers (SAS Institute, Inc., 1990b) was performed on parental body weights and feed consumption (in g/day) and on parental and weanling necropsy organ weights. If examination of pertinent study data did not provide a plausible biologically sound reason for inclusion of the data flagged as "outlier," the data were excluded from summarization and analysis and were designated as outliers. If feed consumption data for a given animal for a given observational interval (e.g., sd 0-7, 7-14, 14-21, 21-28, 28-35, etc., during the prebreed exposure period) were designated outliers or "unrealistic" (e.g., negative value), then summarized data encompassing this period (e.g., sd 0-70 for the prebreed exposure period) was not included in this value.

For all statistical tests, the significance limit of 0.05 (one- or two-tailed) was used as the criterion for significance.

Personnel

The evaluation of BBP for reproductive toxicity in CD® (Sprague-Dawley) rats was conducted at RTI, Research Triangle Park, NC, under contract to The European Council for Plasticizers and Intermediates (ECPI). Dr. Robert Barter of Solutia was the Study Monitor from study start until January 5, 2001. Mr. J.H. Butala (consultant to ECPI through Ferro, a member company) was Study Monitor from January 25, 2001 until the end of this contract (see Appendix V, Protocol Amendment No. 4), and Dr. David Cadogan (ECPI) was the Sponsor's Representative.

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 Analyst); Ms. M.V. Cheesborough (Rieth), Ms. E.C. Seiffer, Ms. L.B. Pelletier, and Ms. D.A. Wenzel (Study Team); Ms. V.I. Wilson, Ms. C. N. Beauman, Ms. N.M. Smith, Ms. N.M. Kuney, Ms. M.P. Gower, and Mr. W.P. Ross. 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, Mr. K. Ford and Mr. R.A. Price. Analyses of dosed feed formulations were performed by Ms. D.R. Brine (Analytical Chemistry Task Leader), Mr. G.M. West, Ms. C.B. Harris, and Mr A.K. Khaled. Analyses of the test material were provided by the Sponsor. Animal care was provided by Dr. D.B. Feldman, DVM, ACLAM, Veterinarian, and Mr. F.N. Ali, Manager of RTI Animal Research Facility personnel. Andrological assessments were

performed by Dr. P.A. Fail, Ms. C.D. Sloan, Ms. K.D. Vick, Ms. A.R. Bellafaire, and Mr. T.W. Wiley. Histologic preparations were made by Ms. T-Y. Chang (HT-ASCP), and pathologic evaluation of histologic slides was by Dr. J.C. Seely, DVM, ACVP, EPL, Inc., Research Triangle Park, NC. RTI Quality Assurance personnel were Mr. D.L. Brodish, Manager (to 12/31/01), Ms. D.J. Smith, Manager (from 01/01/02), Ms. C.D. Keller, Ms. D. D. Rowe, Ms. D.B. Wells, Mr. B.L. McCluney, Ms. M.D. Phillips, Ms. E.D. Shinwald, and Ms. P.D. Hall.

The final report was prepared by Dr. R.W. Tyl, with assistance from Ms. C.B. Myers and Ms. M.C. Marr on data compilation and statistical analyses, and by Mr. T.W. Wiley on data entry. Ms. C.B. Myers provided Figures 2 through 18 and text figures B and C. Ms. M.C. Marr was responsible for assistance in writing the final report Materials and Methods, all transfer of custody procedures for histology and pathology, and for archiving the study records. The individual scientist reports were prepared and signed by the author(s) and included as appendices to the final report.

The protocol and six amendments detailing the design and conduct of the study are presented in Appendix V of this final report. The protocol was signed by the Study Director on May 4, 2000. The historical control dataset for reproductive toxicity studies in CD® rats for the performing laboratory is presented in Appendix IV.

Storage of Records

All original data sheets, records, biological specimens, blocks, and slides for the present study will be 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 ECPI.

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. This study was performed in compliance with U.S. EPA Toxic Substances Control Act (TSCA) GLP standards (U.S. EPA, 1989), and the U.S.

EPA OPPTS Testing Guidelines (U.S. EPA, 1998). The RTI Quality Assurance Unit audited all in-life, necropsy, and postnecropsy critical phases and the raw, individual, and summary data for this final report.

The toxicology laboratories at RTI are operated in compliance with TSCA Good Laboratory Practice Standards (U.S. EPA, 1989), 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

Prior to initiation of the study, dosed feed formulations, encompassing the range of dosage concentrations employed in the range-finding study (700-12,000 ppm), were found to be homogeneous and stable for at least 54 days under freezer conditions (approximately -20°C), so formulations were used within the stability limits established and stored frozen. Concurrent with the start of this study, homogeneity and stability were evaluated at 700 and 12,000 ppm (homogeneity and cageside stability for at least 12 days), and frozen storage stability for 54 days was confirmed. All dietary formulations that were used were analyzed at 90.9–107% of target dietary concentrations prior to use, except for the initial formulation for the 11,250 group (mixed on July 5, 2000), which assayed at 86.5% of target. It was remixed on July 11, 2000, assayed at 107%, and used. No BBP was detected in the control feed formulations, with an estimated detection limit of 35 ppm (Table 1 and Appendix I).

Fate of F0 Animals

One F0 male each at 750 and 11,250 ppm died on study. Therefore, there were 30, 29, 30, and 29 F0 males that were evaluated at scheduled sacrifice at 0, 750, 3750, and 11,250 ppm, respectively. One F0 female each at 0 and 750 ppm died on study. Therefore, there were 29, 29, 30, and 30 F0 females that were evaluated at scheduled sacrifice at 0, 750, 3750, and 11,250 ppm, respectively. None of the F0 deaths were considered treatment related (Table 2).

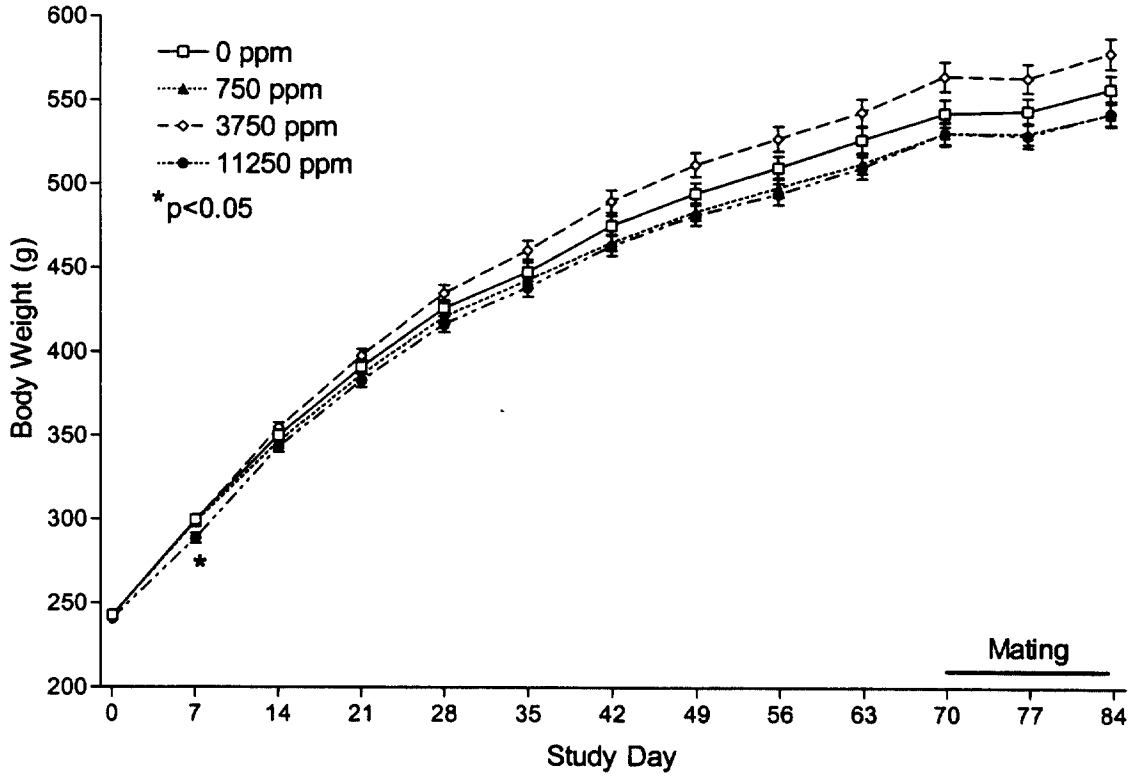
F0 Prebreed

F0 Males

F0 male body weights were significantly reduced only on study day (sd) 7 at 11,250 ppm (Table 3 and Figure 1). The male body weight change was significantly reduced for sd 0-7 at 11,250 ppm, and significantly increased for sd 7-14 at 3750 ppm and for sd 63-70 at 3750 and 11,250 ppm. There were no significant differences among groups for body weight change for the entire prebreed period (sd 0-70) at any dietary dose (Table 3).

Figure 1

F_0 Male Body Weights During the Prebreed and Mating Periods



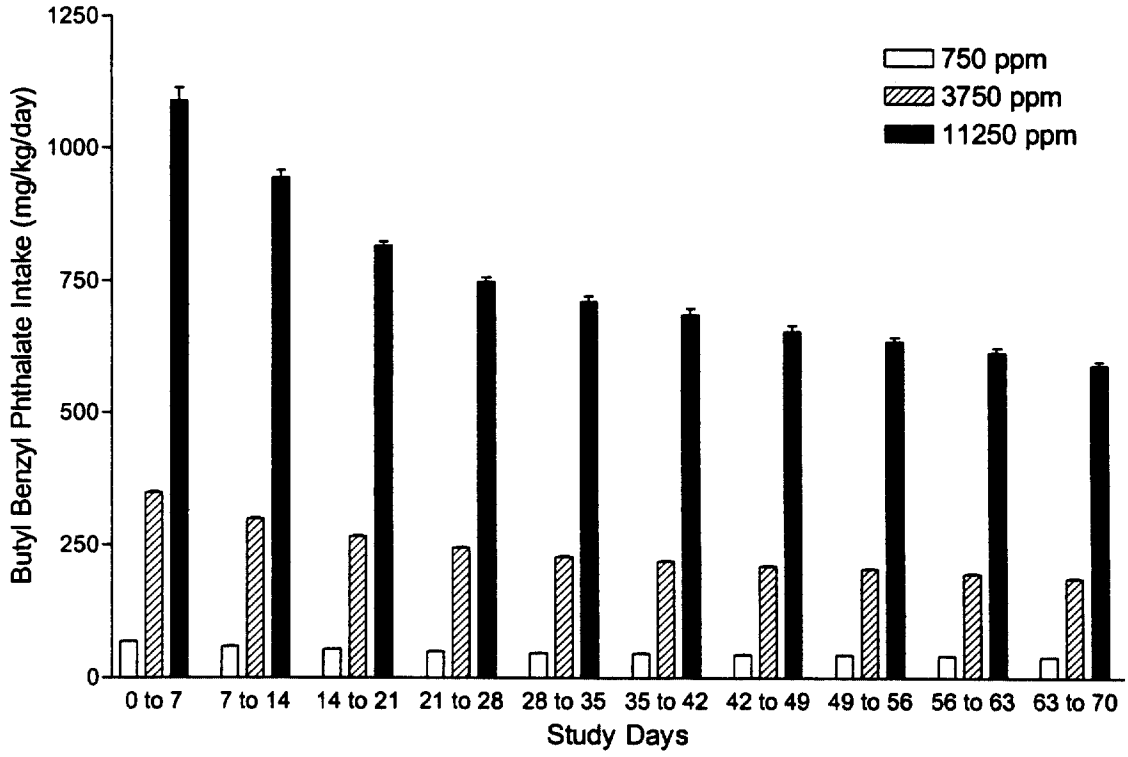
There were no significant effects of treatment on F0 male feed consumption expressed as g/day. When the data were expressed as g/kg body weight/day, feed consumption was significantly increased at 11,250 ppm for sd 0-7 and 7-14, and significantly decreased at 3750 ppm for sd 42-49. Percent food efficiency was variable, with increases and decreases observed at 3750 and 11,250 ppm. Intake of BBP as mg/kg/day exhibited the expected incremental increases across groups, based on dietary concentrations of BBP: 3750 ppm is five times 750

ppm, and the BBP intake was approximately five-fold higher at 3750 ppm than at 750 ppm, 11,250 ppm is three times 3750 ppm, and the BBP intake was approximately three-fold higher at 11,250 ppm than at 3750 ppm. BBP intake also exhibited the expected decreases within groups over time, as the feed consumption and dietary concentrations remained constant, and the body weights increased over two-fold during the ten-week prebreed period (Table 4 and Figure 2).

Clinical observations of F0 males during this period were unremarkable. None of the findings exhibited a dose-response pattern of incidence or severity (Table 5).

Figure 2

F_0 Male Butyl Benzyl Phthalate Intake During the Prebreed Period



F0 Females

In contrast to the F0 males, F0 female body weights were significantly reduced at 11,250 ppm from sd 7 through sd 70 (Table 6 and Figure 3). Body weight change was significantly reduced at 11,250 ppm for sd 0-7, 42-49, and 0-70. Body weight change was significantly increased at 750 ppm for sd 21-28 and at 3750 and 11,250 ppm for sd 49-56 (Table 6).

There were no significant effects of treatment on F0 female feed consumption when expressed as g/day or g/kg/day. Food efficiency was significantly reduced at 11,250 ppm for sd 0-7 and 42-49, and significantly increased at 750 ppm for sd 21-28 and at 3750 and 11,250 ppm for sd 49-56. Intake of BBP in F0 females as mg/kg/day exhibited the same patterns as in the F0 males, with appropriate incremental increases across dietary doses and appropriate reductions over time within dietary doses, based on increasing body weights (Table 7 and Figure 4).

Clinical observations of F0 females indicated only that alopecia increased in all BBP-exposed groups, with no dose response pattern. None of the other findings appeared treatment related (Table 8).

There were no effects of treatment on the percentage of females cycling, percentage of females with an abnormal cycle, or in cycle length in days with mean values of 4.39, 4.58, 4.81, and 4.39 days at 0, 750, 3750, and 11,250 ppm, respectively (Table 9).

Figure 3

F_0 Female Body Weights During the Prebreed Period

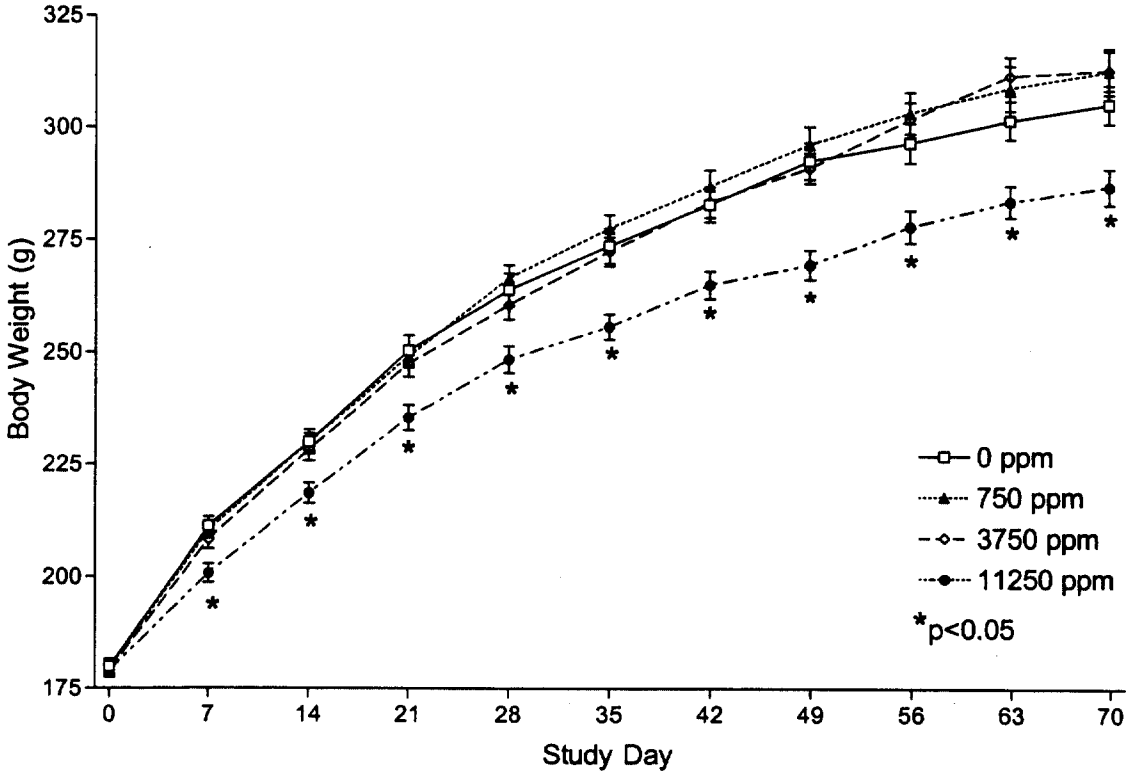
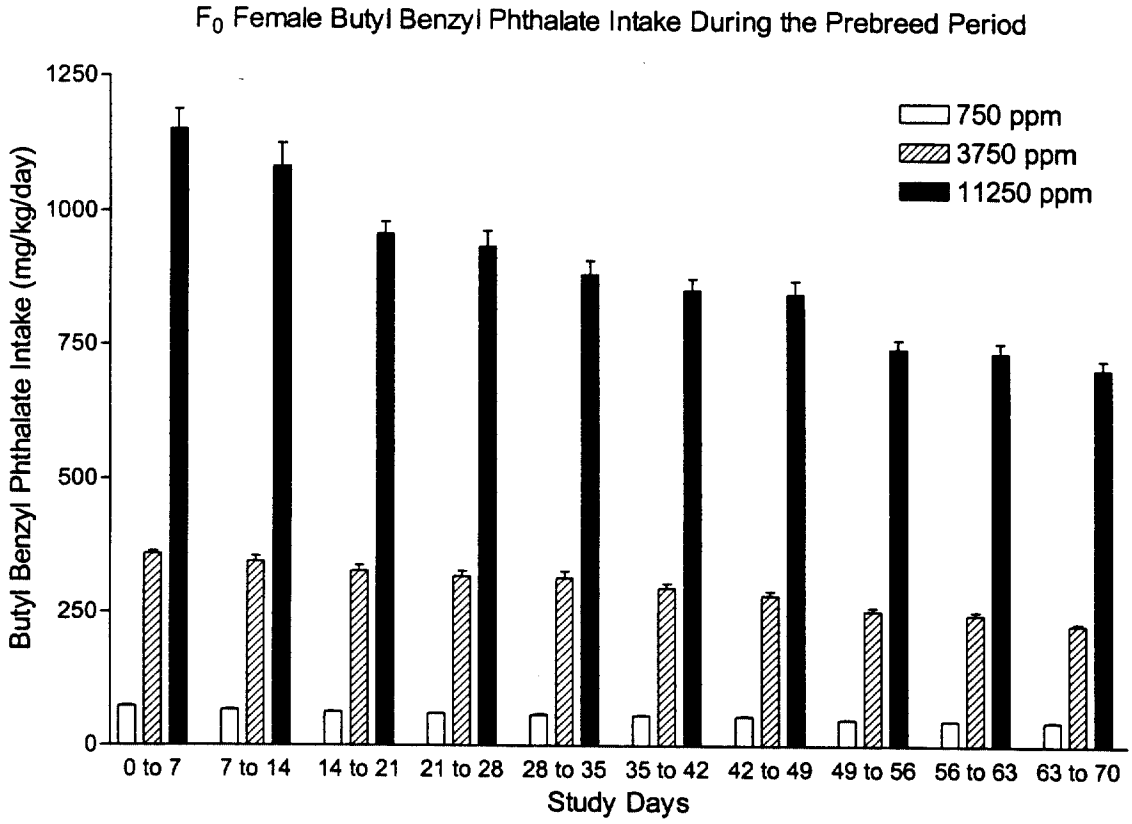


Figure 4



F0 Gestation

F0 maternal body weights during gestation were significantly reduced at 11,250 ppm for gd 7, 14, and 20 (Table 10 and Figure 5). Maternal gestational weight change was significantly reduced at 11,250 ppm for gd 7-14. There were no significant effects of treatment on body weight change for the entire gestational period (gd 0-20) (Table 10).

F0 maternal gestational feed consumption as g/day was unaffected at any BBP dietary dose. When feed consumption was expressed as g/kg/day, it was significantly increased at 11,250 ppm for sd 14-20 and unaffected at all other doses and intervals. Feed efficiency was significantly decreased at 750 and 11,250 ppm for gd 7-14. BBP intake during gestation (gd 0-20) averaged 0, 49.3, 255.0, and 782.2 mg/kg/day at 0, 750, 3750, and 11,250 ppm, respectively (Table 11 and Figure 6).

Clinical observations during gestation were noteworthy only for the incidence of alopecia, which was increased in all BBP-exposed groups and highest at 11,250 ppm (Table 12).

Figure 5

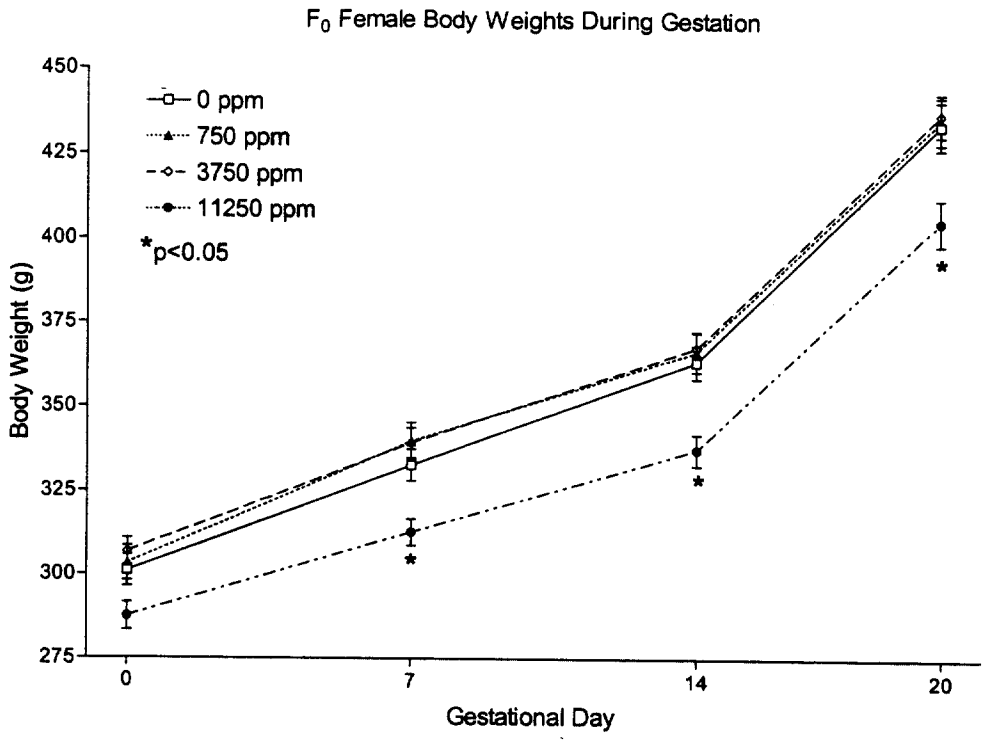
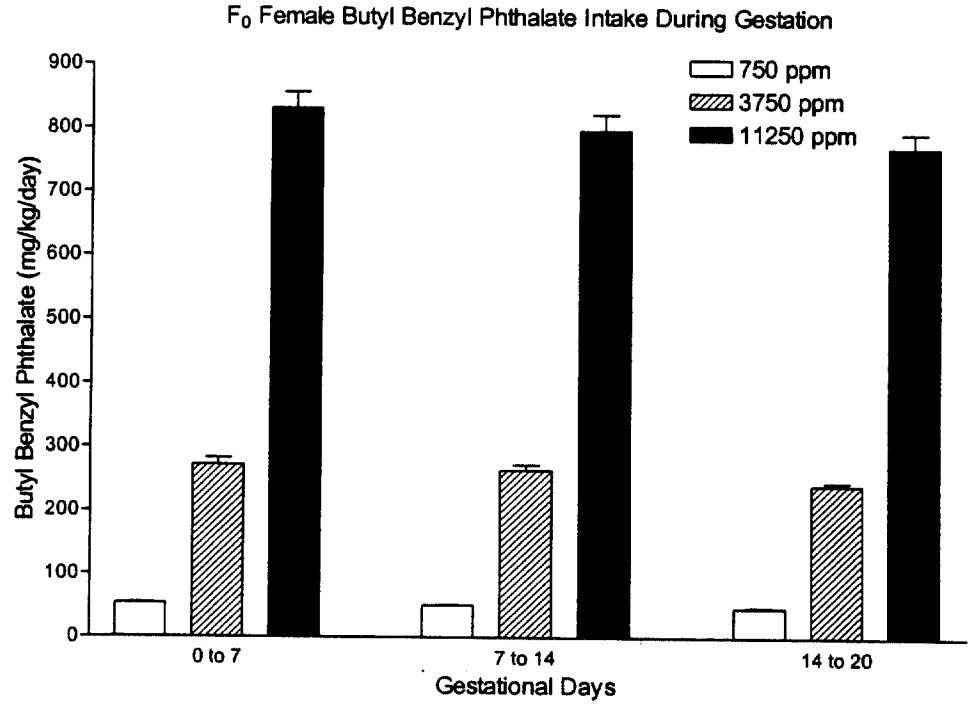


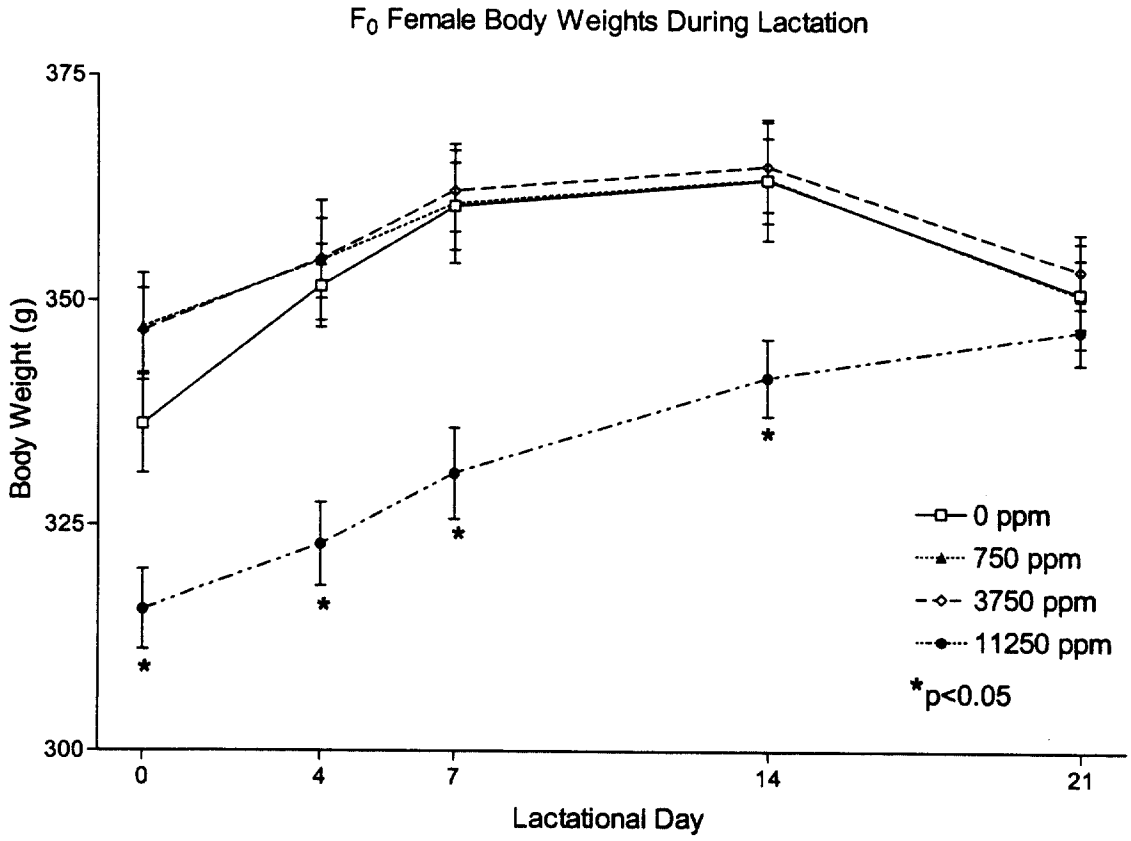
Figure 6



F0 Lactation

F0 maternal lactational body weights were significantly reduced on pnd 0, 4, 7, and 14 (but not on pnd 21) at 11,250 ppm. There were no significant effects at 750 or 3750 ppm (Table 13 and Figure 7). Maternal lactational body weight change was significantly increased at 11,250 ppm for pnd 14-21 and 0-21. It was unaffected at all other dose levels and intervals (Table 13).

Figure 7



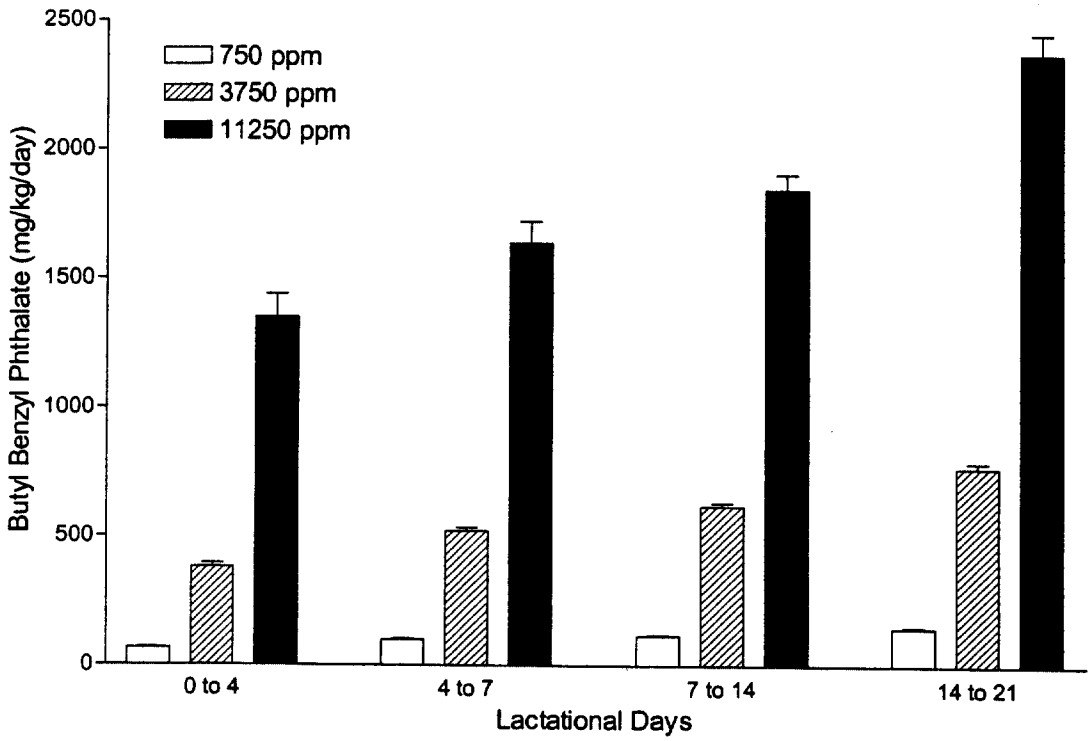
F0 maternal lactational feed consumption as g/day was unaffected at all BBP dietary doses. Feed consumption as g/kg/day was significantly increased at 11,250 ppm for pnd 0-4. There were no other significant effects. Food efficiency was increased at 11,250 ppm for pnd 14-21 and 0-21. It was unaffected at all other dietary levels and for all other intervals. Mean intake of BBP for pnd 0-21 was 0, 116.6, 610.7, and 1905.2 mg/kg/day at 0, 750, 3750, and 11,250 ppm, respectively. The "maternal" intake values were confounded by the pups self-

feeding, beginning on pnd 10 during the second week of postnatal life (unpublished observations), with large increases in apparent maternal intake in all BBP groups for pnd 14-21 (Table 14 and Figure 8).

Maternal clinical observations of note during lactation were limited to alopecia, the incidence of which was increased in all BBP-exposed groups and highest at 11,250 ppm (Table 15).

Figure 8

F_0 Female Butyl Benzyl Phthalate Intake During Lactation



F0 Reproductive Indices

There were no effects of dietary exposure to BBP on any F0 reproductive indices during the production of F1 offspring. Mating, fertility, pregnancy, and gestational indices were all equivalent across groups. Gestational length was equivalent across all groups, as was precoital interval (time from pairing to copulation in days). There were also no differences across groups for the number of total implantation sites per litter, percent postimplantation loss per litter, or number of total, dead, and live pups per litter at birth (pnd 0) (Table 16).

F1 Lactation

There were 26, 27, 28, and 27 live litters on pnd 0 at 0, 750, 3750, and 11,250 ppm, respectively. One dam (no. 78) at 750 ppm was found dead on pnd 17, and her litter was euthanized. This was clearly not treatment or dose related. Live birth and stillbirth indices were unaffected, as were all survival indices during lactation (4-, 7-, 14-, 21-day and lactational [pnd 4, postcull – pnd 21] indices) (Table 17).

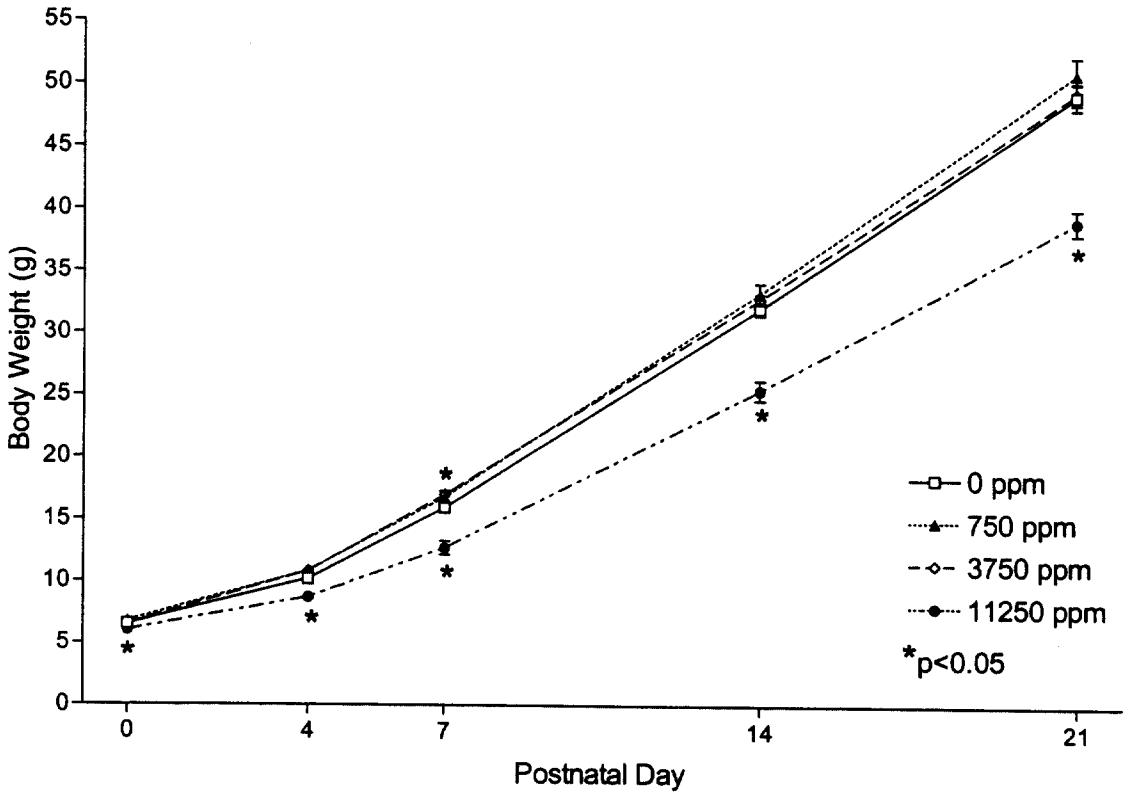
The mean number of live pups per litter throughout lactation was unaffected. Mean F1 male anogenital distance per litter on pnd 0 was significantly reduced (shortened) at 3750 and 11,250 ppm, with a dose-related pattern, as absolute values, or when adjusted for individual body weights (by analysis of covariaince, ANCOVA with body weight as the covariate). Mean F1 female anogenital distance per litter on pnd 0 (absolute or adjusted) was unaffected at any BBP dietary level. Mean F1 pup body weights per litter (sexes combined or separately) were significantly reduced at 11,250 ppm on pnd 0, 4, 7, 14, and 21. Total, male, and female body weights per litter were significantly increased at 3750 ppm on pnd 7. No other intervals or BBP dose levels were affected (Table 17 and Figure 9). The sex ratio (percent male pups/litter) was unaffected at any dose for any interval (Table 17).

Examination of F1 pups on pnd 11-13, for the presence of ventral areolae and/or nipples, indicated that there were no nipples in any males at 0 ppm (with no nipples ever observed to date in control male preweanling CD® (SD) rats in the performing laboratory). There were also no treatment-related increases in the number of areolae per male at 0, 750, or 3750 ppm (with the control incidences of areolae in preweanling CD® (SD) males 0-2.3% in the performing laboratory; Appendix IV). The incidence of nipples per male was significantly increased at 11,250 ppm, expressed as number of nipples per male ($p<0.001$) or as the percentage of males with one or more nipples ($p<0.001$). There were also 4.10% males at 750 ppm with nipples, not significantly different from the control group value (0.0%). The incidence of areolae per male was also significantly increased at 11,250 ppm, expressed as the number of areolae per male ($p<0.01$) and as percent of males with one or more areolae ($p<0.001$) (Table 17).

F1 pup clinical observations during lactation indicated that F1 pup mortality for pnd 0-4 was 24, 21, 15, and 41 pups, and for pnd 5-21 was 1, 3 (plus four euthanized when their dam died), 2, and 7 at 0, 750, 3750, and 11,250 ppm, respectively (Table 18).

Figure 9

F_1 Pup Body Weights During Lactation



Necropsy findings of F1 pups found dead or euthanized moribund on pnd 0-21 included the usual findings: pups which died on pnd 0 exhibited open (fetal state) or closed ductus arteriosus (postnatal state), no air (fetal state) or air (postnatal state) in lungs, no or little milk in stomach, and autolysis of abdominal and/or thoracic organs. Pups that died or were sacrificed moribund on pnd 2-21 usually exhibited closed ductus arteriosus, air in lungs, milk in stomach, autolysis of organs, and varying degrees of cannibalization by the dam. Culled pups on pnd 4, which were necropsied (per protocol), typically exhibited distended ureter(s) and full urinary bladder. However, two male pups at 11,250 ppm, necropsied on pnd 4, exhibited displaced testis (or testes), not typical of control findings but indicative of *in utero* exposure to an anti-androgen (Table 19).

Postnatal Day 21 F1 Pup Necropsy

The number of F1 males necropsied on pnd 21 at weaning (up to three/sex/litter and any additional males with retained nipples on pnd 11-13) were 68, 68, 78, and 76 at 0, 750, 3750, and 11,250 ppm, respectively. F1 male body weight was significantly reduced at 11,250 ppm. All organs weighed exhibited significantly reduced absolute weights at 11,250 ppm: thymus, spleen, brain, paired testes, and paired epididymides. At 3750 ppm, absolute brain and paired testes weights were significantly increased. Relative (organ weight relative to terminal body weight) spleen and paired testes weights were significantly reduced at 11,250 ppm. Relative brain weight at 11,250 ppm and relative paired testes weight at 3750 ppm were both significantly increased versus the control values (Table 20).

The number of F1 females necropsied on pnd 21 at weaning (up to three/sex/litter) were 73, 68, 81, and 67 at 0, 750, 3750, and 11,250 ppm, respectively. F1 female body weight was significantly reduced at 11,250 ppm. All organs weighed exhibited significantly reduced absolute weights at 11,250 ppm: thymus, spleen, brain, paired ovaries, and uterus. Relative spleen weight was also reduced at 11,250 ppm, and relative brain weight was significantly increased at 11,250 ppm. Relative thymus, paired ovaries, and uterus weights were unaffected (Table 20).

Necropsy findings of F1 weanling males at 11,250 ppm included abnormal epididymides: caput and/or corpus and/or cauda missing, caput and/or corpus reduced in size, entire epididymis missing (right, left, or bilateral) or reduced in size (right, left, or bilateral), and abnormal testes: missing, reduced in size, and/or undescended. In addition, only at 11,250 ppm, nipples were present in F1 males: two to six nipples per male (12 is the usual count for females) in a total of 20 male pups (Table 21).

Other necropsy observations (not treatment related) included renal hydronephrosis in one F1 female and one F1 male at 0 ppm, in two males at 750 ppm, and in two males at 11,250 ppm; sacral vertebra missing in one F1 female at 750 ppm; tail missing in one F1 male at 11,250 ppm; and one F1 male at 750 ppm with missing right eye (Table 21).

F0 Parental Holding Period

Males

F0 males were held after mating, with dietary exposures continuing. There were no effects of treatment on F0 male body weights or weight change (Table 22), on feed consumption in g/day, or food efficiency in any group (Table 23). Feed consumption in g/kg/day was slightly ($p<0.05$) reduced at 3750 ppm for sd 98-105. Intake of BBP was 38.01-35.79 at 750 ppm, 183.37-174.39 at 3750 ppm, and 573.50-549.47 mg/kg/day at 11,250 ppm over the three-week holding period (Table 23). There were no treatment- or dose-related clinical observations in F0 males during this period (Table 24).

Females

F0 females that were not pregnant, and/or did not deliver live litters, or whose litters died prior to pnd 21, are included here. The data were not analyzed statistically. As with the F0 males, F0 females exhibited no differences among groups in body weights or body weight change (Table 25), feed consumption in g/day or g/kg/day, or in food efficiency (Table 26). With a limited number of females (0-3) per group, BBP intake was 47.99-40.49 at 750 ppm, 247.32-226.25 at 3750 ppm, and 665.88-794.44 mg/kg/day at 11,250 ppm over the four-week holding period (Table 26). There were no treatment- or dose-related clinical observations in F0 females during this period. Findings were limited to alopecia in one female each at 0 and 11,250 ppm (Table 27).

Necropsy of F0 Parental Animals

Males

At scheduled sacrifice, mean body weights were equivalent across all groups. Absolute weights of the brain, pituitary, thyroid, pancreas, spleen, paired adrenals, paired testes, paired epididymides, prostate, and paired seminal vesicles with coagulating glands were all equivalent across groups. Absolute liver weight was significantly increased at 11,250 ppm, and absolute paired kidney weights were significantly increased at 3750 and 11,250 ppm. The relative weights of the brain, thyroid, pancreas, spleen, paired adrenals, paired testes, paired epididymides, prostate, and seminal vesicles with coagulating glands were all equivalent across all groups. Relative pituitary weight was significantly reduced at 3750 ppm, and relative liver and paired kidney weights were both significantly increased at 11,250 ppm. For the F0 male

parents, there were no effects on percent motile or progressively motile sperm, epididymal sperm concentration, percent abnormal epididymal sperm (2.12, 2.17, 2.33, and 2.11% at 0, 750, 3750, and 11,250 ppm, respectively), testicular homogenization-resistant spermatid head counts, or efficiency of daily sperm production. Daily sperm production, per se, was significantly increased at 750 ($p<0.05$) and 3750 ($p<0.05$) ppm, with no significant effects at 11,250 ppm (Table 28).

Gross necropsy findings of F0 parental males exhibited no treatment- or dose-related pattern of incidence or severity in males at scheduled sacrifice or at unscheduled sacrifice for those that died on study or were sacrificed moribund. Treatment-related histopathologic findings were limited to the liver. "Cytologic Alteration, Hepatocyte" was observed in 8 of 11 livers at 11,250 ppm and 0 of 20-21 at 0, 750, and 3750 ppm. There were no treatment-related histopathologic findings in any other F0 male organ, including the testis and epididymis (Table 29).

Females

At scheduled sacrifice, mean body weights of F0 females were equivalent across all groups. Absolute weights of the brain, pituitary, thyroid, pancreas, spleen, and paired adrenals were equivalent across all groups. Absolute liver weight was significantly increased at 11,250 ppm, and absolute paired kidney weights were significantly increased at 3750 ppm. Absolute paired ovarian and uterine weights were significantly decreased at 11,250 ppm. The relative weights of the brain, pituitary, thyroid, pancreas, spleen, and paired adrenals were equivalent across all groups. Relative liver weight was significantly increased at 11,250 ppm, and relative paired kidney weights were significantly increased at 3750 and 11,250 ppm. Relative paired ovarian and uterine weights were significantly reduced at 11,250 ppm. Paired ovarian primordial follicle counts from ten F0 females each at 0 and 11,250 ppm were equivalent between the groups. There were no significant differences among F0 females in any groups as to the stage of the estrous cycle at necropsy (Table 30).

Gross necropsy findings of F0 parental females included only the incidence of alopecia as possibly treatment and/or dose related, with increased incidences at 3750 and 11,250 ppm in females at scheduled sacrifice. Gross findings of F0 females at unscheduled sacrifice were limited to dams at 0 and 750 ppm. These were considered neither treatment or dose related.

Treatment-related histopathologic findings were limited to the liver. "Cytologic Alteration, Hepatocyte" was observed in 9 of 10 female livers examined at 11,250 ppm, in 2 of 20 examined at 3750 ppm, in 0 of 21 at 750 ppm, and in 1 of 21 at 0 ppm (Table 31).

Fate of F1 Postweanling Offspring

One F1 male at 3750 ppm was euthanized moribund during prebreed, and one F1 male each at 750 and 3750 ppm and two males at 11,250 ppm were found dead or euthanized moribund during mating. Therefore, 30, 29, 28, and 28 F1 males survived to scheduled sacrifice at 0, 750, 3750, and 11,250 ppm, respectively. No F1 females died or were sacrificed moribund, so 30 females per group for all groups survived to scheduled sacrifice (Table 32).

F1 Acquisition of Puberty

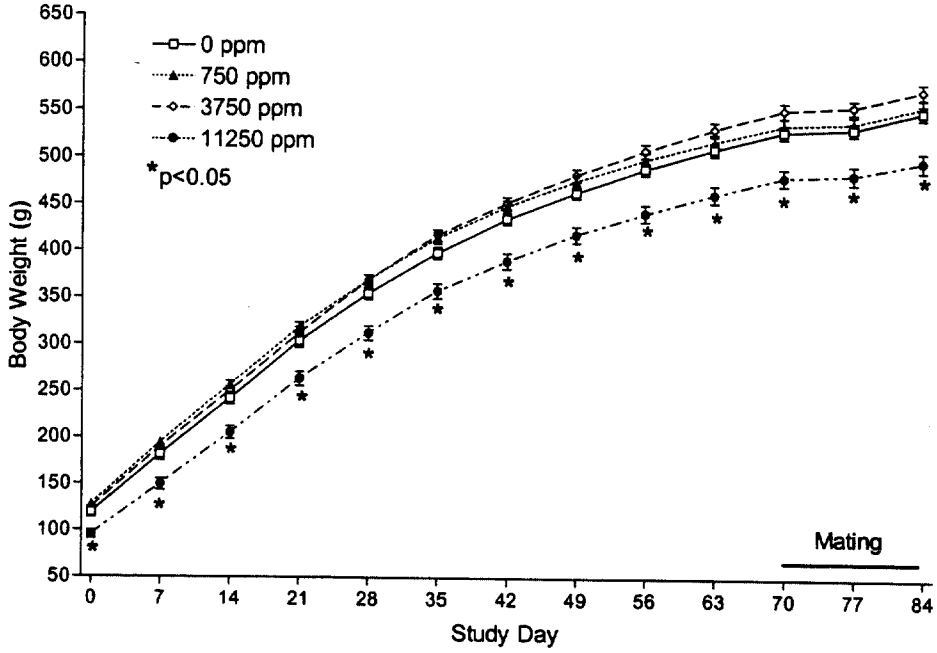
During the F1 prebreed exposure period, 30 F1 females and 30 F1 males per group were assessed for acquisition of vaginal patency (VP; opening) or preputial separation (PPS), respectively. The time in days at acquisition of both VP and PPS was significantly delayed ($p<0.001$; the animals were older at acquisition of puberty) at 11,250 ppm, when the data were expressed as absolute age of acquisition in days or as age of acquisition adjusted for body weight (by analysis of covariance, ANCOVA, with body weight at acquisition as the covariate). The body weights of both the F1 females and males at acquisition were unaffected in any group (Table 33).

F1 Males

F1 male body weights were significantly reduced only at 11,250 ppm throughout the entire prebreed and mating period (through sd 84) (Table 34 and Figure 10). Body weight change was significantly reduced only at 11,250 ppm for sd 0-7 and 7-14 and for the entire prebreed period (sd 0-70) (Table 34). F1 male feed consumption, expressed as g/day, was significantly reduced at 11,250 ppm for sd 0 to 7 (week 1), 7 to 14 (week 2), 14 to 21 (week 3) , 21 to 28 (week 4), 28 to 35 (week 5), 35 to 42 (week 6), and 56 to 63 (week 9), as well as for the entire prebreed period (sd 0 to 70) (Table 35). When expressed as g/kg/day, there were significant increases in feed consumption at 11,250 ppm for sd 7 to 14, 21 to 28, 42 to 49, and for the entire prebreed period (0-70) at 11,250 ppm. Feed efficiency was significantly increased at 11,250 ppm for gd 28-35 and unaffected for any other dose and interval (Table 35).

Figure 10

F_1 Male Body Weights During the Prebreed and Mating Periods

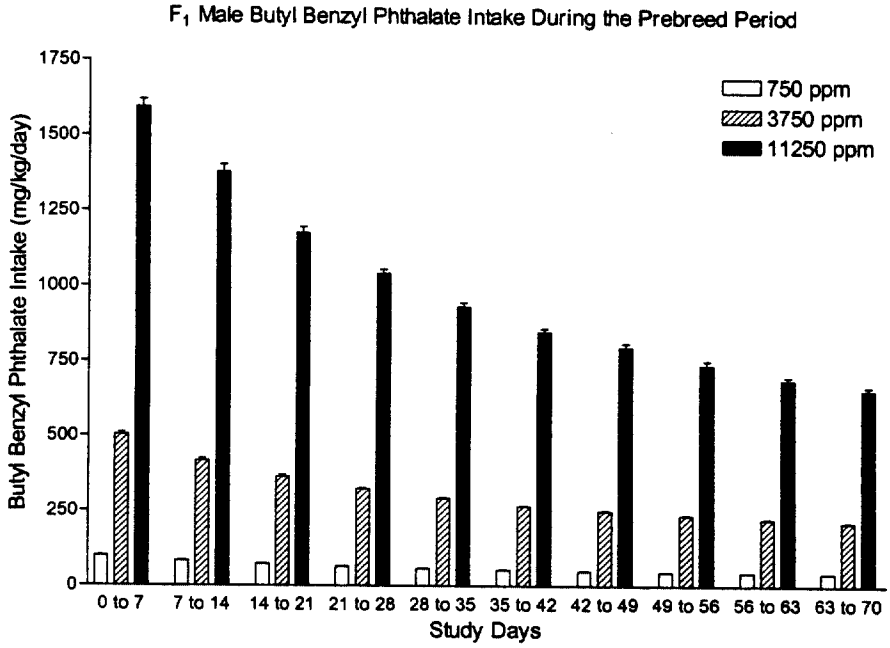


BBP intake, expressed as mg/kg/day, exhibited the expected increases across groups, averaging approximately 56.64, 282.48, and 891.83 mg/kg/day at 750, 3750, and 11,250 ppm, respectively, for the entire prebreed period (sd 0–70). F1 male BBP intake also exhibited the expected decreases over time within groups: 99.32 (first week) – 42.14 (last week) mg/kg/day at 750 ppm, 505.10 – 210.98 mg/kg/day at 3750 ppm, and 1594.35 – 655.70 mg/kg/day at 11,250 ppm (Table 35 and Figure 11).

Clinical observations of the F1 males during the prebreed period consisted of alopecia and chromodacryorrhea seen in all groups. One male at 3750 ppm was emaciated on sd 19, 20, and 21 and had abnormal positioning of both rear legs, and was subsequently euthanized moribund on sd 21. One male at 11,250 ppm was found to have a palpable abdominal mass on sd 75, with blood coming from penis, and was therefore euthanized. Another male at 11,250 ppm (also on sd 75) was noted to have diarrhea, was pale, and prone. One male at 11,250 ppm was slightly dehydrated on sd -6 during the prebreed holding period, and four males at 11,250 ppm were noted as very small during the prebreed holding period. Nipples were present on four males at 11,250 ppm, and two males exhibited hypospadias at 11,250 ppm. Other clinical signs noted in high dose males included pale appearance (one male), pale paws (one male),

piloerection (one male), and rust-colored fur (one male). Rough coat and sore(s) were noted in two different males at 3750 ppm during the prebreed and mating period (Table 36).

Figure 11



F1 Females

F1 weekly body weights were significantly reduced at 11,250 ppm for all ten weeks of prebreed. F1 female body weight change was significantly reduced at 11,250 ppm only for sd 28 to 35. There was no effect on female F1 body weights or weight change for any other time period or dose during the prebreed period (Table 37 and Figure 12).

F1 female feed consumption values, expressed as g/day, were significantly increased at 750 ppm for sd 0-7 and 7-14 but for no other time period or dose. When feed consumption was expressed as g/kg/day, there were significant increases in feed consumption at 11,250 ppm for sd 0-7, 7-14, 56-63, 63-70, and for the entire prebreed period (sd 0-70). Percent food efficiency was significantly increased for sd 35-42 and decreased for sd 28-35 at 11,250 ppm (Table 38).

BBP intake of the F1 females during the ten-week prebreed period was approximately 65.24, 316.79, and 1048.15 mg/kg/day at 750, 3750, and 11,250 ppm, respectively. BBP exhibited the expected decreases over time within groups: 98.93 (week 1) – 49.83 (week 10)

mg/kg/day at 750 ppm, 499.11 – 244.54 mg/kg/day at 3750 ppm, and 1622.04 – 851.05 mg/kg/day at 11,250 ppm (Table 38 and Figure 13).

Figure 12

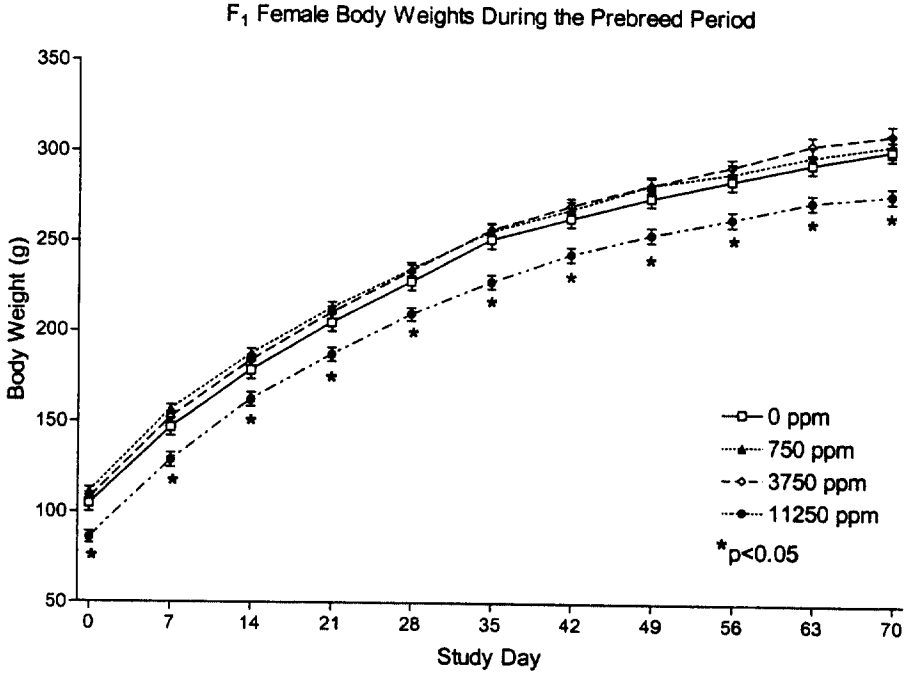
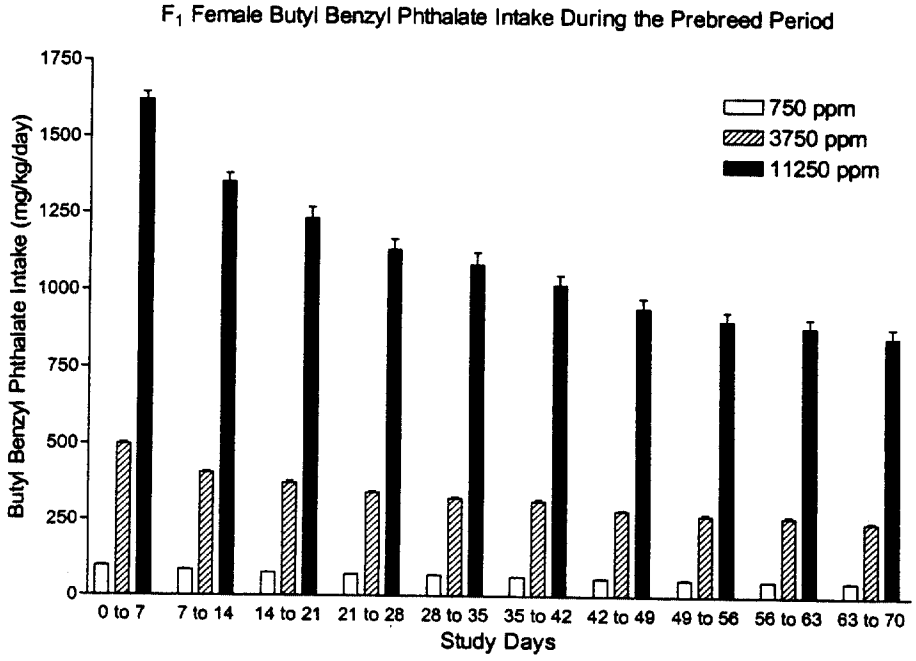


Figure 13



Clinical observations of the F1 females during the prebreed and mating period included alopecia in one, three, and two females at 0, 3750, and 11,250 ppm, respectively, and chromodacryorrhea in one and four females at 0 and 3750 ppm, respectively. Other observations included red ears (two females at 750 ppm and five at 11,250 ppm), rust-colored fur in two females at 11,250 ppm, scabs and sores in one female at 750 ppm and two at 3750 ppm, and one female at 3750 ppm with a shoulder wound. One female at 3750 ppm had malocclusion which required trimming the teeth. One female at 3750 ppm had dried blood present at the urethral opening, one female had torn skin with abrasion at 750 ppm, and one female at 11,250 ppm had blood present in her vaginal smear. Three females at 11,250 ppm were noted as small during the holding period or early prebreed period and were given water dishes (Table 39).

Vaginal cyclicity was evaluated for F1 females for 21 consecutive days during the last three weeks of the prebreed period. One female each in the 750 and 3750 ppm groups was not cycling. Five, two, nine and two females at 0, 750, 3750, and 11,250 ppm, respectively, had abnormal cycles. Cycle length was equivalent across all groups, with a mean duration of 4.38, 4.27, 4.75, and 4.55 days at 0, 750, 3750, and 11,250 ppm, respectively (Table 40).

Gestation – F1 Females for F2 Litters

The number of females that were detected sperm positive and confirmed pregnant were 28, 28, 24, and 16 at 0, 750, 3750, and 11,250 ppm, respectively. F1 maternal body weights were significantly reduced at 11,250 ppm for gd 7, 14, and 20. F1 maternal body weight change was significantly reduced at 11,250 ppm for gd 0-7, 7-14, 14-20, and for the entire gestational period (gd 0-20), and significantly increased at 750 ppm for gd 0-7 (Table 41 and Figure 14).

Maternal gestational feed consumption, expressed as g/day, was not affected for any measurement period during gestation or for gd 0-20. When expressed as g/kg/day, there was a significant increase for gd 0-7 and 0-20 at 11,250 ppm and at 750 ppm for gd 0-7. Percent food efficiency was significantly decreased at 11,250 ppm for gd 0-7, 7-14, and 0-20. Maternal gestational intake of BBP (gd 0-20) was approximately 51.24, 247.36, and 821.13 mg/kg/day for 750, 3750, and 11,250 ppm, respectively (Table 42 and Figure 15).

Figure 14

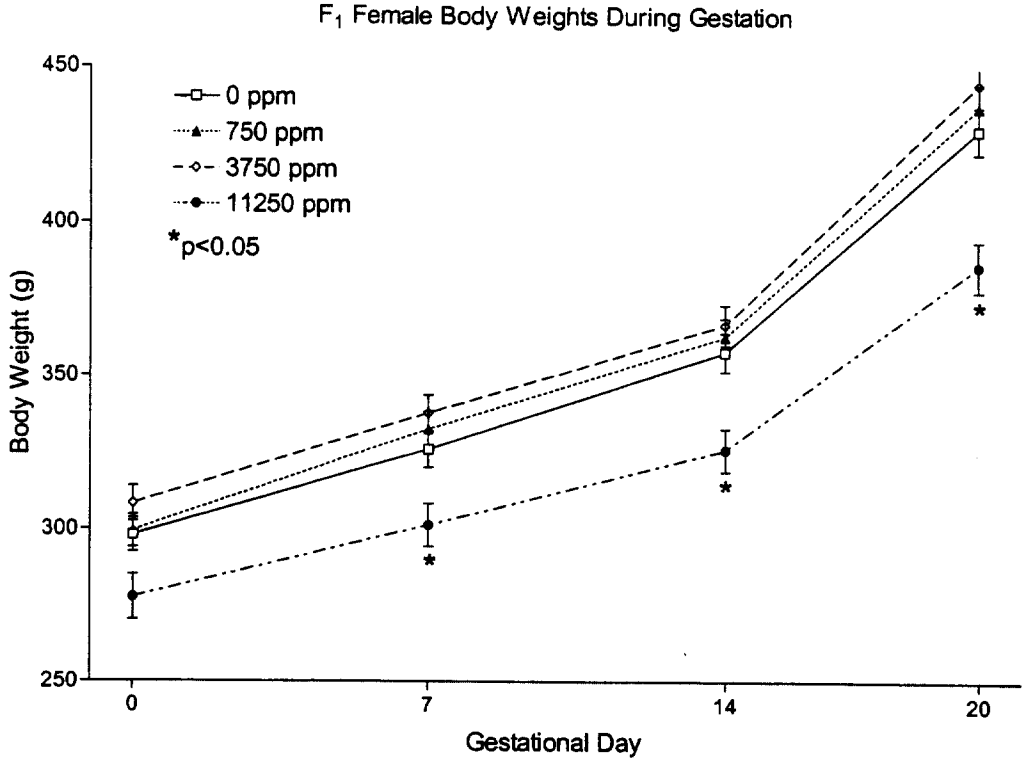
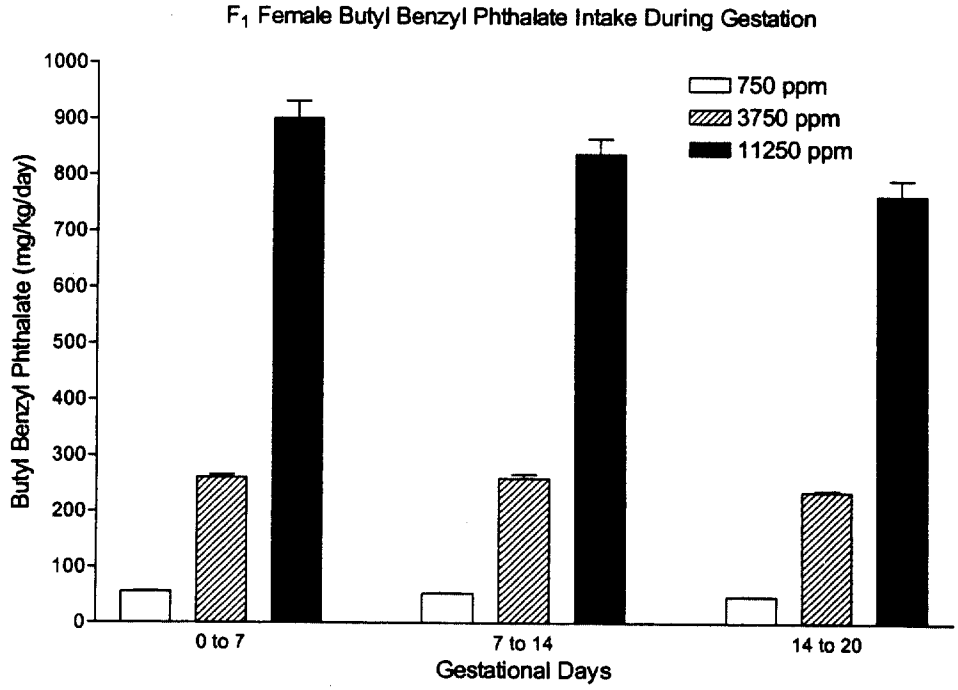


Figure 15



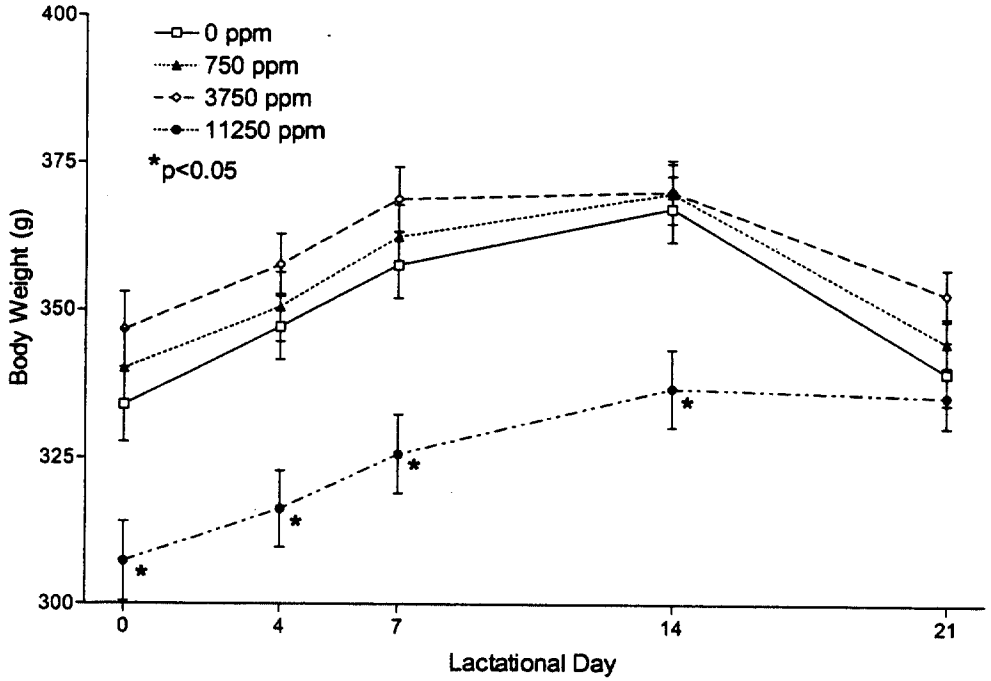
Clinical observations of F1 dams during gestation included alopecia in one, five, and three dams in the 0, 3750, and 11,250 ppm groups, respectively. Chromodacryorrhea was seen in two dams each at 0, 750, 3750, and 11,250 ppm. Red ears were seen in two dams each at 750 and 11,250 ppm. Rust-colored fur was seen in one dam at 3750 ppm and in two dams at 11,250 ppm. One dam at 11,250 ppm exhibited a scab, and one dam at 3750 ppm had her teeth trimmed (Table 43).

Lactation- F1 Females for F2 Litters

The number of females which were confirmed pregnant and delivered live litters (all confirmed pregnant female delivered live litters, and some females were pregnant that were not detected sperm positive or copulation plug positive) were 29, 28, 26, and 17 at 0, 750, 3750, and 11,250 ppm, respectively. Maternal lactational body weights (Table 44 and Figure 16) were statistically significantly reduced at pnd 0, 4, 7, and 14 (but not pnd 21) at 11,250 ppm. Maternal lactational body weight change was significantly increased for pnd 14-21 and for the entire lactation period (pnd 0-21) at 11,250 ppm (Table 44).

Figure 16

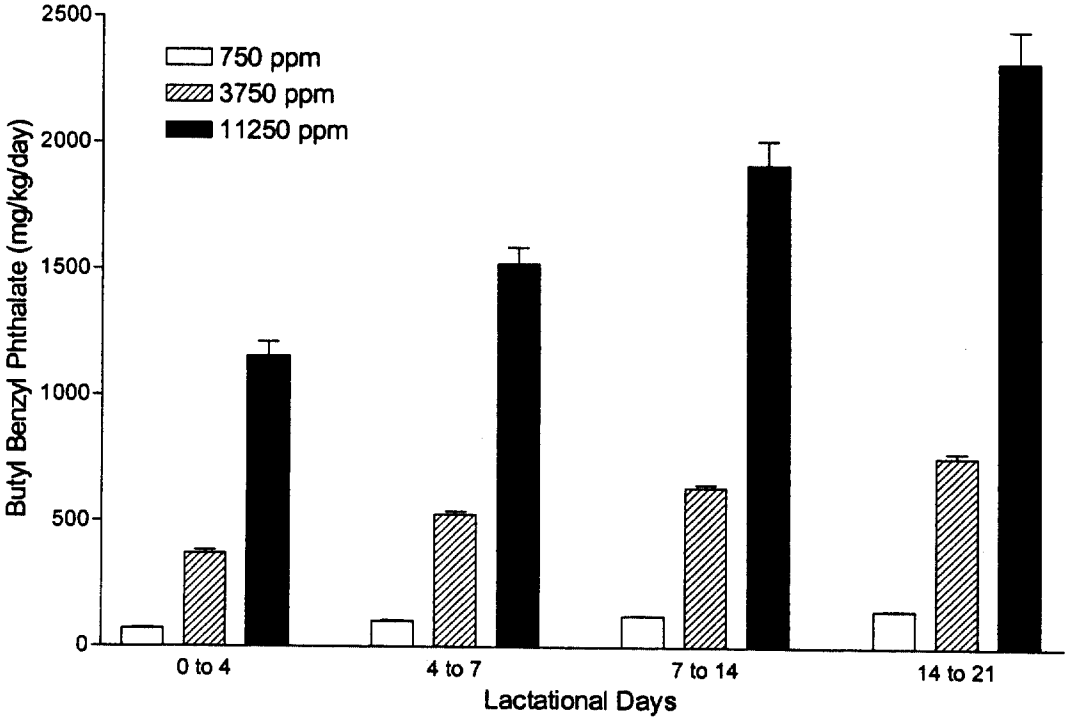
F_1 Female Body Weights During Lactation



Maternal lactational feed consumption as g/day was significantly reduced at 11,250 ppm for pnd 4-7. When expressed as g/kg/day, there was no difference for any time interval for any group. Maternal lactational food efficiency for pnd 14-21 and 0-21 was significantly higher than the control value at 11,250 ppm. Maternal lactational intake of BBP for the entire lactational period (pnd 0-21) was approximately 121.02, 620.04, and 1885.12 mg/kg/day at 750, 3750, and 11,250 ppm, respectively, confounded by the pups self-feeding beginning during the second week of postnatal life (Table 45 and Figure 17).

Figure 17

F_1 Female Butyl Benzyl Phthalate Intake During Lactation



Clinical observations of the F1 dams during lactation included alopecia in four, six, and four dams at 0, 3750, and 11,250 ppm, respectively. Bloody discharge, the day after delivery, was observed in one dam at 750 ppm. Chromodacryorrhea (two dams each at 0, 3750, and 11,250 and three dams at 750 ppm), red ears (two each at 750 and 11,250 ppm and one at 3750 ppm), piloerection (one dam at 3750 ppm), rust-colored fur (two, one, and three dams at 750,

3750, and 11,250 ppm, respectively), and one dam (at 3750 ppm) that needed to have her teeth trimmed were considered nontreatment related (Table 46).

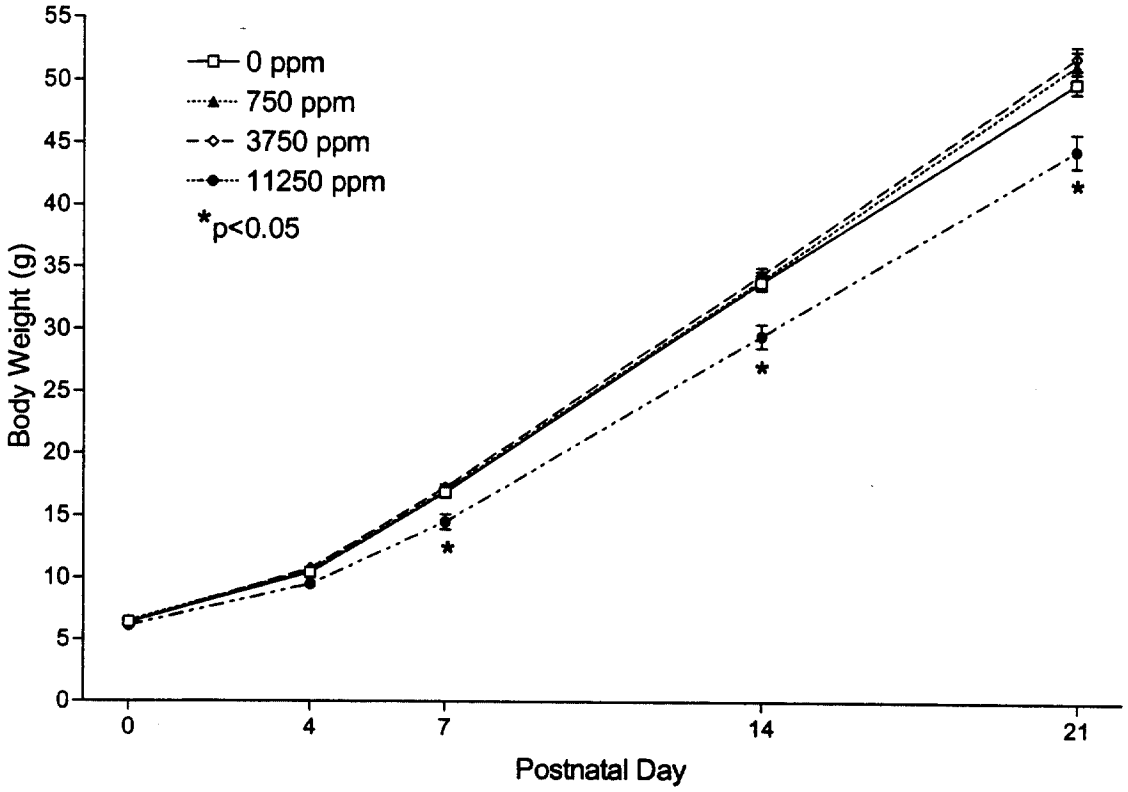
Reproductive and lactational indices for F1 animals producing F2 litters are presented in Table 47. F1 male and female mating and fertility indices were significantly reduced at 11,250 ppm. Gestational index was unaffected across groups. Precoital interval (in days) was also equivalent across all groups. Gestational length in days was equivalent across groups, with mean values of 22.0, 22.1, 22.0, and 22.0 days at 0, 750, 3750, and 11,250 ppm, respectively. The percent prenatal (postimplantation) loss was unaffected by treatment, however the number of implantation sites per dam was significantly reduced at 11,250 ppm. The number of live pups per litter on pnd 0 and the total number of pups per litter on pnd 0 were significantly reduced at 11,250 ppm. The number of litters on pnd 0 were 29, 28, 26, and 17 at 0, 750, 3750, and 11,250 ppm, respectively. On pnd 21, there was no change in the number of live litters from pnd 0. The lactational pup survival index (pnd 4 postcull to pnd 21) was 99.0% (0 ppm), 98.2% (750 ppm), 100.0% (3750 ppm), and 94.1% (11,250 ppm) (Table 47).

F2 pup sex ratio (percent males per litter) was equivalent across all groups throughout lactation. The mean number of live pups per litter was significantly reduced on pnd 0 and 4 (precull) at 11,250 ppm. However, live litter sizes were equivalent across all dose groups for the remainder of the lactational period (Table 48). Average F2 male anogenital distance was statistically significantly reduced at 3750 and 11,250 ppm. When the male anogenital distance was statistically analyzed by Analysis of Covariance (ANCOVA), with each male body weight on pnd 0 as the covariate, the values in the 3750 and 11,250 ppm groups were still significantly reduced. Average F2 female anogenital distance was equivalent across all groups, per se, and when adjusted for body weight by ANCOVA. Mean F2 body weights by litter for all pups or separately by sex were unaffected by treatment on pnd 0 and 4. However, mean body weights by litter for all pups or separately by sex were significantly reduced on pnd 7, 14, and 21 at 11,250 ppm. There was no effect on pup body weight at any interval measured during lactation at 750 or 3750 ppm (Table 48 and Figure 18).

The average number of nipples and areolae per male pup were significantly increased only at the high dose. The percentage of male pups with one or more areolae was also statistically significantly increased at 11,250 ppm (Table 48).

Figure 18

F_2 Pup Body Weights During Lactation



Clinical observations of F2 pups during the lactational period (pnd 0-21) included found dead, missing, presumed dead, euthanized moribund, hematomas, pale, pale and bleeding at umbilical area, tail bent, no milk band, cyanotic and bite marks on anogenital area, face, jaw and leg, and microphthalmia or opaque eye. The number of pups found dead, missing, and presumed dead on pnd 0 through 4 were 28, 22, 17, and 13 at 0, 750, 3750, and 11,250 ppm, respectively. The number of pups with the same criteria on pnd 5 through 21 were three, five, zero, and seven at 0, 750, 3750, and 11,250 ppm, respectively. There were, therefore, no treatment-related increases in F2 pup losses (Table 49).

Gross necropsy of F2 pups which died and were available for examination (Table 50) included 21 at 0 ppm, 20 at 750 ppm, 10 at 3750 ppm, and 16 at 11,250 ppm. The findings included patent (open) ductus arteriosus and no milk in the stomach in the pups examined on pnd 0, closed ductus and air in the lungs of pups examined from pnd 1-21, no milk in the stomach, occasional hydroureter(s)/distended ureter(s), liver half normal size (one at 750 ppm), and yellow

yellow areas in liver and kidneys dark in color (one pup at 750 ppm). These are all usual findings in dead pups during the lactational period.

Gross necropsy findings on the pnd 4 culled pups included distended ureters, hydroureters, and hydronephrosis across all treatment groups. This is not considered unusual in the early postnatal period in which the kidneys are still developing. One male, however, exhibited hypospadias at 11,250 ppm, indicative of exposure to an anti-androgen (Table 50).

Necropsy – F2 Weanlings

Necropsy body and organ weights of F2 male weanlings (pnd 21), up to three per sex per litter, are presented in Table 51. A total of 86, 83, 78, and 54 F2 male weanlings were necropsied at 0, 750, 3750, and 11,250 ppm, respectively. Five additional male pups were included in the 11,250 ppm evaluation, because they were not scheduled for necropsy but were positive for retained nipples and were therefore evaluated. F2 male weanling body weight was significantly reduced at 11,250 ppm (89% of the control value). Absolute male thymus, spleen, and paired testes weights were also significantly reduced at 11,250 ppm, most likely due to the reduced body weight at this dose. Relative spleen and testes weights were significantly reduced at 11,250 ppm, while relative brain weight was significantly increased at this dose.

Necropsy body and organ weights of F2 female weanlings are also presented in Table 51, with 87, 83, 77, and 43 F2 females necropsied at 0, 750, 3750, and 11,250 ppm, respectively. F2 female weanling body weight was significantly reduced at 11,250 ppm (89% of the control value). Absolute thymus, spleen, and paired ovaries weights were significantly decreased at 11,250 ppm. Absolute uterine weight was increased at 3750 ppm, and absolute brain weight was unaffected. Relative spleen weight was significantly reduced at 11,250 ppm (and increased at 750 ppm), while relative brain weight was significantly increased at this dose (Table 51).

A summary of gross necropsy findings in F2 weanlings is presented in Table 52. F2 female weanlings exhibited hydronephrosis in one or both kidneys (one each at 0, 750, and 3750 ppm), kinked tail (one at 3750 ppm), multiple red foci on thymus (one at 3750 ppm), and one with an umbilical hernia involving only fat at 11,250 ppm. One, one, seven, and one male pup had hydronephrosis (right) at 0, 750, 3750, and 11,250 ppm, respectively. One pup at 3750 ppm had an incompletely open eyelid, one pup at 0 ppm had a depression in his skull with the brain appearing flat underneath, and two pups at 11, 250 ppm had red tails. Twenty male weanlings

exhibited missing epididymides (full or caput or corpus) at 11,250 ppm. Five male weanlings had seminal vesicles missing or reduced in size also at 11,250 ppm. One male at 750 ppm had a missing testis (right). Thirteen male pups at 11,250 ppm had one to six retained nipples at necropsy (Table 52).

F1 Parental Holding Period

F1 males were held after mating until delivery of all F2 litters (sd 84-105), with dietary exposures continuing. Body weight was significantly reduced at sd 84, 91, 98, and 105 at 11,250 ppm. Body weight change, however, was equivalent across groups (Table 53). F1 male feed consumption was unaffected when expressed in g/day, but when expressed in g/kg/day, it was significantly increased at 11,250 ppm during the entire postmating holding period. Percent food efficiency was equivalent across all groups. BBP intake was approximately 39.40 (first week) – 35.83 (last/third week) mg/kg/day at 750 ppm, 191.66 – 176.93 mg/kg/day at 3750 ppm, and 618.45 – 570.38 mg/kg/day at 11,250 ppm (Table 54).

Clinical observations of F1 males during this period (Table 55) included alopecia in one male at 0 ppm, three males at 750 ppm, and one male each at 3750 and 11,250 ppm. Chromodacryorrhea was seen in two males each at 750 and 3750 ppm. One male at 3750 ppm had red ears, and one male each had rust-colored fur at 0, 750, and 3750 ppm. None of these findings were considered treatment related.

F1 females who were not pregnant (and/or did not deliver live litters) were held through sd 112. The data from these retained F1 females during this period, one to 13 per group, were not analyzed statistically but are presented for completeness. F1 female body weights and weight changes are presented in Table 56. Body weights appear to be reduced at 11,250 ppm, and body weight changes are variable across all groups.

F1 female feed consumption in g/day and g/kg/day and food efficiency, with one to 13 per group, is presented in Table 57; data are variable. BBP intake in mg/kg/day during this period was 44.10 (week 1) – 45.56 (week 4) mg/kg/day at 750 ppm, 214.59 – 221.63 mg/kg/day at 3750 ppm, and 833.16 – 738.80 mg/kg/day at 11,250 ppm (Table 57).

Clinical observations of the F1 females during this period were limited to alopecia (one female at 0 ppm) and chromodacryorrhea, red ears, and rust-colored fur in one to three dams each at 11,250 ppm (Table 58).

Necropsy – F1 Parental Animals

A summary of F1 male terminal body weight, organ weights, and andrological assessments is presented in Table 59. Body weight at sacrifice was significantly reduced at 11,250 ppm. Absolute brain weight was significantly decreased at 11,250 ppm, absolute liver and paired kidney weights were increased at 3750 ppm (but not at 11,250 ppm), and absolute pancreas weight was increased at 3750 and 11,250 ppm. Absolute paired testes, paired epididymides, prostate, and seminal vesicles weights were all significantly decreased at 11,250 ppm. Paired absolute adrenal, pituitary, thyroid, and spleen weights were unaffected. Relative prostate weight was significantly reduced at 11,250 ppm. Relative liver and paired kidney weights were increased at 3750 ppm. Relative brain, pituitary, liver, pancreas, and paired adrenal weights were significantly increased at 11,250 ppm. Relative thyroid and spleen weights were unaffected. There were significant decreases at 11,250 ppm for percent motile and progressively motile sperm and for epididymal sperm concentrations. There were no effects on testicular spermatid head concentration, daily sperm production (DSP), efficiency of DSP, or percent abnormal sperm in any group. The mean values for percent abnormal sperm were 5.99, 2.77, 5.61, and 3.27% at 0, 750, 3750, and 11,250 ppm, respectively (Table 59). The increased mean incidences at 0 and 3750 ppm were due to four and three males at 0 and 3750 ppm, respectively, with increased incidences of percent abnormal sperm. All affected males, except for two at 3750 ppm, sired live litters (see individual animal data tables A-69, A-52, and A-53 in Appendix II, Volume II for details).

Gross observations for F1 males, which were examined at scheduled sacrifice, included reproductive organ findings predominantly at 11,250 ppm: epididymis missing or reduced in size, seminal vesicles missing or reduced in size, testis missing or reduced in size, and the presence of one or more nipples. Two males at 11,250 ppm had hypospadias. One male at 0 ppm and two males at 3750 ppm also had epididymides reduced in size. One male at 0 ppm and three males at 3750 ppm had testes missing or reduced in size. One male at 11,250 ppm had an abscess on the preputial gland, and one male at 11,250 ppm had a missing coagulating gland. Nontreatment-related findings included alopecia, chromodacryorrhea, red ears, and rust-colored fur (Table 60 and Appendix II). Gross findings for F1 males at unscheduled death were exclusively in the high dose group (except for one male at 750 ppm with hydronephrosis) and

included enlarged adrenal gland, missing reproductive organs (including epididymides and/or testis), hydronephrosis of the kidney, hemorrhagic prostate or prostatitis, and distended urinary bladder. Treatment-related histopathologic findings were observed in the epididymis: aspermia in one of 30 at 0 ppm, zero of seven at 750 ppm, two of 30 at 3750 ppm, and eight of 24 at 11,250 ppm; and chronic epididymal inflammation in one, zero, one, and four at 0, 750, 3750, and 11,250 ppm, respectively. The testis also exhibited treatment-related findings: seminiferous tubule atrophy in 3, 0, 2, and 15; seminiferous tubule degeneration in 0, 0, 2, and 1; and dilatation of the rete testis duct in 0, 0, 0, and 7 out of 30, 7, 29, and 29 males examined at 0, 750, 3750, and 11,250 ppm, respectively. There were no other treatment-related histopathologic findings in any F1 male organ, including the liver, based on examination of 20, 21, 22, and 12 livers at 0, 750, 3750, and 11,250 ppm, respectively (Table 60).

A summary of F1 female terminal body and organ weights, ovarian primordial follicle counts, and stage of estrus at demise is presented in Table 61. F1 body weight at sacrifice was significantly reduced at 11,250 ppm. Absolute and relative uterine weights were increased at 11,250 ppm, and absolute paired kidney weights were increased at 3750 ppm, with all other organs unaffected by treatment. Paired ovarian primordial follicle counts (ten females each at 0 and 11,250 ppm) were equivalent between the two groups. Stage of estrus at demise was equivalent across dose groups (Table 61).

At scheduled sacrifice, F1 female findings included alopecia in the control, mid, and high dose group; chromodacryorrhea, one each at 0, 750, and 3750 ppm; red ears (two at 750 ppm, one 3750, and five at 11,250 ppm); hydronephrosis of the kidney (left, right, or bilateral) in three dams at 0 ppm, three at 750 ppm, one at 3750 ppm, and two at 11,250 ppm. One dam each at 0 ppm and 11,250 ppm had cysts on the kidneys. One dam at 0 ppm had irregular surface of the liver, one dam at 11,250 exhibited a firm white mass in the neck area, and one dam each at 3750 and 11,250 ppm had blood or cyst on the ovary. Rust-colored fur was seen in all doses except the control group. Two dams at 3750 ppm had a fluid-filled vagina or uterus; one dam had a cyst in the uterus, and three exhibited fluid-filled uteri at 11,250 ppm. One dam at 750 ppm had an enlarged thyroid gland. Treatment-related histopathologic findings were limited to the liver. "Cytologic Alteration, Hepatocyte" was observed in five of nine livers examined at 11,250 ppm and in no livers out of 20/group at 0, 750, and 3750 ppm (Table 62).

DISCUSSION

The present study evaluated exposure of CD® (Sprague-Dawley) rats to BBP administered in the diet *ad libitum* at 0, 750, 3750, and 11,250 ppm for two generations, one litter per generation. The justification for these target dietary concentrations was as follows: the low concentration (750 ppm) was selected to provide a BBP intake of 50 mg/kg/day, a dose of DBP established by Mylchreest et al. (1998a,b; 1999) as a NOAEL when administered by gavage during gestation and lactation. The top concentration (11,250 ppm) was selected to provide a BBP intake of 750 mg/kg/day, a dose established by Gray et al. (1998, 2000, 2001) as a dose of BBP when administered by gavage, which resulted in a very high incidence of male offspring reproductive and urinary tract malformations when administered from gd 14 to pnd 3. The mid concentration (3750 ppm) was selected to provide a BBP intake of 250 mg/kg/day (an intermediate intake which is five times the low intake and one-third the high intake values).

F0 and F1 Systemic Toxicity

The top dietary concentration (11,250 ppm) resulted in F0 and F1 male and female systemic toxicity, expressed as reduced body weights and weight changes, increased absolute and relative liver weights, increased relative liver weights in F1 males (also increased absolute and relative liver weights in F1 males at 3750 ppm), with no effects on liver weights in F1 females, and histopathologic lesions in the liver in F0 (but not F1) males and in F0 and F1 females (also seen in F0 females at 3750 ppm). Absolute and relative kidney weights were increased in F0 males (with absolute but not relative kidney weights also increased at 3750 ppm in F0 males), no kidney weight changes in F1 males at 11,250 ppm (but increased absolute and relative kidney weights in F1 males at 3750 ppm), increased relative kidney weight (but not absolute weight) in F0 females at 11,250 ppm (and absolute and relative kidney weights increased in F0 females at 3750 ppm), with absolute kidney weights increased at 3750 ppm, and no effects on relative weights at any dietary concentration in F1 females. The relative and absolute and/or relative kidney weights were not accompanied by any evidence of treatment-related histopathologic lesions in either sex, in either generation, in any dose group.

Since the phthalates as a class are known inducers of proliferation of peroxisomes (membrane-bound organelles) in the liver, the observations of increased absolute and relative liver weights and the presence of hepatic histopathologic findings from BBP exposure are

expected. The histopathologic findings in the liver are described by the study veterinary pathologist (see summary tables and Appendix III) as "Cytologic Alteration, Hepatic." This term "comprised a spectrum of histologic changes that included diffuse cytomegaly and variable karyomegaly, reduced cytoplasmic glycogen, increased cytoplasmic eosinophilia, and increased cytoplasmic granularity" (Appendix III, Pathology Report, page 7). The author continued: "These hepatocellular changes are typical of phthalates since they are often perioxisome proliferators, the eosinophilic granularity representing increased number of peroxisomes. In this study, all of the [affected high dose] animals had minimal change that was characterized by the subtle to slight presence of the aforementioned spectrum of changes. Unequivocal evidence of hepatocellular cytologic alteration was not confirmed in the mid and low dose F0 and F1 males and females" (Appendix III, Pathology Report, page 7). The NOAEL for parental systemic toxicity is therefore 3750 ppm (250 mg/kg/day). A summary of F0 and F1 adult systemic toxicity is presented in Text Table D.

Text Table D. Summary of F0 and F1 Adult Systemic Toxicity

	F0				F1			
Butyl Benzyl Phthalate, ppm	0	750	3750	11250	0	750	3750	11250
MALES								
Death	0	1	0	1	0	1	2	2
Prebreed Exposure								
Body Weights	---	---	↑[a]	↓[a]	---	---	---	↓
Weight Change	---	---	↑[a]	↑↓[a]	---	---	---	↓[a]
Clinical Observations	---	---	---	---	---	---	---	---
Feed Consumption								
g/day	---	---	---	---	---	---	---	↓[a]
g/kg/day	---	---	↓[a]	↑[a]	---	---	---	↑[a]
Food Efficiency	---	---	↑[a]	↑↓[a]	---	---	---	↑[a]
Postmate Holding								
Body Weights	---	---	---	---	---	---	---	↓
Weight Change	---	---	---	---	---	---	---	---
Clinical Observations	---	---	---	---	---	---	---	---

(continued)

Text Table D (continued)

Butyl Benzyl Phthalate, ppm	F0				F1			
	0	750	3750	11250	0	750	3750	11250
Feed Consumption								
g/day	---	---	---	---	---	---	---	---
g/kg/day	---	---	↓[a]	---	---	---	---	↑
Food Efficiency	---	---	---	---	---	---	---	---
Necropsy								
Body Weight	---	---	---	---	---	---	---	↓
Organ Weights:								
Liver - A	---	---	---	↑	---	---	↑	---
- R	---	---	---	↑	---	---	↑	↑
Kidney - A	---	---	↑	↑	---	---	↑	---
- R	---	---	---	↑	---	---	↑	---
Adrenal Gland - A	---	---	---	---	---	---	---	---
- R	---	---	---	---	---	---	---	↑
Spleen - A	---	---	---	---	---	---	---	---
- R	---	---	---	---	---	---	---	---
Brain - A	---	---	---	---	---	---	---	↓
- R	---	---	---	---	---	---	---	↑
Thyroid - A	---	---	---	---	---	---	---	---
- R	---	---	---	---	---	---	---	---
Pancreas - A	---	---	---	---	---	---	↑	↑
- R	---	---	---	---	---	---	---	↑
Pituitary - A	---	---	---	---	---	---	---	---
- R	---	---	↓	---	---	---	---	↑
Gross Findings	---	---	---	---	---	---	---	↑
Histopathology: Liver	---	---	---	8[b]	---	---	---	---

(continued)

Text Table D (continued)

Butyl Benzyl Phthalate, ppm	F0				F1			
	0	750	3750	11250	0	750	3750	11250
FEMALES								
Deaths	1	1	0	0	0	0	0	0
<u>Prebreed Exposure</u>								
Body Weights	---	---	---	↓[a]	---	---	---	↓
Weight Change	---	↑[a]	↑[a]	↑↓[a]	---	↑[a]	---	↓[a]
Clinical Observations	---	---	---	---	---	---	---	---
Feed Consumption								
g/day	---	---	---	---	---	↑[a]	---	---
g/kg/day	---	---	---	---	---	---	---	↑[a]
Food Efficiency	---	↑[a]	↑[a]	↑↓[a]	---	---	---	↑[a]
<u>Gestation</u>								
Body Weights	---	---	---	↓[a]	---	---	---	↓[a]
Weight Change	---	---	---	↓[a]	---	↑[a]	---	↓
Clinical Observations	---	---	---	---	---	---	---	---
Feed Consumption								
g/day	---	---	---	---	---	---	---	---
g/kg/day	---	---	---	↑[a]	---	↑[a]	---	↑[a]
Food Efficiency	---	↓[a]	---	↓[a]	---	---	---	↓[a]
<u>Lactation</u>								
Body Weights	---	---	---	↓[a]	---	---	---	↓[a]
Weight Change	---	---	---	↑[a]	---	---	---	↑[a]
Clinical Observations	---	---	---	---	---	---	---	---
Feed Consumption								
g/day	---	---	---	---	---	---	---	↓[a]
g/kg/day	---	---	---	↑[a]	---	---	---	---
Food Efficiency	---	---	---	↑[a]	---	---	---	↑[a]

(continued)

Text Table D (continued)

Butyl Benzyl Phthalate, ppm		F0				F1			
		0	750	3750	11250	0	750	3750	11250
Necropsy									
Body Weight		---	---	---	---	---	---	---	↓
Organ Weights:									
Liver	- A	---	---	---	↑	---	---	---	---
	- R	---	---	---	↑	---	---	---	---
Kidney	- A	---	---	↑	---	---	---	↑	---
	- R	---	---	↑	↑	---	---	---	---
Adrenal Gland	- A	---	---	---	---	---	---	---	---
	- R	---	---	---	---	---	---	---	---
Spleen	- A	---	---	---	---	---	---	---	---
	- R	---	---	---	---	---	---	---	---
Thyroid	- A	---	---	---	---	---	---	---	---
	- R	---	---	---	---	---	---	---	---
Brain	- A	---	---	---	---	---	---	---	---
	- R	---	---	---	---	---	---	---	---
Pancreas	- A	---	---	---	---	---	---	---	---
	- R	---	---	---	---	---	---	---	---
Pituitary	- A	---	---	---	---	---	---	---	---
	- R	---	---	---	---	---	---	---	---
Gross Findings		---	---	---	---	---	---	---	---
Histopathology: Liver		1[b]	---	2[b]	9[b]	---	---	---	5[b]

[a] Occasional changes, not significant for every interval measured

[b] The number of animals with histopathologic findings in the liver

KEY: ↑ = statistically significant increase

↓ = statistically significant decrease

--- = no statistically significant difference

A = absolute organ weight in grams

R = organ weight relative to terminal body weight in percentage

F0 and F1 Parental Male and Female Reproductive Toxicity

There were no reproductive effects in F0 males at any dietary dose, since they began exposure to BBP after they had achieved puberty. Also, as expected, F0 females exhibited no effects on any parameters of estrous cycling, mating, or gestation. They did exhibit reduced absolute and relative weights of paired ovaries and uterus at necropsy at 11,250 ppm (Text Table E).

Text Table E

Summary of F0 and F1 Parental Male and Female Reproductive Toxicity

	F0				F1			
Butyl Benzyl Phthalate, ppm	0	750	3750	11250	0	750	3750	11250
FEMALES								
Vaginal cyclicity	---	---	---	---	---	---	---	---
Estrous cycle length, days	4.39	4.58	4.81	4.39	4.38	4.27	4.75	4.55
Precoital interval, days	3.1	2.5	2.9	3.1	2.8	2.7	2.7	2.9
Indices:								
Mating	---	---	---	---	---	---	---	↓
Fertility	---	---	---	---	---	---	---	↓
Gestational	---	---	---	---	---	---	---	---
Gestational length, days	22.2	22.2	22.0	22.0	22.0	22.1	22.0	22.0
No. implant sites/litter	---	---	---	---	---	---	---	↓
Prenatal mortality index	---	---	---	---	---	---	---	---
(% postimplantation loss/litter)								
No. total pups/litter, pnd 0	---	---	---	---	---	---	---	↓
No. live pups/litter, pnd 0	---	---	---	---	---	---	---	↓
No. dead pups/litter, pnd 0	---	---	---	---	---	---	---	---

(continued)

Text Table E (continued)

Butyl Benzyl Phthalate, ppm	F0				F1			
	0	750	3750	11250	0	750	3750	11250
Necropsy								
Weights: Paired ovaries - A	---	---	---	↓	---	---	---	---
- R	---	---	---	↓	---	---	---	---
Uterus - A	---	---	---	↓	---	---	---	↑
- R	---	---	---	↓	---	---	---	↑
Stage of estrus at necropsy	---	---	---	---	---	---	---	---
Ovarian follicle count[a]	---			---	---			---
Reproductive system:								
Gross findings: uterus fluid filled	---	---	---	---	0	0	1	3
Histopathology	---	---	---	---	---	---	---	---
MALES								
Indices:								
Mating	---	---	---	---	---	---	---	↓
Fertility	---	---	---	---	---	---	---	↓
Pregnancy	---	---	---	---	---	---	---	---
Necropsy								
Weights: Testes - A	---	---	---	---	---	---	---	↓
- R	---	---	---	---	---	---	---	---
Epididymides - A	---	---	---	---	---	---	---	↓
- R	---	---	---	---	---	---	---	---
Prostate - A	---	---	---	---	---	---	---	↓
- R	---	---	---	---	---	---	---	↓
Seminal vesicles with - A	---	---	---	---	---	---	---	↓
coagulating gland - R	---	---	---	---	---	---	---	---
Reproductive system:								
Gross findings	---	---	---	---	---	---	---	↑
Histopathology: testis	---	---	---	---	3	0	4	23
epididymis	---	---	---	---	2	0	3	15

(continued)

Text Table E (continued)

Butyl Benzyl Phthalate, ppm	F0				F1			
	0	750	3750	11250	0	750	3750	11250
Andrological Assessments								
Epididymal sperm concentration	---	---	---	---	---	---	---	↓
% Motile sperm	---	---	---	---	---	---	---	↓
% Progressively motile sperm	---	---	---	---	---	---	---	↓
TSHC	---	---	---	---	---	---	---	---
DSP	---	---	---	---	---	---	---	---
Efficiency of DSP	---	---	---	---	---	---	---	---
% of abnormal sperm[b]	2.12	2.17	2.33	2.11	5.99[b]	2.77	5.61[b]	3.27

KEY: --- = no statistically significant change
↑ = statistically significant increase
↓ = statistically significant decrease
* $p<0.05$ versus control value
A = absolute organ weight in grams
R = organ weight relative to terminal body weight in %
DSP = Daily sperm production
TSHC = Testicular homogenization-resistant spermatid head counts

[a] Ovarian primordial follicle counts were performed only on control and high dose F0 and F1 females.

[b] There were no statistically significant differences in any BBP exposed group compared to the control group for "% of abnormal sperm." The mean values in F1 groups above 3.50% were due to four and three males in each designated group with increased numbers of malformed sperm. Of the four F1 males affected at 0 ppm, all sired live litters. Of the three F1 males affected at 3750 ppm, one sired a live litter. See text for explanation and summary tables for variance terms.

F1 parental females at 11,250 ppm (exposed since they were gametes) exhibited reduced mating and fertility indices (due to the F1 males which also exhibited reductions in these indices), reduced implantations, and total and live pups per litter on pnd 0 (with no increase in dead pups per litter). They also exhibited an increased incidence of fluid-filled uterus (cause and consequence, if any, unknown). They exhibited increased absolute and relative uterine weight (most likely due to the retention of fluid in this organ), with no histopathologic lesions in female reproductive tract organs. They also exhibited reduced ovarian weights at 11,250 ppm (Text Table E). Since the last step in the synthesis of 17ß-estradiol (E2; the mammalian endogenous estrogen) is conversion of testosterone or androstenedione to E2, the effects on female ovaries may be due to diminished precursor androgen to produce E2 in the ovaries.

F1 parental males at 11,250 ppm (exposed since they were gametes) exhibited reduced mating and fertility indices, reduced absolute (but not relative) testes, epididymides, seminal vesicles/coagulating gland weights, and reduced absolute and relative prostate weights. They exhibited reduced epididymal sperm number, motility, progressive motility, and increased gross and histopathologic findings in the testis and epididymis. In the next section on offspring toxicity, the incidence of F1 and F2 male reproductive malformations identified on pnd 4 and 21 necropsies (for F1 and F2 offspring males) and at adult necropsy (for F1 offspring males) is presented in tabular and graphical format.

F1 and F2 Offspring Toxicity

The summary of F1 and F2 offspring toxicity parameters is presented in Text Table F. As expected, the gestation, birth, and lactational survival of the F1 litters (generated from F0 parents, which began their exposure after they had acquired puberty) were unaffected. The number of F1 litters on pnd 0 were 26, 27, 28, and 27 at 0, 750, 3750, and 11,250 ppm, respectively. Pup body weights per litter were reduced at 11,250 ppm on pnd 0, 4, 7, 14, and 21 (and increased at 3750 ppm on pnd 7). However, the F1 male pups (exposed beginning as gametes through their gestation) did exhibit reduced anogenital distance in a dose-related manner at 3750 and 11,250 ppm. As expected, F1 female pup anogenital distance was unaffected. Although pup body weights confound anogenital distance (larger pups have larger distances, and smaller pups have shorter distances; Gallavan et al., 1999), anogenital distance is under DHT control (Quigley et al., 1995; Gilbert, 1997) and is the most sensitive parameter in animals exposed *in utero* to an antiandrogen (McIntyre et al., 2001). The findings in this study, that only male anogenital distance was affected in both offspring generations (shortened in a dose-related pattern) and at a dose in the presence of reduced body weight (11,250 ppm for F1 males) and at doses in the absence of reduced body weight (11,250 ppm for F2 males and 3750 ppm for F1 and F2 males), support the interpretation of treatment-related effects on this parameter at these doses. However, the biological significance of this finding at 3750 ppm is unclear due to the absence of any effects on male reproductive development, structures, or functions at this dose. Historical control values for F2 male anogenital distance (Volume III, Appendix IV) are 1.96-2.25 mm. The F1 and F2 male mean anogenital distances at 3750 ppm in this study were 1.89 and 1.99 mm, respectively (F2 mean within historical range, F1 mean below historical range). Also, the

large extent of reduction at 11,250 ppm (0.35 mm in F1 and 0.28 mm in F2 males) versus the extent of reduction at 3750 ppm (0.17 mm in F1 and 0.06 mm in F2 males) is consistent with the interpretation of an effect, but not an adverse effect. F1 offspring also exhibited a significant increase in the percent of male pups with one or more nipples, with one or more areolae, and in the number of nipples and areolae per male at 11,250 ppm.

Text Table F

Summary of F1 and F2 Offspring Toxicity Parameters

	F1				F2			
Butyl Benzyl Phthalate, ppm	0	750	3750	11,250	0	750	3750	11,250
LACTATION								
No. litters, pnd 0	26	27	28	27	29	28	26	17
Stillbirth index	---	---	---	---	---	---	---	---
Life birth index	---	---	---	---	---	---	---	---
No. total pups/litter, pnd 0	---	---	---	---	---	---	---	↓
No. live pups/litter, pnd 0	---	---	---	---	---	---	---	↓
Survival indices:								
4-day	---	---	---	---	---	---	---	---
7-day	---	---	---	---	---	---	---	---
14-day	---	---	---	---	---	---	---	---
21-day	---	---	---	---	---	---	---	---
Lactational index	---	---	---	---	---	---	---	---
Sex ratio (% males/litter)	---	---	---	---	---	---	---	---
Pup body weights/litter:								
pnd 0	---	---	---	↓	---	---	---	---
pnd 4	---	---	---	↓	---	---	---	---
pnd 7	---	---	↑	↓	---	---	---	↓
pnd 14	---	---	---	↓	---	---	---	↓
pnd 21	---	---	---	↓	---	---	---	↓
Anogenital distance/litter, pnd 0								
Males (mm)	2.06	2.01	1.89***	1.71***	2.05	2.05	1.99*	1.77***
Females (mm)	0.96	0.97	0.92	0.92	0.98	0.97	0.96	0.99

(continued)

Text Table F (continued)

	F1				F2			
Butyl Benzyl Phthalate, ppm	0	750	3750	11,250	0	750	3750	11,250
Body weight/litter, pnd 0 (g)								
Males	---	---	---	↓	---	---	---	---
Females	---	---	---	↓	---	---	---	---
% male pups with one or more nipples	0	4.10	0	19.23***	0	0	0	16.46
Number of nipples per male	0	0	0	0.72***	0	0	0	0.51*
% male pups with one or more areolae	2.63	4.10	0.76	32.31***	2.13	5.07	5.43	72.15***
Number of areolae per male	0.07	0.00	0.02	1.29**	0.05	0.12	0.19	3.14***
WEANLING NECROPSY								
Males								
Terminal body weight	---	---	---	↓	---	---	---	↓
Weights: Thymus – A	---	---	---	↓	---	---	---	↓
– R	---	---	---	---	---	---	---	---
Spleen – A	---	---	---	↓	---	---	---	↓
– R	---	---	---	↓	---	---	---	↓
Brain – A	---	---	↑	↓	---	---	---	---
– R	---	---	---	↑	---	---	---	↑
Testes – A	---	---	↑	↓	---	---	---	↓
– R	---	---	↑	↓	---	---	---	↓
Epididymides – A	---	---	---	↓	---	---	---	---
– R	---	---	---	---	---	---	---	---
Gross findings	---	---	---	---	---	---	---	↑
Females								
Terminal body weight	---	---	---	↓	---	---	---	↓
Weights: Thymus – A	---	---	---	↓	---	---	---	↓
– R	---	---	---	---	---	---	---	---
Spleen – A	---	---	---	↓	---	---	---	↓
– R	---	---	---	↓	---	↑	---	↓

(continued)

Text Table F (continued)

Butyl Benzyl Phthalate, ppm	F1 0	F1 750	F1 3750	F1 11,250	F2 0	F2 750	F2 3750	F2 11,250
Brain – A	---	---	---	↓	---	---	---	---
– R	---	---	---	↑	---	---	---	↑
Ovaries – A	---	---	---	↓	---	---	---	↓
– R	---	---	---	---	---	---	---	---
Uterus – A	---	---	---	↓	---	---	↑	---
– R	---	---	---	---	---	---	---	---
Gross findings	---	---	---	---	---	---	---	---
POSTWEAN[b]								
Males								
Age at PPS (days)	40.9	41.3	40.6	45.2*** [a]				
Weight at acquisition (g)	208.17	217.23	212.83	207.63				
Adjusted age at PPS (days)	41.0	41.1	40.6	45.4*** [a]				
Females								
Age at VP (days)	31.4	32.0	31.2	34.1*** [a]				
Weight at acquisition (g)	108.25	114.56	109.10	106.30				
Adjusted age at VP (days)	31.5	31.6	31.2	34.4*** [a]				

NOT DONE[b]

KEY: --- = no statistically significant change

↑ = statistically significant increase

↓ = statistically significant decrease

* = $p<0.05$, ** $p<0.01$, *** $p<0.001$ versus control group value

PPS = preputial separation in males

VP = vaginal patency in females

A = absolute organ weight in grams

R = organ weight relative to terminal body weight in %

a Value (age at acquisition) is statistically significantly different from control group value by ANOVA and by analysis of covariance (ANCOVA), with body weight at acquisition as the covariate.

b Postwean evaluations were performed on F1 offspring only. The study was completed at the weaning of the F2 litters. They were therefore terminated at weaning.

At necropsy of the F1 weanlings, both F1 males and F1 females at 11,250 ppm exhibited reduced terminal body weights, reduced absolute (but not relative) thymus weights, reduced absolute and relative spleen weights, and reduced absolute and increased relative brain weights (F1 males also exhibited increased absolute brain weight at 3750 ppm). F1 male weanlings also exhibited reduced absolute and relative testes weights at 11,250 ppm (and increased absolute and relative testes weight at 3750 ppm), and decreased absolute epididymal weight, with relative epididymal weight unaffected. F1 weanling females exhibited reduced absolute ovarian and uterine weights, with relative weights of both organs unaffected. There were no gross findings in any organ examined in either sex at any dose.

Acquisition of puberty was significantly delayed in F1 offspring of both sexes at 11,250 ppm when statistically evaluated by ANOVA (analysis of variance) or by ANCOVA (analysis of covariance), with body weight at acquisition as the covariate. In the present study, age at acquisition of preputial separation and of vaginal patency was significantly delayed for F1 males and females at 11,250 ppm using ANOVA or ANCOVA, with body weight at acquisition as the covariate. Acquisition of developmental landmarks is dependent on both age and weight, i.e., heavier animals acquire the landmark earlier, while lighter animals acquire the landmark later, but lighter animals do acquire the landmark (unless there is another cause for the delay) and in many cases acquire the landmark at a lighter weight than the heavier animals. In the present study, the body weights of the male and female F1 offspring at acquisition were not reduced at 11,250 ppm. Therefore, it is unlikely that reduced body weights resulted in or confounded the observed delay in acquisition of vaginal patency and preputial separation. However, the animals at 11,250 ppm were <u>older</u> at acquisition than the animals at 0, 750, or 3750 ppm and therefore were comparable in weight to the <u>younger</u> animals at acquisition in the lower dose groups. Acquisition of both landmarks in both sexes was delayed. The only endocrine-mediated mechanism currently known to result in delays in puberty in both sexes would be interference with steroidogenesis, thereby reducing testosterone (and DHT) levels in males and estrogen levels in females, and some phthalates have been shown to interfere with steroidogenesis.

The U.S. EPA (1996, p. 56295) has also recognized that "body weight at puberty may provide a means to separate specific delays in puberty from those that are related to general delays in development." The delays in vaginal patency in females and in preputial separation in males at 11,250 ppm in this study were relatively minor (2.7 days for females and 4.3 days for

males) and are typical of those observed in the performing laboratory when systemic toxicity expressed as reduced body weights appears to be the sole cause (e.g., Tyl et al., 1999, 2001).

In addition, Kennedy and Mitra (1963) showed that body weight and food intake are factors in the initiation of puberty in the rat. Carney et al. (1998) fed SD pregnant dams at 70% or 50% of control feed intake levels from gd 7 through weaning of offspring. Feed restriction resulted in reduced body weights in dams and offspring and delayed vaginal patency and preputial separation (by one day at 70% and by six days at 50% restriction for both parameters). The most conservative interpretation for the delays in acquisition of puberty in the F1 males and females is that it is due to effects on steroidogenesis, confounded by systemic toxicity at 11,250 ppm.

Text Table G presents the incidence of urinary tract and reproductive tract malformations in F1 male and female offspring on pnd 4 and 21 and as adults. The F1 males at 11,250 ppm exhibited a significant increase in reproductive tract malformations on pnd 21 and as adults (with a slight but not statistically significant increase on pnd 4). The F1 males did not exhibit any increases in urinary tract malformations. As expected, F1 females did not exhibit any reproductive tract malformations and no treatment-related increases in urinary tract malformations, at any dose at any time. See Text Figure B for a graphical representative of F1 male reproductive tract malformations on pnd 4 and 21 and as adults.

F2 litters, developing in F1 dams (sired by F1 males) exhibited reductions in the number of total and live pups per litter on pnd 0 at 11,250 ppm. The number of F2 litters on pnd 0 were 29, 28, 26, and 17 at 0, 750, 3750, and 11,250 ppm, respectively. Survival during lactation was unaffected, as was sex ratio. Pup body weights were unaffected on pnd 0 and 4 and reduced at 11,250 ppm for pnd 7, 14, and 21. As with the F1 litters, F2 males exhibited treatment-related reductions in anogenital distance on pnd 0 at 3750 and 11,250 ppm, with no effects on pup body weights at this time. F2 female anogenital distance at birth was unaffected. The F2 males also exhibited significant increases at 11,250 ppm in the percentage of male pups with one or more nipples and with one or more areolae, and in the number of nipples and areolae per male.

Text Table G

Analyses of Incidence of Urinary Tract and Reproductive Tract Malformations in F1 and F2 Offspring

	BBP in Diet (ppm)			
	0	750	3750	11250
F1 OFFSPRING				
Pnd 4 Culls				
Males				
No. examined	48	38	51	45
% with UTM[a]	8.33	2.63	3.92	2.22
% with RTM[b]	0.00	0.00	0.00	4.44
Females				
No. examined	55	37	45	30
% with UTM	5.45	13.51	13.33	3.33
% with RTM	0.00	0.00	0.00	0.00
Pnd 21 Necropsies				
Males				
No. examined	68	68	78	76[c]
% with UTM[a]	1.47	2.94	0.00	2.63
% with RTM[b]	0.00	0.00	0.00	32.89***
Females				
No. examined	73	68	81	67
% with UTM	1.47	0.00	0.00	0.00
% with RTM	0.00	0.00	0.00	0.00
Adult Necropsies				
Males				
No. examined	30	30	30	30
% with UTM[a]	16.67	26.67	20.00	16.67
% with RTM[b]	3.33	0.00	10.00	53.33***
Females				
No. examined	30	30	30	30
% with UTM	10.0	10.0	3.33	6.67
% with RTM	0.00	0.00	0.00	0.00

(continued)

Text Table G (continued)

	BBP in Diet (ppm)			
	0	750	3750	11250
F2 OFFSPRING				
Pnd 4 Culls				
Males				
No. examined	61	61	47	14
% with UTM[a]	1.64	1.64	8.51	7.14
% with RTM[b]	0.00	0.00	0.00	7.14
Females				
No. examined	55	47	56	15
% with UTM	14.55	14.89	28.57	6.67
% with RTM	0.00	0.00	0.00	0.00
Pnd 21 Necropsies				
Males				
No. examined	86	83	78	54
% with UTM[a]	1.16	1.20	8.97	1.85
% with RTM[b]	0.00	1.20	0.00	24.07**
Females				
No. examined	87	83	77	43
% with UTM	1.15	1.20	1.30	0.00
% with RTM	0.00	0.00	0.00	0.00

[a] Percentage of the animals examined which had one or more gross urinary tract malformations (UTM), including hydroureter, hydronephrosis, malformed urinary bladder, and/or thinned wall of urinary bladder.

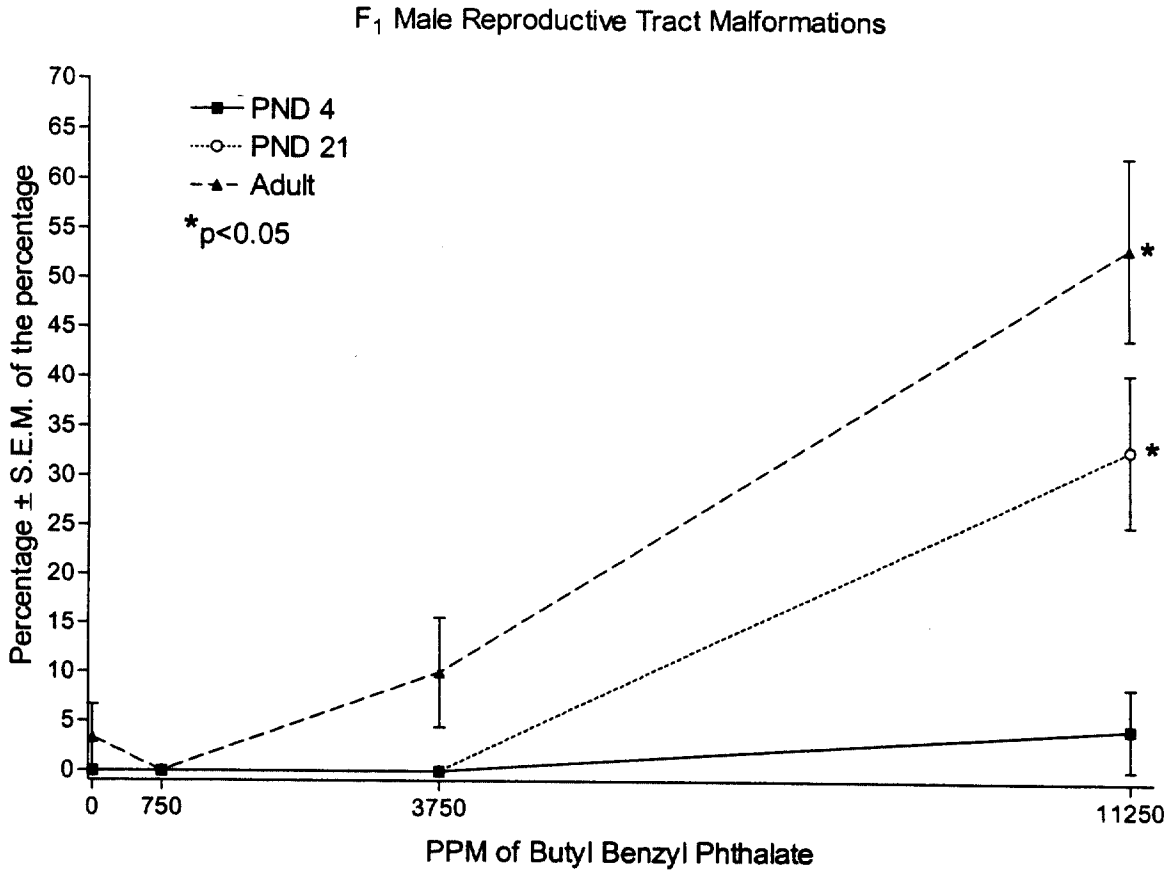
[b] Percentage of the animals examined which had one or more gross reproductive tract malformations (RTM), including hypospadias, missing reproductive organs or portion of organs, abnormal organ size and/or shape.

[c] Two additional F1 male weanlings were included because they had nipples as preweanlings.

** = $p<0.01$ versus control value

*** = $p<0.001$ versus control value

Text Figure B

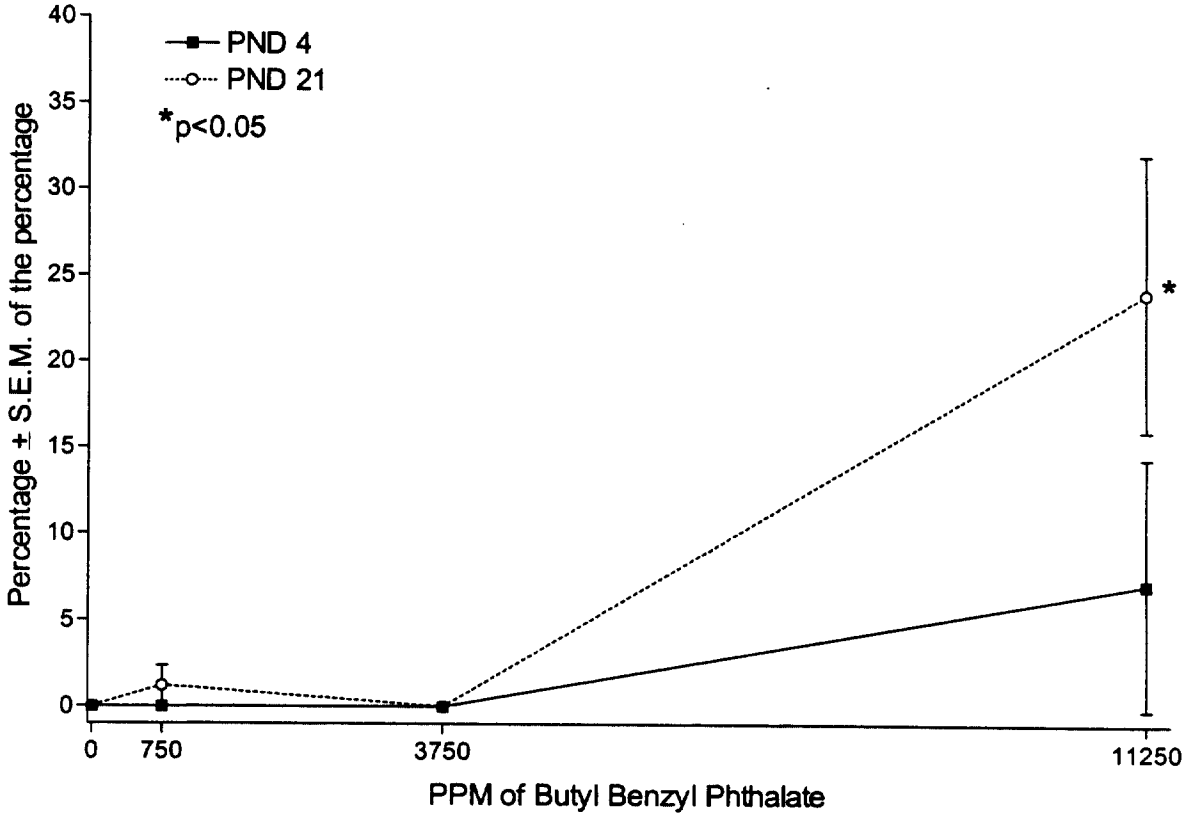


At the F2 weanling necropsy, both F2 males and females at 11,250 ppm exhibited reduced terminal body weights, reduced absolute (but not relative) thymus weights, reduced absolute and relative spleen weights, and increased relative (with no effect on absolute) brain weights. The F2 males also exhibited reduced absolute and relative testes weights, with no effects on absolute or relative epididymal weights and increased incidence of gross findings in the male reproductive organs, all at 11,250 ppm only. F2 female weanlings exhibited reduced absolute (with no effect on relative) ovarian weights at 11,250 ppm and increased absolute uterine weight (with no effect on relative uterine weight) at 3750 ppm, with no effects on uterine weight at 11,250 ppm. There were no treatment-related gross findings in the female weanlings (Text Table F). There were no postweaning evaluations since the study was completed at the weaning of the F2 litters. The F2 litters were therefore terminated at weaning.

Text Table G presents the incidence of urinary tract and reproductive tract malformations in F2 males and females on pnd 4 and 21. The F2 females exhibited no reproductive tract malformations at any dose at any time, and no treatment-related changes in incidence of urinary tract malformations at any dose or time. The F2 males exhibited no treatment-related changes in incidence of urinary tract malformations at any dose or time. The F2 males at 11,250 ppm did exhibit an increased incidence (not statistically significant) of reproductive tract malformations on pnd 4 and significantly increased incidence of reproductive tract malformations on pnd 21. Text Figure C presents the incidence of F2 male reproductive tract malformations by dose over time.

Text Figure C

F_2 Male Reproductive Tract Malformations



The results of the present study are reasonably consistent with the results of Nagao et al. (2000) and confirm and extend the findings of Gray et al. (2000) on perinatal exposure to BBP and to other phthalates (Gray et al., 1999).

CONCLUSIONS

Dietary exposure of CD® (SD) rats to BBP for two generations, one litter per generation, at 0, 750, 3750, and 11,250 ppm, resulted in:

- F0 and F1 parental systemic toxicity at 11,250 ppm
  - -- Reduced body weights and weight gains
  - -- Increased absolute and relative liver weights
  - -- Histopathologic lesions in livers
- F1 (but not F0) parental reproductive toxicity at 11,250 ppm
  - -- Reduced mating and fertility indices in F1 parents to make F2 offspring
  - -- Reduced number of implantations and total and live pups per litter on pnd 0 for F2 offspring
  - -- Reduced F1 male epididymal sperm motility, progressive motility, sperm concentrations
  - -- Increased incidence of F1 adult male reproductive tract malformations (from perinatal exposure)
  - -- Increased gross and histopathologic lesions in F1 adult male testes and epididymides (from perinatal exposure)
- F1 and F2 offspring toxicity at 3750 and 11,250 ppm
  - -- Reduced body weights in F1 and F2 pups during lactation at 11,250 ppm
  - -- Shortened anogenital distance in F1 and F2 males at 3750 and 11,250 ppm
  - -- Delayed acquisition of puberty in F1 males and females at 11,250 ppm
  - -- Retention of nipples and areolae in preweanling F1 and F2 males at 11,250 ppm
  - -- Male reproductive system malformations at 11,250 ppm

Therefore, in rats, under the conditions of this study:

- The F0 and F1 parental systemic and reproductive NOAEL is 3750 ppm (equivalent to approximately 250 mg/kg/day)
- The F1 and F2 offspring reproductive toxicity NOAEL also is 3750 ppm (equivalent to approximately 250 mg/kg/day)
- The F1 and F2 offspring reproductive toxicity NOEL (no observable effect level) in males is 750 ppm (equivalent to approximately 50 mg/kg/day), based on the significantly shortened anogenital distance in F1 and F2 male pups at birth at 3750 (and at 11,250) ppm, with no effects on reproductive development, structures, or functions at 3750 mg/kg/day

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Protocol Deviations

Temperature and Relative Humidity Excursions

The protocol-mandated temperature range was 64-79°F, and relative humidity was 30-70% for the animal rooms (NRC, 1996). Excursions outside these ranges for the RTI Animal Research Facility animal rooms used in this study were as follows:

Temperature			Relative Humidity		
Date	Duration	°F	Date	Duration	% RH
F0 Animals					
Room 303: F0 males (July 10 to November 9, 2000)					
No excursions			09/17/00	3 consecutive hours	Highest Reading 79.4%
Room 305: F0 females (July 10, - November 22, 2000)					
No excursions			09/17/00	3 consecutive hours	Highest reading 78.7%
F1 Animals					
Room 503: F1 males (November 9, 2000– March 15,2001)					
No excursions				No excursions	
Room 504: F1 females (November 9, 2000 – March 29, 2001)					
No excursions				No excursions	

The NRC *Guide for the Care and Use of Laboratory Animals* (Institute of Laboratory Animal Resources, Commission on Life Sciences, National Research Council, revised 1996) recommends, and the study protocol mandated, a temperature range of 64–79°F (17.8-26.1°C) and relative humidity range of 30-70% for rats. There were no temperature excursions outside the protocol-mandated range in any animal room at any time. There were two excursions for relative humidity, each for three consecutive hours, in duration on the same day in F0 males and F0 female rooms. These relative humidity excursions did exceed the NRC recommended range, but they were few, brief, and minor. These few, brief, and minor excursions in relative humidity did not affect the design, conduct, or conclusions of this study in the Study Director's professional judgment.

Rochelle W. Tyl

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

January 29, 2002
Date

RTI INTERNATIONAL

Quality Assurance Statement

Study Title: Two-Generation Reproductive Toxicity Evaluation of Buty Benzyl Phthalate Administered in the Feed to CD® (Sprague-Dawley) Rats

Sponsor: The European Council for Plasticizers and Intermediates (ECPI)

Study Code: Rt00-BBP

Protocol Number: RTI-761

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	1/7-8/00	1/10/00
Stability Analysis	4/17/00	4/17/00
Sample Preparation (Dose Analysis)	7/6/00	7/6/00
Dose Formulation	7/6/00	7/6/00
Quarantine	7/14/00	7/14/00
Randomization	7/20/00	7/20/00
Body Weights, Feed Consumption, And Clinical Observations	7/26/00	7/26/00
Dose Formulation	9/11/00	9/11/00
Dosing, Vaginal Cytology, And Clinical Observations	9/13/00	9/13/00
Histology	10/5/00	10/05/00
Mating and Gestation	10/10/00	10/10/00
Necropsy	11/6/00	11/6/00
Dose Formulation	11/6/00	11/6/00
Female Necropsy	11/9/00	11/9/00
Vaginal Patency and Preputial Separation	12/1/00	12/1/00
Clinical Observations Data	12/7/00	12/7/00
Clinical Observations	12/21/00	12/21/00
Clinical Observations	1/3/01	1/3/01
Vaginal Cytology	1/22/01	1/22/01

Continued

Inspections and Audits	Inspection and Audit Date(s)	Date Inspection/Audit Report Sent to Study Director and Management
Dose Formulation	1/29/01	1/29/01
Mating and Gestation	2/12/01	2/12/01
Sample Preparation (Dose Analysis Records)	2/27/01	2/27/01
Male Necropsy and Sperm Analysis	3/12/01	3/12/01
Female Necropsy, Pnd 21 Necropsy	3/15/01	3/15/01
Data/Report – Dose Analysis	7/12-13,16, 27, 30/01	8/2/01
Data/Report – Dose Analysis	7/16, 27, 30/01	8/2/01
Data/Report – Dose Analysis	7/16-17, 27, 30/01	8/2/01
Data/Report – Dose Analysis	7/17, 27, 30/01	8/2/01
Data/Report – Dose Analysis	7/17-18, 30/01	8/3/01
Data/Report – Dose Analysis	7/18, 27, 30/01	8/3/01
Data/Report – Dose Analysis	7/19, 27, 30/01	8/3/01
Data Audit – 5-Day Stability	8/1/01	8/6/01
Data Audit – 12-Day Stability	8/2/01	8/6/01
Data Audit – 20- Day Stability	8/2/01	8/6/01
Data Audit – 40-Day Stability	8/3/01	8/6/01
Data Audit – 54-Day Stability	8/6/01	8/6/01
Data Audit – Method Validation	8/6-8/01	8/8/01
Report Audit – Dose Formulation	8/9-10, 13/01	8/13/01
Data Audit	7/15 – 11/27/01	11/27/01
Report Audit	11/16, 20, 28-29; 12/2-9, 11-14, 17/01	12/17/01

Patricia D. Hall
Quality Assurance Specialist

1/31/02
Date

Approval:

Doris J. Smith
Quality Assurance Manager

1-31-02
Date

Table 1. Analyses of Dosed Feed (page 1 of 1)

Group No.	Rx Code	Color Code	Target Conc. (ppm)	Date(s) of Formulation	Analytical Results	
					Actual Conc. (ppm)	% of Target
1	02322	Green	0.0	07/05/00	ND[a]	NA[b]
				08/14/00	ND	NA
				09/11/00	ND	NA
				10/09/00	ND	NA
				11/06/00	--[c]	--
				12/04/00	ND	NA
				01/02/01	--	--
				01/29/01	--	--
				02/26/01	ND	NA
2	15072	Yellow	750.0	07/05/00	682 (2.1)[d]	90.9
				08/14/00	755 (1.9)	101
				09/11/00	775 (5.6)	103
				10/09/00	740 (1.0)	98.7
				11/06/00	--	--
				12/04/00	709 (0.6)	94.5
				01/02/01	--	--
				01/29/01	--	--
				02/26/01	703 (1.0)	93.7
3	27002	Red	3750.0	07/05/00	3750 (5.3)	95.2
				08/14/00	4020 (1.6)	107
				09/11/00	3890 (0.90)	104
				10/09/00	3610 (1.2)	96.3
				11/06/00	--	--
				12/04/00	3800 (0.5)	101
				01/02/01	--	--
				01/29/01	--	--
				02/26/01	3810 (1.5)	102
4	66181	Blue	11,250.0	07/05/00	9730 (1.8)	86.5[e]
				remix 07/11/00	12,000 (0.48)	107
				08/14/00	11,000 (0.90)	97.8
				09/11/00	11,600 (0.50)	103
				10/09/00	10,500 (1.1)	93.3
				11/06/00	--	--
				12/04/00	10,700 (1.1)	95.1
				01/02/01	--	--
				01/29/01	--	--
				02/26/01	10,600 (3.3)	94.2

[a] ND = not detectable. The estimated limit of detection is 35 ppm.
[b] NA = not applicable.
[c] Dosed feeds (all levels) formulated on 11/06/00, 01/02/01, and 01/29/01 were not scheduled for analysis in the protocol and so were not analyzed.
[d] The data are presented as mean of three determinations (% relative standard deviation).
[e] The initial formulation was not acceptable since the analytical concentration was outside the acceptable range (within ±10% of target) at 86.5% of target (with six replicate determinations). As indicated, it was remixed on 07/11/00. The remix was analyzed as within acceptable range (107% of target) and was presented to the animals beginning on 07/19/00.

Table 2. Summary of the Fate of the F_0 Males and Females (page 1 of 1)

	Butyl Benzyl Phthalate (ppm in the feed)			
	0	750	3750	11250
MALES	30	30	30	30
Phase of Study				
Prebreed	0	1[a]	0	0
Mating	0	0	0	0
Holding Period after Mating	0	0	0	1[b]
Scheduled Sacrifice	30	29	30	29
FEMALES	30	30	30	30
Phase of Study				
Prebreed	0	0	0	0
Mating	0	0	0	0
Gestation	0	0	0	0
Lactation	0	1[c]	0	0
Holding Period after Mating	1[d]	0	0	0
Scheduled Sacrifice	29	29	30	30

[a]Male 63 was found dead on study day 40.
[b]Male 21 was found dead on study day 109.
[c]Female 78 was found dead on postnatal day 17 (study day 110).
[d]Female 52 was found dead on study day 103.

Table 3. Summary and Statistical Analysis of the F_0 Male Body Weights and Weight Changes During the Prebreed and Mating Periods (page 1 of 4)

	Butyl Benzyl Phthalate (ppm in the feed)			
	0	750	3750	11250
No. Males on Study	30	30	30	30
Body Weight (sd 0) (g)[a]	242.7 ± 1.6 N=30	242.4 ± 1.7 N=30	242.0 ± 1.6 N=30	240.7 ± 1.7 N=30
Body Weight (sd 7) (g)[a]	299.8 ‡‡ ± 2.2 §§§ N=30	298.2 ± 2.1 N=30	299.0 ± 2.1 N=30	288.8 ** ± 3.0 N=30
Body Weight (sd 14) (g)[a]	350.1 ± 3.1 N=30	346.4 ± 2.9 N=30	354.8 ± 3.1 N=30	343.2 ± 3.3 N=30
Body Weight (sd 21) (g)[a]	391.3 ± 4.0 N=30	386.6 ± 3.7 N=30	397.7 ± 4.0 N=30	383.0 ± 4.0 N=30
Body Weight (sd 28) (g)[a]	426.1 ‡ ± 4.8 N=30	420.9 ± 4.3 N=30	434.9 ± 5.0 N=30	416.4 ± 4.7 N=30
Body Weight (sd 35) (g)[a]	447.8 ‡ ± 5.6 N=30	442.7 ± 4.8 N=30	460.6 ± 5.6 N=30	438.5 ± 5.4 N=30
Body Weight (sd 42) (g)[a]	475.4 ‡‡ ± 6.0 N=30	465.2 ± 4.8 N=29[b]	489.9 ± 6.7 N=30	463.3 ± 5.9 N=30
Body Weight (sd 49) (g)[a]	494.7 ‡‡ ± 6.6 N=30	483.8 ± 5.3 N=29	511.9 ± 7.1 N=30	481.6 ± 6.1 N=30
Body Weight (sd 56) (g)[a]	510.2 ‡‡ ± 6.8 N=30	498.3 ± 5.7 N=29	527.6 ± 7.5 N=30	494.4 ± 6.4 N=30

(continued)

Table 3. Summary and Statistical Analysis of the F_0 Male Body Weights and Weight Changes During the Prebreed and Mating Periods (page 2 of 3)

	Butyl Benzyl Phthalate (ppm in the feed)			
	0	750	3750	11250
Body Weight (sd 63) (g)[a]				
	527.0 ‡‡ ± 7.6 N=30	512.7 ± 5.9 N=29	543.5 ± 8.1 N=30	510.4 ± 6.7 N=30
Body Weight (sd 70) (g)[a]				
	543.1 ‡‡ ± 8.1 N=30	531.1 ± 6.4 N=29	565.2 ± 8.8 N=30	531.0 ± 7.2 N=30
Body Weight (sd 77) (g)[a]				
	544.5 ‡‡ ± 7.8 N=30	531.1 ± 6.4 N=29	564.3 ± 8.6 N=30	529.7 ± 7.1 N=30
Body Weight (sd 84) (g)[a]				
	557.9 ‡‡ ± 8.5 N=30	543.0 ± 6.5 N=29	579.5 ± 9.3 N=30	543.4 ± 7.6 N=30
Body Weight Change (sd 0 to 7) (g)[a]				
#	57.1 ††† ± 1.2 ŸŸŸ N=30	55.8 ± 0.9 N=30	57.0 ± 1.2 N=30	48.1 ÞÞÞ ± 1.9 N=30
Body Weight Change (sd 7 to 14) (g)[a]				
	50.3 ‡‡‡ ± 1.3 §§ N=30	48.1 ± 1.1 N=30	55.8 * ± 1.5 N=30	54.4 ± 1.6 N=30
Body Weight Change (sd 14 to 21) (g)[a]				
	41.1 ± 1.1 N=30	40.3 ± 1.3 N=30	43.0 ± 1.3 N=30	39.7 ± 1.1 N=30
Body Weight Change (sd 21 to 28) (g)[a]				
	34.8 ± 1.2 N=30	34.2 ± 1.9 N=30	37.2 ± 1.4 N=30	33.4 ± 1.0 N=30
Body Weight Change (sd 28 to 35) (g)[a]				
	21.7 ± 1.2 N=30	21.8 ± 1.6 N=30	25.7 ± 1.1 N=30	22.2 ± 1.0 N=30

(continued)

Table 3. Summary and Statistical Analysis of the F_0 Male Body Weights and Weight Changes During the Prebreed and Mating Periods (page 3 of 4)

	Butyl Benzyl Phthalate (ppm in the feed)			
	0	750	3750	11250
Body Weight Change (sd 35 to 42) (g)[a]				
	27.6 ‡‡ ± 1.1 N=30	24.8 ± 1.0 N=29[b]	29.3 ± 1.3 N=30	24.7 ± 0.8 N=30
Body Weight Change (sd 42 to 49) (g)[a]				
	19.3 ‡ ± 0.9 N=30	18.6 ± 0.9 N=29	22.0 ± 0.8 N=30	18.3 ± 0.7 N=30
Body Weight Change (sd 49 to 56) (g)[a]				
	15.5 ± 0.7 § N=30	14.5 ± 0.9 N=29	15.8 ± 0.9 N=30	12.8 ± 1.0 N=30
Body Weight Change (sd 56 to 63) (g)[a]				
	16.7 ± 1.0 N=30	14.4 ± 0.6 N=29	15.8 ± 1.0 N=30	16.0 ± 0.7 N=30
Body Weight Change (sd 63 to 70) (g)[a]				
	16.1 ‡‡‡ ± 1.1 §§ N=30	18.4 ± 0.9 N=29	21.8 *** ± 1.2 N=30	20.6 ** ± 0.7 N=30
Body Weight Change (sd 0 to 70) (g)[a]				
	300.3 ‡‡ ± 7.5 N=30	288.7 ± 5.9 N=29	323.2 ± 8.4 N=30	290.3 ± 6.4 N=30
Body Weight Change (sd 70 to 77) (g)[a]				
	1.5 ± 1.4 N=30	-0.0 ± 1.2 N=29	-0.9 ± 1.3 N=30	-1.3 ± 1.1 N=30
Body Weight Change (sd 77 to 84) (g)[a]				
	13.4 ± 1.3 N=30	11.9 ± 0.9 N=29	15.2 ± 1.4 N=30	13.7 ± 1.1 N=30

(continued)

Table 3. Summary and Statistical Analysis of the F_0 Male Body Weights and Weight Changes During the Prebreed and Mating Periods (page 4 of 4)

[a]Reported as the mean ± S.E.M.; sd=study day with study day 0 being the first day of exposure.
[b]Decrease in N is due to male 63 being found dead on study day 40.
[#]Levene's test for homogeneity of variances was significant ($p<0.05$), therefore robust regression methods were used to test all treatment effects.
‡$p<0.05$; ANOVA Test.
‡‡$p<0.01$; ANOVA Test.
‡‡‡$p<0.001$; ANOVA Test.
§$p<0.05$; Test for Linear Trend.
§§$p<0.01$; Test for Linear Trend.
§§§$p<0.001$; Test for Linear Trend.
*$p<0.05$; Dunnett's Test.
**$p<0.01$; Dunnett's Test.
***$p<0.001$; Dunnett's Test.
†††$p<0.001$; Wald Chi-square Test for overall treatment effect in robust regression model.
ÞÞÞ$p<0.001$; Individual t-test for pairwise comparisons to control in robust regression model.
ŸŸŸ$p<0.001$; Linear trend test in robust regression model.

Table 4. Summary and Statistical Analysis of the F_0 Male Feed Consumption, Percent Food Efficiency and Butyl Benzyl Phthalate Intake During the Prebreed and Mating Periods (page 1 of 6)

	Butyl Benzyl Phthalate (ppm in the feed)			
	0	750	3750	11250
No. Males on Study	30	30	30	30
Feed Consumption (sd 0 to 7) (g/day)[a]				
#	24.6 ± 0.3 N=30	24.8 ± 0.3 N=29[b]	25.1 ± 0.3 N=30	25.8 ± 0.6 N=23[c]
Feed Consumption (sd 7 to 14) (g/day)[a]				
	25.9 ± 0.4 N=30	25.7 ± 0.3 N=30	26.1 ± 0.4 N=30	26.5 ± 0.4 N=30
Feed Consumption (sd 14 to 21) (g/day)[a]				
	26.7 ± 0.4 N=30	26.6 ± 0.3 N=29[b]	26.7 ± 0.4 N=29[d]	26.2 ± 0.3 N=29[b]
Feed Consumption (sd 21 to 28) (g/day)[a]				
	27.3 ± 0.5 N=30	26.7 ± 0.3 N=30	27.2 ± 0.4 N=30	26.6 ± 0.3 N=30
Feed Consumption (sd 28 to 35) (g/day)[a]				
	27.0 ± 0.5 N=30	26.8 ± 0.3 N=30	27.3 ± 0.5 N=30	26.9 ± 0.4 N=30
Feed Consumption (sd 35 to 42) (g/day)[a]				
	28.1 ± 0.5 N=30	27.9 ± 0.3 N=29[e]	27.9 ± 0.5 N=30	27.6 ± 0.4 N=29[b]
Feed Consumption (sd 42 to 49) (g/day)[a]				
	28.1 ± 0.5 N=30	27.4 ± 0.3 N=29	28.1 ± 0.5 N=30	27.4 ± 0.4 N=30
Feed Consumption (sd 49 to 56) (g/day)[a]				
	28.1 ± 0.4 N=30	28.1 ± 0.3 N=29	28.4 ± 0.5 N=30	27.5 ± 0.4 N=30
Feed Consumption (sd 56 to 63) (g/day)[a]				
	27.8 ± 0.4 N=30	27.9 ± 0.3 N=29	28.0 ± 0.5 N=30	27.4 ± 0.4 N=30

(continued)

Table 4. Summary and Statistical Analysis of the F_0 Male Feed Consumption, Percent Food Efficiency and Butyl Benzyl Phthalate Intake During the Prebreed and Mating Periods (page 2 of 6)

	Butyl Benzyl Phthalate (ppm in the feed)			
	0	750	3750	11250
Feed Consumption (sd 63 to 70) (g/day)[a]				
	27.7 ± 0.5 N=30	27.3 ± 0.4 N=29	27.8 ± 0.5 N=30	27.3 ± 0.4 N=30
Feed Consumption (sd 0 to 70) (g/day)[a]				
	27.1 ± 0.4 N=30	26.8 ± 0.3 N=27[f]	27.3 ± 0.4 N=29[f]	26.8 ± 0.4 N=21[f]
Feed Consumption (sd 77 to 84) (g/day)[a,g]				
	27.6 ± 0.7 N=27	27.6 ± 0.3 N=25	27.9 ± 0.5 N=26	28.3 ± 0.5 N=26
Feed Consumption (sd 0 to 7) (g/kg/day)[a]				
#	90.7 † ± 0.9 Ÿ N=30	91.9 ± 0.7 N=29[b]	92.9 ± 0.7 N=30	96.8 Þ ± 2.2 N=23[c]
Feed Consumption (sd 7 to 14) (g/kg/day)[a]				
#	79.6 † ± 0.9 ŸŸ N=30	79.8 ± 0.6 N=30	79.7 ± 0.8 N=30	83.9 ÞÞ ± 1.3 N=30
Feed Consumption (sd 14 to 21) (g/kg/day)[a]				
	72.1 ± 0.8 N=30	72.3 ± 0.4 N=29[b]	70.9 ± 0.7 N=29[d]	72.5 ± 0.8 N=29[b]
Feed Consumption (sd 21 to 28) (g/kg/day)[a]				
	66.9 ± 0.9 N=30	66.1 ± 0.6 N=30	65.2 ± 0.6 N=30	66.5 ± 0.7 N=30
Feed Consumption (sd 28 to 35) (g/kg/day)[a]				
	61.8 ± 0.7 N=30	62.2 ± 0.5 N=30	60.8 ± 0.6 N=30	63.1 ± 1.1 N=30
Feed Consumption (sd 35 to 42) (g/kg/day)[a]				
	60.8 ‡ ± 0.7 N=30	61.6 ± 0.6 N=29[e]	58.5 ± 0.5 N=30	61.0 ± 1.0 N=29[b]

(continued)

Table 4. Summary and Statistical Analysis of the F_0 Male Feed Consumption, Percent Food Efficiency and Butyl Benzyl Phthalate Intake During the Prebreed and Mating Periods (page 3 of 6)

	Butyl Benzyl Phthalate (ppm in the feed)			
	0	750	3750	11250
Feed Consumption (sd 42 to 49) (g/kg/day)[a]				
#	57.9 †† ± 0.6 N=30	57.9 ± 0.5 N=29	56.1 Þ ± 0.4 N=30	58.2 ± 1.0 N=30
Feed Consumption (sd 49 to 56) (g/kg/day)[a]				
	55.9 ‡ ± 0.5 N=30	57.2 ± 0.6 N=29	54.7 ± 0.5 N=30	56.5 ± 0.8 N=30
Feed Consumption (sd 56 to 63) (g/kg/day)[a]				
	53.6 ‡‡ ± 0.5 N=30	55.3 ± 0.6 N=29	52.3 ± 0.5 N=30	54.7 ± 0.8 N=30
Feed Consumption (sd 63 to 70) (g/kg/day)[a]				
	51.8 ‡ ± 0.5 N=30	52.4 ± 0.6 N=29	50.1 ± 0.4 N=30	52.5 ± 0.7 N=30
Feed Consumption (sd 0 to 70) (g/kg/day)[a]				
	63.4 ± 0.6 N=30	63.9 ± 0.4 N=27[f]	62.1 ± 0.4 N=29[f]	64.1 ± 1.0 N=21[f]
Feed Consumption (sd 77 to 84) (g/kg/day)[a,g]				
	50.1 ± 0.8 N=27	51.7 ± 0.6 N=25	49.0 ± 0.5 N=26	52.5 ± 1.1 N=26
Percent Food Efficiency (sd 0 to 7)[a]				
#	33.2 ††† ± 0.6 ŸŸŸ N=30	32.0 ± 0.4 N=29[b]	32.4 ± 0.5 N=30	28.7 ÞÞÞ ± 0.8 N=23[c]
Percent Food Efficiency (sd 7 to 14)[a]				
#	27.7 ††† ± 0.5 Ÿ N=30	26.7 ± 0.4 N=30	30.5 ÞÞÞ ± 0.5 N=30	29.4 ± 0.8 N=30
Percent Food Efficiency (sd 14 to 21)[a]				
	22.0 ± 0.5 N=30	21.7 ± 0.6 N=29[b]	23.0 ± 0.5 N=29[d]	21.5 ± 0.5 N=29[b]

(continued)

Table 4. Summary and Statistical Analysis of the F_0 Male Feed Consumption, Percent Food Efficiency and Butyl Benzyl Phthalate Intake During the Prebreed and Mating Periods (page 4 of 6)

	Butyl Benzyl Phthalate (ppm in the feed)			
	0	750	3750	11250
Percent Food Efficiency (sd 21 to 28)[a]				
	18.2 ± 0.5 N=30	18.2 ± 0.9 N=30	19.4 ± 0.6 N=30	17.9 ± 0.4 N=30
Percent Food Efficiency (sd 28 to 35)[a]				
	11.4 ± 0.5 N=30	11.5 ± 0.8 N=30	13.4 ± 0.5 N=30	11.8 ± 0.5 N=30
Percent Food Efficiency (sd 35 to 42)[a]				
	14.0 ‡‡ ± 0.5 N=30	12.7 ± 0.5 N=29[e]	14.9 ± 0.5 N=30	13.0 ± 0.4 N=29[b]
Percent Food Efficiency (sd 42 to 49)[a]				
	9.8 ‡ ± 0.4 N=30	9.6 ± 0.4 N=29	11.1 * ± 0.3 N=30	9.5 ± 0.3 N=30
Percent Food Efficiency (sd 49 to 56)[a]				
	7.9 ± 0.4 § N=30	7.3 ± 0.4 N=29	7.9 ± 0.4 N=30	6.6 ± 0.5 N=30
Percent Food Efficiency (sd 56 to 63)[a]				
	8.5 ± 0.4 N=30	7.4 ± 0.3 N=29	8.0 ± 0.5 N=30	8.4 ± 0.3 N=30
Percent Food Efficiency (sd 63 to 70)[a]				
	8.2 ‡‡‡ ± 0.5 §§§ N=30	9.6 ± 0.4 N=29	11.1 *** ± 0.5 N=30	10.8 *** ± 0.3 N=30
Percent Food Efficiency (sd 0 to 70)[a]				
	15.8 ‡‡‡ ± 0.3 N=30	15.3 ± 0.2 N=27[f]	16.9 ** ± 0.3 N=29[f]	15.6 ± 0.3 N=21[f]
Percent Food Efficiency (sd 77 to 84)[a,g]				
	7.4 ± 0.4 N=27	6.8 ± 0.4 N=25	8.3 ± 0.5 N=26	7.7 ± 0.5 N=26

(continued)

Table 4. Summary and Statistical Analysis of the F_0 Male Feed Consumption, Percent Food Efficiency and Butyl Benzyl Phthalate Intake During the Prebreed and Mating Periods (page 5 of 6)

	Butyl Benzyl Phthalate (ppm in the feed)			
	0	750	3750	11250
Butyl Benzyl Phthalate Intake (sd 0 to 7) (mg/kg/day)[a]		68.93 ± 0.53 N=29[b]	348.19 ± 2.67 N=30	1089.47 ± 24.75 N=23[c]
Butyl Benzyl Phthalate Intake (sd 7 to 14) (mg/kg/day)[a]		59.82 ± 0.42 N=30	299.04 ± 2.85 N=30	943.58 ± 14.18 N=30
Butyl Benzyl Phthalate Intake (sd 14 to 21) (mg/kg/day)[a]		54.22 ± 0.33 N=29[b]	265.90 ± 2.47 N=29[d]	815.67 ± 8.52 N=29[b]
Butyl Benzyl Phthalate Intake (sd 21 to 28) (mg/kg/day)[a]		49.60 ± 0.42 N=30	244.46 ± 2.08 N=30	748.29 ± 8.24 N=30
Butyl Benzyl Phthalate Intake (sd 28 to 35) (mg/kg/day)[a]		46.67 ± 0.39 N=30	228.07 ± 2.23 N=30	709.97 ± 11.89 N=30
Butyl Benzyl Phthalate Intake (sd 35 to 42) (mg/kg/day)[a]		46.20 ± 0.45 N=29[e]	219.48 ± 1.87 N=30	686.39 ± 11.31 N=29[b]
Butyl Benzyl Phthalate Intake (sd 42 to 49) (mg/kg/day)[a]		43.39 ± 0.36 N=29	210.38 ± 1.58 N=30	654.91 ± 11.30 N=30
Butyl Benzyl Phthalate Intake (sd 49 to 56) (mg/kg/day)[a]		42.91 ± 0.41 N=29	204.95 ± 1.71 N=30	635.48 ± 8.64 N=30
Butyl Benzyl Phthalate Intake (sd 56 to 63) (mg/kg/day)[a]		41.47 ± 0.46 N=29	196.27 ± 1.85 N=30	615.47 ± 8.63 N=30

(continued)

Table 4. Summary and Statistical Analysis of the F_0 Male Feed Consumption, Percent Food Efficiency and Butyl Benzyl Phthalate Intake During the Prebreed and Mating Periods (page 6 of 6)

	Butyl Benzyl Phthalate (ppm in the feed)			
	0	750	3750	11250
Butyl Benzyl Phthalate Intake (sd 63 to 70) (mg/kg/day)[a]				
		39.27 ± 0.46 N=29	188.00 ± 1.41 N=30	590.68 ± 7.72 N=30
Butyl Benzyl Phthalate Intake (sd 0 to 70) (mg/kg/day)[a]				
		47.94 ± 0.33 N=27[f]	232.93 ± 1.59 N=29[f]	720.69 ± 10.98 N=21[f]
Butyl Benzyl Phthalate Intake (sd 77 to 84) (mg/kg/day)[a]				
		38.75 ± 0.47 N=25	183.74 ± 1.90 N=26	591.00 ± 12.81 N=26

[a]Reported as the mean ± S.E.M. sd = study day with study day 0 being the first day of exposure.
[b]Decrease in N is due to the feed consumption value for one male being a statistical outlier and it was therefore removed.
[c]Decrease in N is due to one or more males scratching the feed out of the feed jar and therefore an accurate feed weight could not be obtained.
[d]Decrease in N is due to the feed weight for one male inadvertently not being recorded.
[e]Decrease in N is due to male 63 being found dead on study day 40.
[f]Decrease in N is due to interim feed consumption value(s) for one or more males being missing and therefore the overall feed consumption value could not be calculated.
[g]Includes those males that had mated with a female. Statistical analyses were not performed on these endpoints since not all males were represented.
[#]Levene's test for homogeneity of variances was significant ($p<0.05$), therefore robust regression methods were used to test all treatment effects.
‡$p<0.05$; ANOVA Test.
‡‡$p<0.01$; ANOVA Test.
‡‡‡$p<0.001$; ANOVA Test.
§$p<0.05$; Test for Linear Trend.
§§§$p<0.001$; Test for Linear Trend.
*$p<0.05$; Dunnett's Test.
**$p<0.01$; Dunnett's Test.
***$p<0.001$; Dunnett's Test.
†$p<0.05$; Wald Chi-square Test for overall treatment effect in robust regression model.
††$p<0.01$; Wald Chi-square Test for overall treatment effect in robust regression model.
†††$p<0.001$; Wald Chi-square Test for overall treatment effect in robust regression model.
Þ$p<0.05$; Individual t-test for pairwise comparisons to control in robust regression model.
ÞÞ$p<0.01$; Individual t-test for pairwise comparisons to control in robust regression model.
ÞÞÞ$p<0.001$; Individual t-test for pairwise comparisons to control in robust regression model.
Ÿ$p<0.05$; Linear trend test in robust regression model.
ŸŸ$p<0.01$; Linear trend test in robust regression model.
ŸŸŸ$p<0.001$; Linear trend test in robust regression model.

Table 5. Summary of the F_0 Male Clinical Observations During the Prebreed and Mating Periods
(page 1 of 10)

A. Clinical Observations Summarized by Group

	Butyl Benzyl Phthalate (ppm in the feed)			
Observation	0	750	3750	11250
Alopecia	6	2	4	8
Chromodacryorrhea		1	1	2
Found dead		1		
Rust colored fur	3		1	2
Scab(s)			1	
Sore(s)			1	2
Stressed during ear tagging, mouth bled				1
Toe: torn nail, right rear paw			1	

B. Clinical Observations Summarized by Group and Day

		Butyl Benzyl Phthalate (ppm in the feed)			
Day[a]	Observation[b]	0	750	3750	11250
7	Alopecia: limb(s)				1
8	Alopecia: limb(s)	1			1
9	Alopecia: limb(s)	1			1
10	Alopecia: limb(s)	1			1
11	Alopecia: limb(s)	1			1
12	Alopecia: limb(s)	1			1
	Sore(s): neck				1
13	Alopecia: limb(s)	1			1
	Sore(s): neck				1
14	Alopecia: limb(s)	1			1
	Rust colored fur: limb(s)	1			
	neck and shoulder(s)	1			
	Sore(s): neck				1
15	Alopecia: limb(s)	1			1
	Rust colored fur: limb(s)	1			
	neck and shoulder(s)	1			
	Sore(s): neck				1
16	Alopecia: limb(s)	1			1
	Chromodacryorrhea: eye, left				1
	Rust colored fur: limb(s)	1			
	neck and shoulder(s)	1			
	Sore(s): neck				1
17	Alopecia: limb(s)	1			1
	Chromodacryorrhea: eye, left				1
	Rust colored fur: limb(s)	1			
	neck and shoulder(s)	1			
	Sore(s): neck				1

(continued)

Table 5. Summary of the F_0 Male Clinical Observations During the Prebreed and Mating Periods
(page 2 of 10)

Day[a]	Observation[b]	Butyl Benzyl Phthalate (ppm in the feed)			
		0	750	3750	11250
18	Alopecia: limb(s)	1			1
	Chromodacryorrhea: eye, left				1
	Rust colored fur: limb(s)	1			
	neck and shoulder(s)	1			
	Sore(s): neck				1
19	Alopecia: limb(s)	1			1
	Chromodacryorrhea: eye, left				1
	Rust colored fur: limb(s)	1			
	neck and shoulder(s)	1			
	Sore(s): neck				1
20	Alopecia: limb(s)	1			1
	Chromodacryorrhea: eye, left				1
	Rust colored fur: limb(s)	1			
	neck and shoulder(s)	1			
	Sore(s): neck, treated with 0.2% nitrofurazone powder				1
21	Alopecia: limb(s)	1	1		1
	Chromodacryorrhea: eye, left				1
	Rust colored fur: limb(s)	1			
	neck and shoulder(s)	1			
	Sore(s): neck, treated with povidone iodine (0.75% iodine)				1
22	Alopecia: limb(s)	1	1		1
	Chromodacryorrhea: eye, left, slight				1
	Rust colored fur: limb(s)	1			
	neck and shoulder(s)	1			
	Sore(s): neck, treated with povidone iodine (0.75% iodine)				1
23	Alopecia: limb(s)	1	1		1
	Chromodacryorrhea: eye, left				1
	Rust colored fur: limb(s)	1			
	neck and shoulder(s)	1			
	Sore(s): neck, treated with povidone iodine (0.75% iodine)				1
24	Alopecia: limb(s)	1	1		1
	Chromodacryorrhea: eye, left				1
	Rust colored fur: limb(s)	1			
	neck and shoulder(s)	1			
	Sore(s): neck, treated with povidone iodine (0.75% iodine)				1
25	Alopecia: limb(s)	1	1		1
	Chromodacryorrhea: eye, left				1
	Rust colored fur: limb(s)	1			
	neck and shoulder(s)	1			
	Sore(s): neck, treated with povidone iodine (0.75% iodine)				1
26	Alopecia: limb(s)	1	1		1
	Chromodacryorrhea: eye, left				1
	Rust colored fur: limb(s)	1			
	neck and shoulder(s)	1			
	Sore(s): neck, treated with povidone iodine (0.75% iodine)				1

(continued)

Table 5. Summary of the F_0 Male Clinical Observations During the Prebreed and Mating Periods
(page 3 of 10)

Day[a]	Observation[b]	Butyl Benzyl Phthalate (ppm in the feed)			
		0	750	3750	11250
27	Alopecia: limb(s)	1	1		1
	Chromodacryorrhea: eye, left				1
	Rust colored fur: limb(s)	1			
	neck and shoulder(s)	1			
	Sore(s): neck, treated with povidone iodine (0.75% iodine)				1
28	Alopecia: limb(s)	1	1		1
	Chromodacryorrhea: eye, left				1
	Rust colored fur: limb(s)	1			
	neck and shoulder(s)	1			
	shoulder(s)				1
	Sore(s): neck, treated with povidone iodine (0.75% iodine)				1
29	Alopecia: limb(s)	1	1		2
	Chromodacryorrhea: eye, left				1
	Rust colored fur: limb(s)	1			
	neck and shoulder(s)	1			
	Sore(s): neck, treated with 0.2% nitrofurazone powder				1
30	Alopecia: limb(s)	2	1		3
	Chromodacryorrhea: eye, left				1
	Rust colored fur: limb(s)	1			
	neck and shoulder(s), improving	1			
	Sore(s): neck, treated with 0.2% nitrofurazone powder				1
31	Alopecia: limb(s)	2	1		3
	Chromodacryorrhea: eye, left				1
	Rust colored fur: limb(s)	1			
	neck and shoulder(s)	1			
	Sore(s): neck, treated with 0.2% nitrofurazone powder				1
32	Alopecia: limb(s)	2	1		3
	Chromodacryorrhea: eye, left				1
	Rust colored fur: limb(s)	1			
	neck and shoulder(s)	1			
	Sore(s): neck, treated with 0.2% nitrofurazone powder				1
33	Alopecia: limb(s)	2	1		3
	Chromodacryorrhea: eye, left				1
	Rust colored fur: limb(s)	1			
	neck and shoulder(s), much improved	1			
	Sore(s): neck, treated with 0.2% nitrofurazone powder				1
34	Alopecia: limb(s)	2	1		3
	Chromodacryorrhea: eye, left				1
	nose				1
	Rust colored fur: limb(s)	1			
	neck and shoulder(s), improving	1			
	Sore(s): neck, treated with 0.2% nitrofurazone powder				1

(continued)

Table 5. Summary of the F_0 Male Clinical Observations During the Prebreed and Mating Periods
(page 4 of 10)

Day[a]	Observation[b]	Butyl Benzyl Phthalate (ppm in the feed) 0	750	3750	11250
35	Alopecia: limb(s)	3	1		5
	Chromodacryorrhea: eye, left				1
	Rust colored fur: limb(s)	1			1
	Sore(s): neck, treated with 0.2% nitrofurazone powder				1
36	Alopecia: limb(s)	3	1		5
	Chromodacryorrhea: eye, left				1
	Rust colored fur: limb(s)	1			1
	Sore(s): neck, treated with 0.2% nitrofurazone powder				1
37	Alopecia: limb(s)	3	1		5
	Chromodacryorrhea: eye, left				1
	Rust colored fur: limb(s)	1			1
	Sore(s): neck, treated with 0.2% nitrofurazone powder				1
38	Alopecia: limb(s)	3	1		5
	Chromodacryorrhea: eye, left				1
	Rust colored fur: limb(s)	1			1
	Sore(s): neck, treated with 0.2% nitrofurazone powder				1
39	Alopecia: limb(s)	3	1		5
	Chromodacryorrhea: eye, left				1
	Rust colored fur: limb(s)	1			1
	Sore(s): neck, treated with 0.2% nitrofurazone powder				1
40	Alopecia: limb(s)	4	1		4
	multiple areas				1
	Chromodacryorrhea: eye, left, slight				1
	Found dead		1		
	Rust colored fur: limb(s)	1			1
	Sore(s): neck, treated with 0.2% nitrofurazone powder				1
41	Alopecia: limb(s)	5	1		4
	multiple areas				1
	Chromodacryorrhea: eye, left, gone				1
	Rust colored fur: limb(s)	2			1
	Sore(s): neck, treated with 0.2% nitrofurazone powder				1
42	Alopecia: limb(s)	5	1		4
	multiple areas				1
	Rust colored fur: limb(s)	2			1
	Sore(s): neck, treated with 0.2% nitrofurazone powder				1
43	Alopecia: limb(s)	5	1		4
	multiple areas				1
	Rust colored fur: limb(s)	2			1
	Sore(s): neck, treated with 0.2% nitrofurazone powder				1
44	Alopecia: limb(s)	5	1		4
	multiple areas				1
	Rust colored fur: limb(s)	2			1
	Sore(s): neck, treated with 0.2% nitrofurazone powder				1

(continued)

Table 5. Summary of the F_0 Male Clinical Observations During the Prebreed and Mating Periods
(page 5 of 10)

Day[a]	Observation[b]	Butyl Benzyl Phthalate (ppm in the feed) 0	750	3750	11250
45	Alopecia: limb(s)	5	1		4
	multiple areas				1
	Rust colored fur: limb(s)	2			1
	Sore(s): neck, treated with 0.2% nitrofurazone powder				1
46	Alopecia: limb(s)	5	1		4
	multiple areas				1
	Rust colored fur: limb(s)	2			1
	Sore(s): neck				1
47	Alopecia: limb(s)	5	1		4
	multiple areas				1
	Rust colored fur: limb(s)	2			1
	Sore(s): neck, treated with 0.2% nitrofurazone powder				1
48	Alopecia: limb(s)	5	1		4
	multiple areas				1
	Rust colored fur: limb(s)	2			1
	Sore(s): neck, treated with 0.2% nitrofurazone powder, improving				1
49	Alopecia: limb(s)	5	1	2	3
	limb(s), growing in				1
	multiple areas				1
	Rust colored fur: limb(s)	1		1	1
	limb(s), improving	1			
	Sore(s): neck, treated with 0.2% nitrofurazone powder				1
50	Alopecia: limb(s)	5	1	2	3
	limb(s), growing in				1
	multiple areas				1
	Rust colored fur: limb(s)	2		1	1
	Sore(s): neck, treated with 0.2% nitrofurazone powder				1
51	Alopecia: limb(s)	5	1	2	3
	limb(s), growing in				1
	multiple areas				1
	Rust colored fur: limb(s)	2		1	1
	Sore(s): neck, treated with 0.2% nitrofurazone powder				1
	Stressed during ear tagging, mouth bled				1
52	Alopecia: limb(s)	5	1	2	3
	limb(s), growing in				1
	multiple areas				1
	Chromodacryorrhea: nose			1	
	Rust colored fur: limb(s)	2		1	1
	Sore(s): neck, treated with 0.2% nitrofurazone powder				1
53	Alopecia: limb(s)	5	1	2	3
	multiple areas				1
	Rust colored fur: limb(s)	1		1	1
	limb(s), gone	1			
	Sore(s): neck, treated with 0.2% nitrofurazone powder				1

(continued)

Table 5. Summary of the F_0 Male Clinical Observations During the Prebreed and Mating Periods
(page 6 of 10)

Day[a]	Observation[b]	Butyl Benzyl Phthalate (ppm in the feed) 0	750	3750	11250
54	Alopecia: limb(s)	5	1	2	3
	multiple areas				1
	Rust colored fur: limb(s)	1		1	1
	Sore(s): neck, is one small scab				1
55	Alopecia: limb(s)	5	1	2	3
	multiple areas				1
	Rust colored fur: limb(s)	1		1	1
	Sore(s): neck				1
56	Alopecia: limb(s)	5	1	2	2
	limb(s), growing in				1
	multiple areas				1
	neck				1
	Rust colored fur: limb(s)	1		1	1
	Sore(s): neck				1
	shoulder(s)				1
57	Alopecia: limb(s)	5	1	2	2
	multiple areas				1
	neck				1
	Rust colored fur: limb(s)	1		1	1
	Sore(s): neck				2
58	Alopecia: limb(s)	5	1	2	2
	multiple areas				1
	neck				1
	Rust colored fur: limb(s)	1		1	1
	Sore(s): neck				2
59	Alopecia: limb(s)	5	1	2	2
	multiple areas				1
	neck				1
	Rust colored fur: limb(s)	1		1	1
	Sore(s): neck				2
60	Alopecia: limb(s)	5	1	2	2
	multiple areas				1
	neck				1
	Rust colored fur: limb(s)	1		1	1
	Sore(s): neck				2
61	Alopecia: limb(s)	5	1	2	2
	multiple areas				1
	neck				1
	Rust colored fur: limb(s)	1		1	1
	Sore(s): neck				2
62	Alopecia: limb(s)	5	1	2	2
	multiple areas				1
	neck				1
	Rust colored fur: limb(s)	1		1	1
	Sore(s): neck				2

(continued)

Table 5. Summary of the F_0 Male Clinical Observations During the Prebreed and Mating Periods
(page 7 of 10)

Day[a]	Observation[b]	Butyl Benzyl Phthalate (ppm in the feed) 0	750	3750	11250
63	Alopecia: limb(s)	4	2	2	4
	limb(s), growing in	1			
	multiple areas				1
	neck				1
	Rust colored fur: limb(s)	1		1	1
	Sore(s): neck				1
	neck, healing				1
64	Alopecia: limb(s)	5	2	2	4
	multiple areas				1
	neck				1
	Rust colored fur: limb(s)	1		1	1
	Sore(s): neck				1
	neck, healing				1
65	Alopecia: limb(s)	5	2	2	4
	multiple areas				1
	neck				1
	Rust colored fur: limb(s)	1		1	1
	Sore(s): neck				1
66	Alopecia: limb(s)	5	2	2	4
	multiple areas				1
	neck				1
	Rust colored fur: limb(s)	1		1	1
	Sore(s): neck				1
67	Alopecia: limb(s)	5	2	2	4
	multiple areas				1
	neck				1
	Rust colored fur: limb(s)	1		1	1
	Sore(s): neck				1
68	Alopecia: limb(s)	5	2	2	4
	multiple areas				1
	neck				1
	Rust colored fur: limb(s)	1		1	1
	Sore(s): neck				1
69	Alopecia: limb(s)	5	2	2	4
	multiple areas				1
	neck, growing in				1
	Rust colored fur: limb(s)	1		1	1
	Sore(s): neck				1
70	Alopecia: limb(s)	4	2	2	3
	limb(s), growing in	1			
	multiple areas				2
	neck, growing in				1
	Rust colored fur: limb(s)	1		1	1
	shoulder(s)				1
	Sore(s): neck			1	1

(continued)

Table 5. Summary of the F_0 Male Clinical Observations During the Prebreed and Mating Periods
(page 8 of 10)

Day[a]	Observation[b]	Butyl Benzyl Phthalate (ppm in the feed) 0	750	3750	11250
71	Alopecia: limb(s)	4	2	2	3
	limb(s), growing in	1			
	multiple areas				2
	Chromodacryorrhea: nose		1		
	Rust colored fur: limb(s)	1		1	1
	shoulder(s)				1
	Sore(s): neck			1	
	neck, is one small scab				1
72	Alopecia: limb(s)	4	2	2	3
	multiple areas				2
	Chromodacryorrhea: nose, improving		1		
	Rust colored fur: limb(s)	1		1	1
	shoulder(s)				1
	Scab(s): neck			1	
	Sore(s): neck, healed			1	
	neck, healing				1
73	Alopecia: limb(s)	4	2	2	3
	multiple areas				2
	Chromodacryorrhea: nose		1		
	Rust colored fur: limb(s)	1		1	1
	shoulder(s)				1
	Scab(s): neck			1	
	Sore(s): neck, healing				1
	Toe: torn nail, right rear paw			1	
74	Alopecia: limb(s)	4	2	2	3
	multiple areas				2
	Chromodacryorrhea: nose		1		
	Rust colored fur: limb(s)	1		1	1
	shoulder(s)				1
	Scab(s): neck			1	
	Sore(s): neck, healing				1
75	Alopecia: limb(s)	4	2	2	3
	multiple areas				2
	neck			1	
	Rust colored fur: limb(s)	1		1	1
	shoulder(s), improved				1
	Scab(s): neck, gone			1	
	Sore(s): neck, healing				1
76	Alopecia: limb(s)	4	2	2	3
	multiple areas				2
	neck			1	
	Rust colored fur: limb(s)	1		1	1
	Sore(s): neck, healing				1

(continued)

Table 5. Summary of the F_0 Male Clinical Observations During the Prebreed and Mating Periods
(page 9 of 10)

Day[a]	Observation[b]	Butyl Benzyl Phthalate (ppm in the feed)			
		0	750	3750	11250
77	Alopecia: limb(s)	2	2	2	3
	limb(s), growing in	2			
	multiple areas				2
	neck			1	
	Rust colored fur: limb(s)	1		1	1
	Sore(s): neck, healing				1
78	Alopecia: limb(s)	2	2	2	3
	limb(s), growing in	2			
	multiple areas				2
	neck			1	
	Rust colored fur: limb(s)	1		1	1
	Sore(s): neck, healing				1
79	Alopecia: limb(s)	3	2	2	3
	multiple areas				2
	neck			1	
	Rust colored fur: limb(s)	1		1	1
	Sore(s): neck, healing				1
80	Alopecia: limb(s)	3	2	2	3
	multiple areas				2
	neck			1	
	Rust colored fur: limb(s)	1		1	1
	Sore(s): neck, healing				1
81	Alopecia: limb(s)	3	2	2	3
	multiple areas				2
	neck			1	
	Rust colored fur: limb(s)	1		1	1
	Sore(s): neck, healing				1
82	Alopecia: face			1	
	limb(s)	3	2	2	3
	multiple areas				2
	neck			1	
	side(s)	1			
	Rust colored fur: limb(s)	1		1	1
	Sore(s): neck, bleeding				1
83	Alopecia: face			1	
	limb(s)	3	2	2	3
	multiple areas	1			2
	neck			1	
	Rust colored fur: limb(s)	1		1	1
	Sore(s): neck				1
84	Alopecia: face			1	
	limb(s)	3	2	2	3
	multiple areas	1			2
	neck, growing in			1	
	Rust colored fur: limb(s)	1		1	1
	Sore(s): neck				1

(continued)

Table 5. Summary of the F_0 Male Clinical Observations During the Prebreed and Mating Periods
(page 10 of 10)

[a]Study day.
[b]Clinical observations are tabulated once per day per animal.

Table 6. Summary and Statistical Analysis of the F_0 Female Body Weights and Weight Changes During the Prebreed and Mating Periods (page 1 of 4)

	Butyl Benzyl Phthalate (ppm in the feed)			
	0	750	3750	11250
No. Females on Study	30	30	30	30
Body Weight (sd 0) (g)[a]	179.9 ± 1.7 N=30	179.5 ± 1.6 N=30	179.1 ± 1.6 N=30	179.0 ± 1.6 N=30
Body Weight (sd 7) (g)[a]	211.3 ‡‡ ± 2.0 §§§ N=30	210.3 ± 1.7 N=30	208.3 ± 2.1 N=30	200.8 *** ± 2.1 N=30
Body Weight (sd 14) (g)[a]	230.0 ‡‡ ± 2.7 §§§ N=30	229.9 ± 2.0 N=30	228.3 ± 2.5 N=30	218.5 ** ± 2.3 N=30
Body Weight (sd 21) (g)[a]	250.4 ‡‡ ± 3.4 §§§ N=30	249.0 ± 2.5 N=30	247.4 ± 2.9 N=30	235.4 ** ± 2.8 N=30
Body Weight (sd 28) (g)[a]	263.9 ‡‡‡ ± 3.6 §§§ N=30	266.5 ± 2.9 N=30	260.6 ± 3.3 N=30	248.4 ** ± 3.0 N=30
Body Weight (sd 35) (g)[a]	273.7 ‡‡‡ ± 3.9 §§§ N=30	277.5 ± 3.1 N=30	272.4 ± 3.2 N=30	255.7 *** ± 2.8 N=30
Body Weight (sd 42) (g)[a]	282.9 ‡‡‡ ± 3.9 §§§ N=30	286.9 ± 3.6 N=30	283.4 ± 3.4 N=30	265.0 ** ± 3.1 N=30
Body Weight (sd 49) (g)[a]	292.6 ‡‡‡ ± 4.1 §§§ N=30	296.3 ± 3.9 N=30	291.0 ± 3.4 N=30	269.5 *** ± 3.3 N=30
Body Weight (sd 56) (g)[a]	296.7 ‡‡‡ ± 4.4 §§§ N=30	303.4 ± 4.6 N=30	301.9 ± 3.9 N=30	278.1 ** ± 3.6 N=30

(continued)

Table 6. Summary and Statistical Analysis of the F_0 Female Body Weights and Weight Changes During the Prebreed and Mating Periods (page 2 of 4)

	Butyl Benzyl Phthalate (ppm in the feed)			
	0	750	3750	11250
Body Weight (sd 63) (g)[a]				
	301.8 ‡‡‡ ± 4.3 §§§ N=30	309.0 ± 5.1 N=30	311.7 ± 4.3 N=30	283.7 * ± 3.5 N=30
Body Weight (sd 70) (g)[a]				
	305.4 ‡‡‡ ± 4.3 §§§ N=30	312.8 ± 5.2 N=30	313.0 ± 4.4 N=30	287.0 * ± 4.0 N=30
Body Weight (sd 77) (g)[a,b]				
	324.3 ± 15.5 N=3	341.7 ± 14.0 N=4	332.3 ± 11.2 N=4	302.2 ± 5.2 N=4
Body Weight (sd 84) (g)[a,b]				
	. ± . N=0	357.7 ± 19.8 N=3	356.9 ± 15.8 N=3	320.6 ± 6.6 N=2
Body Weight Change (sd 0 to 7) (g)[a]				
	31.4 ‡‡‡ ± 0.8 §§§ N=30	30.7 ± 0.9 N=30	29.1 ± 1.1 N=30	21.9 *** ± 1.0 N=30
Body Weight Change (sd 7 to 14) (g)[a]				
	18.7 ± 1.1 N=30	19.6 ± 1.1 N=30	20.1 ± 1.2 N=30	17.7 ± 0.9 N=30
Body Weight Change (sd 14 to 21) (g)[a]				
	20.4 ± 1.4 § N=30	19.1 ± 1.2 N=30	19.1 ± 1.1 N=30	16.9 ± 1.0 N=30
Body Weight Change (sd 21 to 28) (g)[a]				
	13.5 ‡‡ ± 0.9 N=30	17.4 * ± 1.0 N=30	13.2 ± 1.5 N=30	13.1 ± 0.7 N=30
Body Weight Change (sd 28 to 35) (g)[a]				
	9.7 ‡ ± 1.2 § N=30	11.0 ± 1.0 N=30	11.8 ± 1.5 N=30	7.3 ± 0.6 N=30

(continued)

Table 6. Summary and Statistical Analysis of the F_0 Female Body Weights and Weight Changes During the Prebreed and Mating Periods (page 3 of 4)

	Butyl Benzyl Phthalate (ppm in the feed)			
	0	750	3750	11250
Body Weight Change (sd 35 to 42) (g)[a]				
	9.3 ± 1.1 N=30	9.5 ± 0.9 N=30	11.0 ± 0.9 N=30	9.3 ± 0.7 N=30
Body Weight Change (sd 42 to 49) (g)[a]				
	9.7 ‡‡‡ ± 0.9 §§§ N=30	9.4 ± 1.0 N=30	7.6 ± 0.9 N=30	4.5 *** ± 0.9 N=30
Body Weight Change (sd 49 to 56) (g)[a]				
	4.1 ‡‡ ± 1.1 § N=30	7.1 ± 1.2 N=30	10.9 *** ± 1.4 N=30	8.6 * ± 1.1 N=30
Body Weight Change (sd 56 to 63) (g)[a]				
#	5.1 ± 1.1 N=30	5.6 ± 1.1 N=30	9.8 ± 1.6 N=30	5.6 ± 0.9 N=30
Body Weight Change (sd 63 to 70) (g)[a]				
#	3.5 ± 1.5 N=30	3.8 ± 1.3 N=30	1.4 ± 2.2 N=30	3.3 ± 1.3 N=30
Body Weight Change (sd 0 to 70) (g)[a]				
	125.4 ‡‡‡ ± 3.4 §§§ N=30	133.3 ± 4.8 N=30	133.9 ± 3.9 N=30	108.1 ** ± 3.3 N=30
Body Weight Change (sd 70 to 77) (g)[a,b]				
	20.7 ± 4.1 N=3	19.8 ± 1.2 N=4	16.8 ± 5.6 N=4	19.0 ± 1.9 N=4
Body Weight Change (sd 77 to 84) (g)[a,b]				
	. ± . N=0	21.0 ± 4.6 N=3	24.9 ± 0.9 N=3	23.6 ± 3.3 N=2

(continued)

Table 6. Summary and Statistical Analysis of the F_0 Female Body Weights and Weight Changes During the Prebreed and Mating Periods (page 4 of 4)

[a]Reported as the mean ± S.E.M. sd = study day with study day 0 being the first day of exposure.
[b]Includes all females that were not found sperm and/or plug positive. Statistical analyses were not performed on these endpoints since not all females were represented.
[#]Levene's test for homogeneity of variances was significant ($p<0.05$), therefore robust regression methods were used to test all treatment effects.
‡$p<0.05$; ANOVA Test.
‡‡$p<0.01$; ANOVA Test.
‡‡‡$p<0.001$; ANOVA Test.
§$p<0.05$; Test for Linear Trend.
§§§$p<0.001$; Test for Linear Trend.
*$p<0.05$; Dunnett's Test.
**$p<0.01$; Dunnett's Test.
***$p<0.001$; Dunnett's Test.

Table 7. Summary and Statistical Analysis of the F_0 Female Feed Consumption, Percent Food Efficiency and Butyl Benzyl Phthalate Intake During the Prebreed Period (page 1 of 6)

	Butyl Benzyl Phthalate (ppm in the feed)			
	0	750	3750	11250
No. Females on Study	30	30	30	30
Feed Consumption (sd 0 to 7) (g/day)[a]				
#	18.8 ± 0.3 N=30	19.1 ± 0.3 N=30	18.5 ± 0.3 N=30	19.6 ± 0.7 N=25[b]
Feed Consumption (sd 7 to 14) (g/day)[a]				
#	19.6 ± 0.4 N=30	19.5 ± 0.3 N=30	20.1 ± 0.6 N=30	20.1 ± 0.7 N=30
Feed Consumption (sd 14 to 21) (g/day)[a]				
	20.2 ± 0.5 N=30	20.0 ± 0.3 N=30	20.7 ± 0.6 N=29[b]	19.3 ± 0.4 N=30
Feed Consumption (sd 21 to 28) (g/day)[a]				
	20.9 ± 0.5 N=30	20.4 ± 0.3 N=30	21.4 ± 0.6 N=30	20.1 ± 0.7 N=30
Feed Consumption (sd 28 to 35) (g/day)[a]				
	20.9 ± 0.6 N=30	20.8 ± 0.5 N=30	22.2 ± 0.9 N=29[b]	19.7 ± 0.6 N=28[c]
Feed Consumption (sd 35 to 42) (g/day)[a]				
	20.8 ± 0.5 N=30	21.2 ± 0.4 N=30	21.9 ± 0.6 N=29[b]	19.8 ± 0.5 N=28[b]
Feed Consumption (sd 42 to 49) (g/day)[a]				
	21.2 ± 0.5 N=30	21.1 ± 0.4 N=30	21.6 ± 0.5 N=29[c]	20.1 ± 0.6 N=28[b]
Feed Consumption (sd 49 to 56) (g/day)[a]				
	19.1 ‡ ± 0.5 § N=29[b]	19.4 ± 0.5 N=30	20.0 ± 0.5 N=29[b]	18.1 ± 0.4 N=28[c]
Feed Consumption (sd 56 to 63) (g/day)[a]				
	18.8 ± 0.6 N=30	19.1 ± 0.4 N=30	20.1 ± 0.6 N=29[b]	18.4 ± 0.5 N=30

(continued)

Table 7. Summary and Statistical Analysis of the F_0 Female Feed Consumption, Percent Food Efficiency and Butyl Benzyl Phthalate Intake During the Prebreed Period (page 2 of 6)

	Butyl Benzyl Phthalate (ppm in the feed)			
	0	750	3750	11250
Feed Consumption (sd 63 to 70) (g/day)[a]				
	18.5 ± 0.5 N=29[b]	18.7 ± 0.4 N=30	19.0 ± 0.4 N=29[b]	17.9 ± 0.5 N=30
Feed Consumption (sd 0 to 70) (g/day)[a]				
	19.8 ‡ ± 0.5 § N=29[d]	19.9 ± 0.3 N=30	20.5 ± 0.5 N=29[d]	18.6 ± 0.3 N=21[d]
Feed Consumption (sd 0 to 7) (g/kg/day)[a]				
#	96.4 ± 1.8 N=30	97.9 ± 1.4 N=30	95.7 ± 1.3 N=30	102.3 ± 3.2 N=25[b]
Feed Consumption (sd 7 to 14) (g/kg/day)[a]				
#	88.9 ± 1.8 N=30	88.6 ± 1.5 N=30	92.1 ± 2.6 N=30	96.2 ± 3.8 N=30
Feed Consumption (sd 14 to 21) (g/kg/day)[a]				
	84.1 ± 1.7 N=30	83.4 ± 1.2 N=30	87.2 ± 2.8 N=29[b]	85.2 ± 2.0 N=30
Feed Consumption (sd 21 to 28) (g/kg/day)[a]				
	81.4 ± 2.3 N=30	79.2 ± 1.1 N=30	84.6 ± 2.6 N=30	83.0 ± 2.6 N=30
Feed Consumption (sd 28 to 35) (g/kg/day)[a]				
	78.0 ± 2.4 N=30	76.7 ± 1.9 N=30	83.7 ± 3.5 N=29[b]	78.3 ± 2.4 N=28[c]
Feed Consumption (sd 35 to 42) (g/kg/day)[a]				
	75.2 ± 2.2 N=30	75.1 ± 1.3 N=30	78.8 ± 2.4 N=29[b]	75.8 ± 1.9 N=28[b]
Feed Consumption (sd 42 to 49) (g/kg/day)[a]				
	73.9 ± 2.1 N=30	72.5 ± 1.3 N=30	75.3 ± 2.1 N=29[c]	75.2 ± 2.2 N=28[b]

(continued)

Table 7. Summary and Statistical Analysis of the F_0 Female Feed Consumption, Percent Food Efficiency and Butyl Benzyl Phthalate Intake During the Prebreed Period (page 3 of 6)

	Butyl Benzyl Phthalate (ppm in the feed)			
	0	750	3750	11250
Feed Consumption (sd 49 to 56) (g/kg/day)[a]				
	64.8 ± 1.9 N=29[b]	64.8 ± 1.2 N=30	67.6 ± 1.7 N=29[b]	66.0 ± 1.6 N=28[c]
Feed Consumption (sd 56 to 63) (g/kg/day)[a]				
	63.1 ± 2.1 N=30	62.2 ± 1.0 N=30	65.6 ± 1.8 N=29[b]	65.5 ± 1.6 N=30
Feed Consumption (sd 63 to 70) (g/kg/day)[a]				
	61.0 ± 1.8 N=29[b]	60.1 ± 1.1 N=30	60.7 ± 1.2 N=29[b]	62.7 ± 1.5 N=30
Feed Consumption (sd 0 to 70) (g/kg/day)[a]				
	75.5 ± 1.9 N=29[d]	75.1 ± 1.0 N=30	77.9 ± 1.8 N=29[d]	74.8 ± 1.3 N=21[d]
Percent Food Efficiency (sd 0 to 7)[a]				
	23.9 ‡‡‡ §§§ ± 0.6 N=30	23.1 ± 0.7 N=30	22.4 ± 0.8 N=30	16.9 *** ± 0.9 N=25[b]
Percent Food Efficiency (sd 7 to 14)[a]				
	13.6 ± 0.7 N=30	14.4 ± 0.7 N=30	14.4 ± 0.8 N=30	12.9 ± 0.7 N=30
Percent Food Efficiency (sd 14 to 21)[a]				
	14.4 ± 0.9 N=30	13.7 ± 0.7 N=30	13.6 ± 0.8 N=29[b]	12.6 ± 0.8 N=30
Percent Food Efficiency (sd 21 to 28)[a]				
	9.4 ‡‡ ± 0.6 N=30	12.2 * ± 0.6 N=30	8.9 ± 1.0 N=30	9.5 ± 0.5 N=30
Percent Food Efficiency (sd 28 to 35)[a]				
	6.8 ± 0.8 N=30	7.5 ± 0.6 N=30	7.6 ± 1.0 N=29[b]	5.5 ± 0.4 N=28[c]

(continued)

Table 7. Summary and Statistical Analysis of the F_0 Female Feed Consumption, Percent Food Efficiency and Butyl Benzyl Phthalate Intake During the Prebreed Period (page 4 of 6)

	Butyl Benzyl Phthalate (ppm in the feed)			
	0	750	3750	11250
Percent Food Efficiency (sd 35 to 42)[a]				
	6.3 ± 0.7 N=30	6.3 ± 0.5 N=30	7.4 ± 0.6 N=29[b]	6.9 ± 0.5 N=28[b]
Percent Food Efficiency (sd 42 to 49)[a]				
	6.5 ‡‡‡ ± 0.6 §§§ N=30	6.2 ± 0.6 N=30	5.0 ± 0.6 N=29[c]	3.2 *** ± 0.6 N=28[b]
Percent Food Efficiency (sd 49 to 56)[a]				
	3.1 ‡‡ ± 0.8 § N=29[b]	5.0 ± 0.8 N=30	7.4 ** ± 1.0 N=29[b]	6.7 * ± 0.9 N=28[c]
Percent Food Efficiency (sd 56 to 63)[a]				
#	3.7 ± 0.7 N=30	4.0 ± 0.7 N=30	6.2 ± 1.1 N=29[b]	4.3 ± 0.7 N=30
Percent Food Efficiency (sd 63 to 70)[a]				
	2.6 ± 1.2 N=29[b]	2.7 ± 0.9 N=30	1.4 ± 1.6 N=29[b]	2.4 ± 1.1 N=30
Percent Food Efficiency (sd 0 to 70)[a]				
	9.2 ‡ ± 0.3 §§ N=29[d]	9.5 ± 0.3 N=30	9.4 ± 0.3 N=29[d]	8.3 ± 0.3 N=21[d]
Butyl Benzyl Phthalate Intake (sd 0 to 7) (mg/kg/day)[a]				
		73.43 ± 1.05 N=30	359.00 ± 4.75 N=30	1151.20 ± 36.44 N=25[b]
Butyl Benzyl Phthalate Intake (sd 7 to 14) (mg/kg/day)[a]				
		66.44 ± 1.12 N=30	345.22 ± 9.66 N=30	1082.15 ± 42.50 N=30
Butyl Benzyl Phthalate Intake (sd 14 to 21) (mg/kg/day)[a]				
		62.53 ± 0.89 N=30	326.97 ± 10.54 N=29[b]	958.00 ± 22.17 N=30

(continued)

Table 7. Summary and Statistical Analysis of the F_0 Female Feed Consumption, Percent Food Efficiency and Butyl Benzyl Phthalate Intake During the Prebreed Period (page 5 of 6)

	Butyl Benzyl Phthalate (ppm in the feed)		
0	750	3750	11250
Butyl Benzyl Phthalate Intake (sd 21 to 28) (mg/kg/day)[a]			
	59.42 ± 0.81 N=30	317.14 ± 9.73 N=30	933.37 ± 29.54 N=30
Butyl Benzyl Phthalate Intake (sd 28 to 35) (mg/kg/day)[a]			
	57.53 ± 1.39 N=30	313.82 ± 13.03 N=29[b]	881.33 ± 26.50 N=28[c]
Butyl Benzyl Phthalate Intake (sd 35 to 42) (mg/kg/day)[a]			
	56.32 ± 0.97 N=30	295.51 ± 9.09 N=29[b]	852.36 ± 21.88 N=28[b]
Butyl Benzyl Phthalate Intake (sd 42 to 49) (mg/kg/day)[a]			
	54.34 ± 0.96 N=30	282.20 ± 7.82 N=29[c]	845.49 ± 25.02 N=28[b]
Butyl Benzyl Phthalate Intake (sd 49 to 56) (mg/kg/day)[a]			
	48.62 ± 0.87 N=30	253.39 ± 6.45 N=29[b]	742.57 ± 17.56 N=28[c]
Butyl Benzyl Phthalate Intake (sd 56 to 63) (mg/kg/day)[a]			
	46.66 ± 0.74 N=30	245.95 ± 6.87 N=29[b]	736.41 ± 17.83 N=30
Butyl Benzyl Phthalate Intake (sd 63 to 70) (mg/kg/day)[a]			
	45.08 ± 0.85 N=30	227.61 ± 4.39 N=29[b]	705.81 ± 17.31 N=30
Butyl Benzyl Phthalate Intake (sd 0 to 70) (mg/kg/day)[a]			
	56.31 ± 0.74 N=30	292.19 ± 6.70 N=29[d]	841.39 ± 14.49 N=21[d]

(continued)

Table 7. Summary and Statistical Analysis of the F_0 Female Feed Consumption, Percent Food Efficiency and Butyl Benzyl Phthalate Intake During the Prebreed Period (page 6 of 6)

[a]Reported as the mean ± S.E.M. sd = study day with study day 0 being the first day of exposure.
[b]Decrease in N is due to one or more females scratching the feed out of the feed jar and therefore an accurate feed weight could not be obtained.
[c]Decrease in N is due to the feed consumption value for one or more females being a statistical outlier and they were therefore removed.
[d]Decrease in N is due to interim feed consumption value(s) for one or more females being missing and therefore the overall feed consumption value could not be calculated.
[#]Levene's test for homogeneity of variances was significant ($p<0.05$), therefore robust regression methods were used to test all treatment effects.
‡$p<0.05$; ANOVA Test.
‡‡$p<0.01$; ANOVA Test.
‡‡‡$p<0.001$; ANOVA Test.
§$p<0.05$; Test for Linear Trend.
§§$p<0.01$; Test for Linear Trend.
§§§$p<0.001$; Test for Linear Trend.
*$p<0.05$; Dunnett's Test.
**$p<0.01$; Dunnett's Test.
***$p<0.001$; Dunnett's Test.

Table 8. Summary of the F_0 Female Clinical Observations During the Prebreed and Mating Periods
(page 1 of 8)

A. Clinical Observations Summarized by Group

Observation	Butyl Benzyl Phthalate (ppm in the feed)			
	0	750	3750	11250
Alopecia	1	6	5	7
Ear(s): red			1	
Eye(s): opaque			1	
Head caught in cage top, sore on top of head and beside left eye, small amount of blood in corner of left eye			1	
Rust colored fur			1	1
Scab(s)			1	
Sore(s)			1	1
Vaginal smear: blood present, no sperm				1

B. Clinical Observations Summarized by Group and Day

Day[a]	Observation[b]	Butyl Benzyl Phthalate (ppm in the feed)			
		0	750	3750	11250
2	Head caught in cage top, sore on top of head and beside left eye, small amount of blood in corner of left eye			1	
3	Eye: opaque, left			1	
	Sore(s): head			1	
4	Eye: opaque, left			1	
	Sore(s): head			1	
5	Eye: opaque, left			1	
	Sore(s): head, healing			1	
6	Eye: opaque, left			1	
	Sore(s): head, healing			1	
7	Alopecia: limb(s)			1	
	Eye: opaque, left			1	
	Sore(s): head			1	
	shoulder(s)				1
8	Alopecia: limb(s)			1	
	Eye: opaque, left			1	
	Sore(s): head			1	
	shoulder(s)				1
9	Alopecia: limb(s)			1	
	Eye: opaque, left			1	
	Sore(s): head, improving			1	
	shoulder(s)				1
10	Alopecia: limb(s)			1	
	Eye: opaque, left			1	
	Sore(s): head, improving			1	
	shoulder(s)				1

(continued)

Table 8. Summary of the F_0 Female Clinical Observations During the Prebreed and Mating Periods
(page 2 of 8)

Day[a]	Observation[b]	Butyl Benzyl Phthalate (ppm in the feed) 0	750	3750	11250
11	Alopecia: limb(s)			1	
	Eye: opaque, left			1	
	Sore(s): head, improving			1	
	shoulder(s)				1
12	Alopecia: limb(s)			1	
	Eye: opaque, left			1	
	Sore(s): head, healed			1	
	shoulder(s), improving				1
13	Alopecia: limb(s)			1	
	Eye: opaque, left			1	
	Sore(s): shoulder(s), improving				1
14	Alopecia: limb(s)			1	
	Eye: opaque, left[c]			1	
15	Alopecia: limb(s)			1	
16	Alopecia: limb(s)			1	
17	Alopecia: limb(s)			1	
18	Alopecia: limb(s)			1	
19	Alopecia: limb(s), growing in			1	
20	Alopecia: limb(s), growing in			1	
23	Alopecia: limb(s), returned			1	
24	Alopecia: limb(s)			1	
25	Alopecia: limb(s)			1	
26	Alopecia: limb(s)			1	
27	Alopecia: limb(s)			1	
28	Alopecia: limb(s)		1	1	
29	Alopecia: limb(s)		1	1	
30	Alopecia: limb(s)		1		2
	limb(s), growing in			1	
31	Alopecia: limb(s)		1		2
	limb(s), growing in			1	
32	Alopecia: limb(s)		1	1	2
33	Alopecia: limb(s)		1	1	2
34	Alopecia: limb(s)		1	1	2

(continued)

Table 8. Summary of the F_0 Female Clinical Observations During the Prebreed and Mating Periods
(page 3 of 8)

Day[a]	Observation[b]	Butyl Benzyl Phthalate (ppm in the feed) 0	750	3750	11250
35	Alopecia: limb(s)	1	1	1	2
	multiple areas		1		
	Rust colored fur: shoulder(s)				1
36	Alopecia: limb(s)	1	1	1	2
	multiple areas		1		
	Rust colored fur: shoulder(s)				1
37	Alopecia: limb(s)	1	1	1	2
	multiple areas		1		
	Rust colored fur: shoulder(s)				1
38	Alopecia: limb(s)	1	1	1	2
	multiple areas		1		
	Rust colored fur: shoulder(s)				1
39	Alopecia: limb(s)	1	1	1	2
	multiple areas		1		
	Rust colored fur: shoulder(s)				1
40	Alopecia: limb(s)	1	1	1	2
	multiple areas		1		
	Rust colored fur: shoulder(s)				1
41	Alopecia: limb(s)	1	1	1	2
	multiple areas		1	1	
	Rust colored fur: shoulder(s)				1
42	Alopecia: limb(s)	1	1	1	1
	limb(s), growing in				1
	multiple areas		2	1	
	Rust colored fur: shoulder(s)				1
43	Alopecia: limb(s)	1	1	1	2
	multiple areas		2	1	
	Ear: red, bilateral			1	
	Rust colored fur: shoulder(s)				1
44	Alopecia: limb(s)	1		1	1
	limb(s), growing in		1		1
	multiple areas		2	1	
	Ear: red, bilateral			1	
	Rust colored fur: shoulder(s)				1
45	Alopecia: limb(s)	1		1	1
	limb(s), growing in		1		1
	multiple areas		2	1	
	Ear: red, bilateral			1	
	Rust colored fur: shoulder(s)				1

(continued)

Table 8. Summary of the F_0 Female Clinical Observations During the Prebreed and Mating Periods
(page 4 of 8)

Day[a]	Observation[b]	Butyl Benzyl Phthalate (ppm in the feed)			
		0	750	3750	11250
46	Alopecia: limb(s)	1		1	1
	limb(s), growing in		1		1
	multiple areas		2	1	
	Ear: red, bilateral			1	
	Rust colored fur: shoulder(s)				1
47	Alopecia: limb(s)	1		1	2
	multiple areas		2	1	
	Ear: red, bilateral			1	
	Rust colored fur: shoulder(s)				1
48	Alopecia: limb(s)	1		1	2
	multiple areas		2	1	
	Ear: red, bilateral			1	
	Rust colored fur: shoulder(s)				1
49	Alopecia: limb(s)	1	2	1	2
	multiple areas		2	1	
	Ear: red, bilateral			1	
	Rust colored fur: shoulder(s)				1
	Scab(s): back			1	
50	Alopecia: limb(s)	1	2	1	2
	multiple areas		2	1	
	Ear: red, bilateral			1	
	Rust colored fur: shoulder(s)				1
	Scab(s): back			1	
51	Alopecia: limb(s)	1	2	1	2
	multiple areas		2	1	
	Ear: red, bilateral			1	
	Rust colored fur: shoulder(s)				1
	Scab(s): back			1	
52	Alopecia: limb(s)	1	2	1	2
	multiple areas		2	1	
	Ear: red, bilateral			1	
	Rust colored fur: shoulder(s)				1
	Scab(s): back			1	
53	Alopecia: abdomen				1
	limb(s)	1	2	1	2
	multiple areas		2	1	
	Ear: red, bilateral			1	
	Rust colored fur: shoulder(s)				1
	Scab(s): back, healing			1	

(continued)

Table 8. Summary of the F_0 Female Clinical Observations During the Prebreed and Mating Periods
(page 5 of 8)

Day[a]	Observation[b]	Butyl Benzyl Phthalate (ppm in the feed) 0	750	3750	11250
54	Alopecia: abdomen				1
	limb(s)	1	2	1	2
	multiple areas		2	1	
	Ear: red, bilateral			1	
	Rust colored fur: shoulder(s)				1
	Scab(s): back, healing			1	
55	Alopecia: abdomen				1
	limb(s)	1	2	1	2
	multiple areas		1	1	
	multiple areas, growing in		1		
	Ear: red, bilateral			1	
	Rust colored fur: shoulder(s)				1
	Scab(s): back, healing			1	
56	Alopecia: abdomen				1
	limb(s)	1	1	1	3
	limb(s), growing in		1		
	multiple areas		1	1	
	multiple areas, growing in		1		
	Ear: red, bilateral			1	
	Rust colored fur: multiple areas			1	
	shoulder(s)				1
	Scab(s): back, healing			1	
57	Alopecia: abdomen				1
	limb(s)	1	2	1	3
	multiple areas		1	1	
	multiple areas, growing in		1		
	Ear: red, bilateral			1	
	Rust colored fur: multiple areas			1	
	shoulder(s)				1
	Scab(s): back			1	
58	Alopecia: abdomen				1
	limb(s)	1	2	1	3
	multiple areas		1	1	
	multiple areas, growing in		1		
	Ear: red, bilateral			1	
	Rust colored fur: multiple areas			1	
	shoulder(s)				1
	Scab(s): back			1	
59	Alopecia: abdomen				1
	limb(s)	1	2	1	3
	multiple areas		1	1	
	multiple areas, growing in		1		
	Ear: red, bilateral			1	
	Rust colored fur: multiple areas			1	
	shoulder(s)				1
	Scab(s): back			1	

(continued)

Table 8. Summary of the F_0 Female Clinical Observations During the Prebreed and Mating Periods
(page 6 of 8)

Day[a]	Observation[b]	Butyl Benzyl Phthalate (ppm in the feed)			
		0	750	3750	11250
60	Alopecia: abdomen				1
	limb(s)	1	2	1	3
	multiple areas		1	1	
	multiple areas, growing in		1		
	Ear: red, bilateral			1	
	Rust colored fur: multiple areas			1	
	shoulder(s)				1
	Scab(s): back			1	
61	Alopecia: abdomen				1
	limb(s)	1	2	1	2
	limb(s), growing in				1
	multiple areas		1	1	
	multiple areas, growing in		1		
	Ear: red, bilateral			1	
	Rust colored fur: multiple areas			1	
	shoulder(s)				1
	Scab(s): back, healing			1	
62	Alopecia: abdomen				1
	limb(s)	1	1	1	3
	multiple areas		3	1	
	Ear: red, bilateral			1	
	Rust colored fur: multiple areas			1	
	shoulder(s)				1
	Scab(s): back, healed			1	
63	Alopecia: abdomen				1
	limb(s)	1	2	2	2
	limb(s), growing in				1
	multiple areas		3	1	
	Ear: red, bilateral			1	
	Rust colored fur: multiple areas			1	
	shoulder(s)				1
64	Alopecia: abdomen				1
	limb(s)	1	2	3	2
	limb(s), growing in				1
	multiple areas		3	1	
	Ear: red, bilateral			1	
	Rust colored fur: multiple areas			1	
	shoulder(s)				1
65	Alopecia: abdomen				1
	limb(s)	1	2	3	2
	limb(s), growing in				1
	multiple areas		3	1	
	Ear: red, bilateral			1	
	Rust colored fur: multiple areas			1	
	shoulder(s)				1

(continued)

Table 8. Summary of the F_0 Female Clinical Observations During the Prebreed and Mating Periods
(page 7 of 8)

Day[a]	Observation[b]	Butyl Benzyl Phthalate (ppm in the feed) 0	750	3750	11250
66	Alopecia: abdomen				1
	limb(s)	1	2	3	3
	multiple areas		3	1	
	Ear: red, bilateral			1	
	Rust colored fur: multiple areas			1	
	shoulder(s)				1
67	Alopecia: abdomen				1
	limb(s)	1	2	3	3
	multiple areas		3	1	
	Ear: red, bilateral			1	
	Rust colored fur: multiple areas			1	
	shoulder(s)				1
68	Alopecia: abdomen				1
	limb(s)	1	2	3	3
	multiple areas		3	1	
	Ear: red, bilateral			1	
	Rust colored fur: multiple areas			1	
	shoulder(s)				1
69	Alopecia: abdomen				1
	limb(s)	1	2	3	3
	multiple areas		3	1	
	Ear: red, bilateral			1	
	Rust colored fur: multiple areas			1	
	shoulder(s)				1
70	Alopecia: abdomen				1
	limb(s)	1		4	5
	limb(s), growing in		2		
	multiple areas		3	1	
	Ear: red, bilateral			1	
	Rust colored fur: multiple areas			1	
	shoulder(s)				1
71	Alopecia: limb(s)	1		3	5
	limb(s), growing in		1		
	multiple areas		2	1	
	Ear: red, bilateral			1	
	Rust colored fur: multiple areas			1	
72	Alopecia: limb(s)	1		1	4
	limb(s), growing in		1		
	multiple areas		1		
	Ear: red, bilateral			1	
	Rust colored fur: multiple areas			1	
	Vaginal smear: blood present, no sperm				1
73	Alopecia: limb(s)	1		1	4
	multiple areas		1		

(continued)

Table 8. Summary of the F_0 Female Clinical Observations During the Prebreed and Mating Periods
(page 8 of 8)

Day[a]	Observation[b]	Butyl Benzyl Phthalate (ppm in the feed)			
		0	750	3750	11250
74	Alopecia: limb(s)	1		1	2
75	Alopecia: limb(s)	1		1	2
76	Alopecia: limb(s)	1		1	2
77	Alopecia: limb(s)	1		1	2
78	Alopecia: limb(s)	1		1	2
79	Alopecia: limb(s)	1		1	2
80	Alopecia: limb(s)	1		1	2
81	Alopecia: limb(s)	1			2
82	Alopecia: limb(s)	1			2
83	Alopecia: limb(s)	1			1
84	Alopecia: limb(s)	1			

[a]Study day.
[b]Clinical observations are tabulated once per day per animal.
[c]No further notation was made unless a change occurred.

Table 9. Summary and Statistical Analysis of the F_0 Female Vaginal Cytology Prior to Mating
(page 1 of 1)

	Butyl Benzyl Phthalate (ppm in the feed)			
	0	750	3750	11250
Number of Females Evaluated	30	30	30	30
Number of Females Cycling	29	28	30	30
Percent of Females Cycling	96.67	93.33	100.00	100.00
Number of Females with an Abnormal Cycle[a]	6	7	3	6
Percent of Females with an Abnormal Cycle[a]	20.69	25.00	10.00	20.00
Cycle Length (days)[a,b]	4.39 ± 0.20 N=30	4.58 ± 0.33 N=30	4.81 ± 0.24 N=30	4.39 ± 0.11 N=30

[a]Includes only those females that were cycling.
[b]Reported as the mean ± S.E.M.

Table 10. Summary and Statistical Analysis of the F_0 Female Body Weights and Weight Changes During Gestation (page 1 of 2)

	Butyl Benzyl Phthalate (ppm in the feed)			
	0	750	3750	11250
No. Sperm Positive, Pregnant Females	27	26	25	26
Body Weight (gd 0) (g)[a]				
	301.0 ‡ ± 4.7 §§ N=27	303.2 ± 5.2 N=26	306.7 ± 4.1 N=25	287.4 ± 4.1 N=26
Body Weight (gd 7) (g)[a]				
	332.7 ‡‡‡ ± 4.7 §§§ N=27	339.8 ± 5.5 N=26	339.3 ± 4.5 N=25	312.7 ** ± 4.0 N=26
Body Weight (gd 14) (g)[a]				
	363.4 ‡‡‡ ± 5.0 §§§ N=27	366.4 ± 5.8 N=26	367.6 ± 4.9 N=25	337.3 ** ± 4.6 N=26
Body Weight (gd 20) (g)[a]				
	434.1 ‡‡ ± 7.1 §§§ N=27	435.6 ± 6.9 N=26	437.2 ± 6.3 N=25	405.4 ** ± 6.9 N=26
Body Weight Change (gd 0 to 7) (g)[a]				
	31.7 ‡ ± 1.1 §§ N=27	36.5 ± 1.5 N=26	32.6 ± 4.3 N=25	25.3 ± 1.1 N=26
Body Weight Change (gd 7 to 14) (g)[a]				
	30.6 ‡‡ ± 1.0 §§ N=27	26.6 ± 1.2 N=26	28.3 ± 1.1 N=25	24.5 ** ± 1.5 N=26
Body Weight Change (gd 14 to 20) (g)[a]				
	70.8 ± 3.0 N=27	69.2 ± 3.0 N=26	69.6 ± 2.8 N=25	68.2 ± 4.1 N=26
Body Weight Change (gd 0 to 20) (g)[a]				
	133.1 ± 3.9 § N=27	132.4 ± 3.7 N=26	130.5 ± 6.4 N=25	118.0 ± 4.5 N=26

(continued)

Table 10. Summary and Statistical Analysis of the F_0 Female Body Weights and Weight Changes During Gestation (page 2 of 2)

[a]Reported as the mean ± S.E.M. gd = gestational day.
‡p<0.05; ANOVA Test.
‡‡p<0.01; ANOVA Test.
‡‡‡p<0.001; ANOVA Test.
§p<0.05; Test for Linear Trend.
§§p<0.01; Test for Linear Trend.
§§§p<0.001; Test for Linear Trend.
**p<0.01; Dunnett's Test.

Table 11. Summary and Statistical Analysis of the F_0 Female Feed Consumption, Percent Food Efficiency and Butyl Benzyl Phthalate Intake During Gestation (page 1 of 3)

	Butyl Benzyl Phthalate (ppm in the feed)			
	0	750	3750	11250
No. Sperm Positive, Pregnant Females	27	26	25	26
Feed Consumption (gd 0 to 7) (g/day)[a]				
	21.9 ± 0.6 N=27	22.6 ± 0.5 N=26	23.4 ± 1.0 N=25	22.1 ± 0.7 N=26
Feed Consumption (gd 7 to 14) (g/day)[a]				
	23.5 ± 0.5 N=27	23.7 ± 0.6 N=26	24.9 ± 0.8 N=25	23.1 ± 0.7 N=25[b]
Feed Consumption (gd 14 to 20) (g/day)[a]				
	24.4 ± 0.6 N=27	25.0 ± 0.6 N=26	25.8 ± 0.6 N=25	25.4 ± 0.7 N=26
Feed Consumption (gd 0 to 20) (g/day)[a]				
	23.2 ± 0.5 N=27	23.7 ± 0.5 N=26	24.6 ± 0.8 N=25	23.4 ± 0.6 N=25[c]
Feed Consumption (gd 0 to 7) (g/kg/day)[a]				
	69.3 ± 1.8 N=27	70.5 ± 1.2 N=26	72.5 ± 3.0 N=25	73.9 ± 2.2 N=26
Feed Consumption (gd 7 to 14) (g/kg/day)[a]				
	67.5 ± 1.4 N=27	67.1 ± 1.0 N=26	70.4 ± 2.2 N=25	70.9 ± 2.2 N=25[b]
Feed Consumption (gd 14 to 20) (g/kg/day)[a]				
	61.3 ‡‡ ± 1.2 §§§ N=27	62.4 ± 1.1 N=26	64.2 ± 1.4 N=25	68.5 ** ± 2.0 N=26
Feed Consumption (gd 0 to 20) (g/kg/day)[a]				
	65.0 ± 1.3 § N=27	65.7 ± 0.9 N=26	68.0 ± 2.1 N=25	69.5 ± 1.9 N=25[c]

(continued)

Table 11. Summary and Statistical Analysis of the F_0 Female Feed Consumption, Percent Food Efficiency and Butyl Benzyl Phthalate Intake During Gestation (page 2 of 3)

	Butyl Benzyl Phthalate (ppm in the feed)			
	0	750	3750	11250
Percent Food Efficiency (gd 0 to 7)[a]				
	20.8 ‡ ± 0.6 §§ N=27	23.1 ± 0.9 N=26	20.4 ± 2.7 N=25	16.6 ± 0.8 N=26
Percent Food Efficiency (gd 7 to 14)[a]				
	18.7 ‡‡ ± 0.6 § N=27	16.1 * ± 0.7 N=26	16.5 ± 0.7 N=25	15.3 ** ± 1.0 N=25[b]
Percent Food Efficiency (gd 14 to 20)[a]				
	48.2 ± 1.6 N=27	46.4 ± 2.0 N=26	45.6 ± 2.1 N=25	44.6 ± 2.9 N=26
Percent Food Efficiency (gd 0 to 20)[a]				
	28.7 ± 0.7 § N=27	28.0 ± 0.8 N=26	26.9 ± 1.4 N=25	25.6 ± 1.1 N=25[c]
Butyl Benzyl Phthalate Intake (gd 0 to 7) (mg/kg/day)[a]				
		52.86 ± 0.94 N=26	271.85 ± 11.24 N=25	831.13 ± 25.07 N=26
Butyl Benzyl Phthalate Intake (gd 7 to 14) (mg/kg/day)[a]				
		50.29 ± 0.77 N=26	264.15 ± 8.32 N=25	797.20 ± 24.79 N=25[b]
Butyl Benzyl Phthalate Intake (gd 14 to 20) (mg/kg/day)[a]				
		46.83 ± 0.80 N=26	240.70 ± 5.18 N=25	770.52 ± 22.79 N=26
Butyl Benzyl Phthalate Intake (gd 0 to 20) (mg/kg/day)[a]				
		49.26 ± 0.70 N=26	254.96 ± 7.79 N=25	782.19 ± 20.90 N=25[c]

(continued)

Table 11. Summary and Statistical Analysis of the F_0 Female Feed Consumption, Percent Food Efficiency and Butyl Benzyl Phthalate Intake During Gestation (page 3 of 3)

[a]Reported as the mean ± S.E.M. gd = gestational day.
[b]Decrease in N is due to one female scratching the feed out of the feed jar and therefore an accurate feed weight could not be obtained.
[c]Decrease in N is due to interim feed consumption value(s) for one female being missing and therefore the overall feed consumption value could not be calculated.
‡$p<0.05$; ANOVA Test.
‡‡$p<0.01$; ANOVA Test.
§$p<0.05$; Test for Linear Trend.
§§$p<0.01$; Test for Linear Trend.
§§§$p<0.001$; Test for Linear Trend.
*$p<0.05$; Dunnett's Test.
**$p<0.01$; Dunnett's Test.

Table 12. Summary of the F_0 Female Clinical Observations During Gestation (page 1 of 5)

A. Clinical Observations Summarized by Group

Observation	Butyl Benzyl Phthalate (ppm in the feed)			
	0	750	3750	11250
Alopecia	1	6	5	10
Ear(s): red			1	
Having trouble delivering	1			
Piloerection	1			1
Rust colored fur			1	1
Sore(s)	1			

B. Clinical Observations Summarized by Group and Day

Day[a]	Observation[b]	Butyl Benzyl Phthalate (ppm in the feed)			
		0	750	3750	11250
0	Alopecia: abdomen				1
	limb(s)			4	6
	limb(s), growing in		2		
	multiple areas		3	1	
	Ear: red, bilateral			1	
	Rust colored fur: multiple areas			1	
	shoulder(s)				1
	Sore(s): face	1			
1	Alopecia: abdomen				1
	limb(s)			4	6
	limb(s), growing in		1		
	multiple areas		3	1	
	Ear: red, bilateral			1	
	Rust colored fur: multiple areas			1	
	shoulder(s)				1
	Sore(s): face	1			
2	Alopecia: abdomen				1
	limb(s)		1	4	6
	multiple areas		3	1	
	Ear: red, bilateral			1	
	Rust colored fur: multiple areas			1	
	shoulder(s)				1
	Sore(s): face, improving	1			
3	Alopecia: abdomen				1
	limb(s)		1	4	6
	multiple areas		3	1	
	Ear: red, bilateral			1	
	Rust colored fur: multiple areas			1	
	shoulder(s)				1
	Sore(s): face, healing	1			

(continued)

Table 12. Summary of the F_0 Female Clinical Observations During Gestation (page 2 of 5)

Day[a]	Observation[b]	Butyl Benzyl Phthalate (ppm in the feed) 0	750	3750	11250
4	Alopecia: abdomen				1
	limb(s)		1	4	6
	multiple areas		3	1	
	Ear: red, bilateral			1	
	Rust colored fur: multiple areas			1	
	shoulder(s)				1
	Sore(s): face	1			
5	Alopecia: abdomen				1
	limb(s)		1	4	5
	limb(s), growing in				1
	multiple areas		3	1	
	Ear: red, bilateral			1	
	Rust colored fur: multiple areas			1	
	shoulder(s)				1
	Sore(s): face	1			
6	Alopecia: abdomen				1
	limb(s)		1	4	5
	limb(s), growing in				1
	multiple areas		3	1	
	neck		1		
	Ear: red, bilateral			1	
	Rust colored fur: multiple areas			1	
	shoulder(s)				1
	Sore(s): face, healed	1			
7	Alopecia: abdomen				1
	limb(s)		1	2	4
	limb(s), growing in			1	1
	multiple areas		3	2	1
	neck		1		
	Ear: red, bilateral			1	
	Rust colored fur: multiple areas			1	
	shoulder(s)				1
8	Alopecia: abdomen				1
	limb(s)		1	3	4
	limb(s), growing in				1
	multiple areas		3	2	1
	neck		1		
	Ear: red, bilateral			1	
	Rust colored fur: multiple areas			1	
	shoulder(s)				1
9	Alopecia: abdomen				1
	limb(s)		1	2	4
	limb(s), growing in				1
	multiple areas		3	2	1
	neck		1		
	Ear: red, bilateral			1	
	Rust colored fur: multiple areas			1	
	shoulder(s)				1

(continued)

Table 12. Summary of the F_0 Female Clinical Observations During Gestation (page 3 of 5)

Day[a]	Observation[b]	Butyl Benzyl Phthalate (ppm in the feed) 0	750	3750	11250
10	Alopecia: abdomen				1
	limb(s)		1	2	4
	limb(s), growing in				1
	multiple areas		3	2	1
	neck		1		
	Ear: red, bilateral			1	
	Rust colored fur: multiple areas			1	
	shoulder(s)				1
11	Alopecia: abdomen				1
	limb(s)		1	2	4
	limb(s), growing in				1
	multiple areas		3	2	2
	neck		1		
	Ear: red, bilateral			1	
	Rust colored fur: multiple areas			1	
	shoulder(s)				1
12	Alopecia: abdomen				1
	limb(s)		1	2	4
	limb(s), growing in				1
	multiple areas		4	2	2
	Ear: red, bilateral			1	
	Rust colored fur: multiple areas			1	
	shoulder(s)				1
13	Alopecia: abdomen				1
	limb(s)		1	2	4
	limb(s), growing in				1
	multiple areas		4	2	2
	Ear: red, bilateral			1	
	Rust colored fur: multiple areas			1	
	shoulder(s)				1
14	Alopecia: abdomen				1
	limb(s)		1	2	5
	limb(s), grown in at all other areas			1	
	limb(s), returned			1	
	multiple areas		3	1	3
	multiple areas, growing in		1		
	Ear: red, bilateral			1	
	Rust colored fur: multiple areas			1	
	shoulder(s)				1
15	Alopecia: abdomen				1
	chest				1
	limb(s)		1	4	4
	limb(s), grown in				1
	multiple areas		3	1	3
	multiple areas, growing in		1		
	Ear: red, bilateral			1	
	Rust colored fur: multiple areas			1	
	shoulder(s)				1

(continued)

Table 12. Summary of the F_0 Female Clinical Observations During Gestation (page 4 of 5)

Day[a]	Observation[b]	Butyl Benzyl Phthalate (ppm in the feed) 0	750	3750	11250
16	Alopecia: abdomen				1
	chest				1
	limb(s)		1	4	4
	multiple areas		3	1	3
	multiple areas, growing in		1		
	Ear: red, bilateral			1	
	Rust colored fur: multiple areas			1	
	shoulder(s)				1
17	Alopecia: abdomen				1
	chest				1
	limb(s)		1	4	4
	multiple areas		4	1	3
	Ear: red, bilateral			1	
	Rust colored fur: multiple areas			1	
	shoulder(s)				1
18	Alopecia: abdomen				1
	chest				1
	limb(s)	1	1	4	4
	multiple areas		3	1	2
	multiple areas, growing in				1
	multiple areas, grown in		1		
	Ear: red, bilateral			1	
	Rust colored fur: multiple areas			1	
	shoulder(s)				1
19	Alopecia: abdomen				1
	chest				1
	limb(s)	1	1	4	4
	multiple areas		3	1	2
	multiple areas, growing in				1
	Ear: red, bilateral			1	
	Rust colored fur: multiple areas, improving			1	
	shoulder(s)				1
20	Alopecia: abdomen				1
	chest				1
	limb(s)		1	4	4
	limb(s), growing in	1			
	multiple areas		3	1	3
	Ear: red, bilateral			1	
	Piloerection				1
	Rust colored fur: multiple areas, improving			1	
	shoulder(s)				1

(continued)

Table 12. Summary of the F_0 Female Clinical Observations During Gestation (page 5 of 5)

Day[a]	Observation[b]	Butyl Benzyl Phthalate (ppm in the feed)			
		0	750	3750	11250
21	Alopecia: abdomen				1
	chest				1
	limb(s)		1	4	3
	limb(s), growing in	1			
	multiple areas		3		3
	Ear: red, bilateral			1	
	Rust colored fur: multiple areas, improving			1	
	shoulder(s)				1
22	Alopecia: chest				1
	limb(s)			1	
	Rust colored fur: multiple areas, improving			1	
23	Alopecia: chest				1
	Having trouble delivering	1			
	Piloerection	1			
24	Alopecia: chest				1

[a]Gestational day.
[b]Clinical observations are tabulated once per day per animal.

Table 13. Summary and Statistical Analysis of the F_0 Maternal Body Weights and Weight Changes During Lactation (page 1 of 2)

	Butyl Benzyl Phthalate (ppm in the feed)			
	0	750	3750	11250
No. Females with Live Litters	27	27	28	27
Body Weight (pnd 0) (g)[a]	336.3 ‡‡‡ ± 5.5 §§§ N=27	347.1 ± 5.9 N=27	346.7 ± 4.7 N=28	315.7 * ± 4.4 N=27
Body Weight (pnd 4) (g)[a] #	351.7 ††† ± 4.6 ŸŸŸ N=26[b]	354.5 ± 6.6 N=27	354.7 ± 4.4 N=28	322.9 ÞÞÞ ± 4.6 N=27
Body Weight (pnd 7) (g)[a]	360.5 ‡‡‡ ± 4.8 §§§ N=26	360.8 ± 6.6 N=27	362.2 ± 4.5 N=28	330.8 *** ± 5.1 N=27
Body Weight (pnd 14) (g)[a]	363.5 ‡‡ ± 4.7 §§§ N=26	363.6 ± 6.7 N=27	365.0 ± 5.0 N=28	341.6 * ± 4.3 N=27
Body Weight (pnd 21) (g)[a] #	351.1 ± 3.8 N=26	350.9 ± 5.8 N=26[c]	353.6 ± 4.1 N=28	346.9 ± 3.7 N=27
Body Weight Change (pnd 0 to 4) (g)[a]	12.0 ± 2.2 N=26	7.4 ± 3.2 N=27	7.9 ± 2.4 N=28	7.1 ± 2.5 N=27
Body Weight Change (pnd 4 to 7) (g)[a]	8.8 ± 2.1 N=26	6.3 ± 1.8 N=27	7.5 ± 2.0 N=28	7.9 ± 2.4 N=27
Body Weight Change (pnd 7 to 14) (g)[a]	3.0 ± 4.3 N=26	2.7 ± 3.6 N=27	2.8 ± 3.4 N=28	10.9 ± 2.5 N=27

(continued)

Table 13. Summary and Statistical Analysis of the F_0 Maternal Body Weights and Weight Changes During Lactation (page 2 of 2)

	Butyl Benzyl Phthalate (ppm in the feed)			
	0	750	3750	11250
Body Weight Change (pnd 14 to 21) (g)[a]				
#	-12.4 †††, ŸŸŸ ± 4.1 N=26	-15.4 ± 3.5 N=26	-11.4 ± 4.3 N=28	5.3 ÞÞÞ ± 2.3 N=27
Body Weight Change (pnd 0 to 21) (g)[a]				
	11.4 ‡‡‡, §§§ ± 2.6 N=26	4.3 ± 3.8 N=26	6.9 ± 3.1 N=28	31.2 *** ± 3.1 N=27

[a]Reported as the mean ± S.E.M. pnd = postnatal day.
[b]Decrease in N is due to the entire litter of female 52 being dead or missing and presumed dead on or before postnatal day 0.
[c]Decrease in N is due to female 78 being found dead on postnatal day 17.
#Levene's test for homogeneity of variances was significant ($p<0.05$), therefore robust regression methods were used to test all treatment effects.
‡‡$p<0.01$; ANOVA Test.
‡‡‡$p<0.001$; ANOVA Test.
§§§$p<0.001$; Test for Linear Trend.
*$p<0.05$; Dunnett's Test.
***$p<0.001$; Dunnett's Test.
†††$p<0.001$; Wald Chi-square Test for overall treatment effect in robust linear regression model.
ÞÞÞ$p<0.001$; Individual t-test for pairwise comparisons to control in robust linear regression model.
ŸŸŸ$p<0.001$; Linear trend test in robust regression model.

Table 14. Summary and Statistical Analysis of F_0 Maternal Feed Consumption, Percent Food Efficiency and Butyl Benzyl Phthalate Intake During Lactation (page 1 of 3)

	Butyl Benzyl Phthalate (ppm in the feed)			
	0	750	3750	11250
No. Females with Live Litters	27	27	28	27
Feed Consumption (pnd 0 to 4) (g/day)[a]				
#	32.9 † ± 1.3 Ÿ N=26[b]	31.0 ± 1.6 N=25[c]	35.5 ± 1.3 N=27[d]	38.2 ± 2.4 N=27
Feed Consumption (pnd 4 to 7) (g/day)[a]				
	49.5 ± 1.7 N=25[e]	47.5 ± 2.6 N=27	49.7 ± 1.3 N=28	47.6 ± 2.5 N=27
Feed Consumption (pnd 7 to 14) (g/day)[a]				
	60.8 ‡ ± 1.7 N=25[e]	55.8 ± 2.1 N=27	59.7 ± 1.1 N=28	55.1 ± 1.7 N=26[c]
Feed Consumption (pnd 14 to 21) (g/day)[a]				
	73.3 ± 2.3 N=26	69.6 ± 2.3 N=26[f]	73.5 ± 1.7 N=28	72.7 ± 2.5 N=27
Feed Consumption (pnd 0 to 21) (g/day)[a]				
	58.2 ± 1.7 N=25[g]	54.8 ± 1.9 N=24[g]	58.2 ± 1.3 N=27[g]	56.0 ± 1.6 N=26[g]
Feed Consumption (pnd 0 to 4) (g/kg/day)[a]				
#	95.1 †† ± 3.6 ŸŸŸ N=26[b]	88.8 ± 4.2 N=25[c]	101.0 ± 3.9 N=27[d]	120.1 ÞÞ ± 7.7 N=27
Feed Consumption (pnd 4 to 7) (g/kg/day)[a]				
	138.9 ± 4.4 N=25[e]	132.3 ± 6.2 N=27	138.9 ± 3.6 N=28	145.7 ± 7.5 N=27
Feed Consumption (pnd 7 to 14) (g/kg/day)[a]				
	168.3 ± 4.9 N=25[e]	154.1 ± 5.4 N=27	164.8 ± 3.5 N=28	164.5 ± 5.3 N=26[c]
Feed Consumption (pnd 14 to 21) (g/kg/day)[a]				
	205.7 ± 6.5 N=26	195.3 ± 7.0 N=26[f]	205.2 ± 5.4 N=28	211.3 ± 7.1 N=27

(continued)

Table 14. Summary and Statistical Analysis of F_0 Maternal Feed Consumption, Percent Food Efficiency and Butyl Benzyl Phthalate Intake During Lactation (page 2 of 3)

	Butyl Benzyl Phthalate (ppm in the feed)			
	0	750	3750	11250
Feed Consumption (pnd 0 to 21) (g/kg/day)[a]				
	164.8 ± 4.7 N=25[g]	155.4 ± 5.5 N=24[g]	162.8 ± 3.8 N=27[g]	169.4 ± 4.9 N=26[g]
Percent Food Efficiency (pnd 0 to 4)[a]				
	8.4 ± 1.6 N=26[b]	2.1 ± 3.4 N=25[c]	5.0 ± 1.7 N=27[d]	4.6 ± 1.8 N=27
Percent Food Efficiency (pnd 4 to 7)[a]				
	6.1 ± 1.4 N=25[e]	3.8 ± 1.5 N=27	4.7 ± 1.3 N=28	5.2 ± 2.1 N=27
Percent Food Efficiency (pnd 7 to 14)[a]				
	0.7 ± 1.1 § N=25[e]	0.5 ± 1.0 N=27	0.8 ± 0.8 N=28	2.9 ± 0.7 N=26[c]
Percent Food Efficiency (pnd 14 to 21)[a]				
#	-2.8 ††† ± 0.8 ŸŸŸ N=26	-3.2 ± 0.7 N=26[f]	-2.4 ± 0.8 N=28	1.1 ÞÞÞ ± 0.4 N=27
Percent Food Efficiency (pnd 0 to 21)[a]				
	1.0 ‡‡‡ ± 0.2 §§§ N=25[g]	0.2 ± 0.3 N=24[g]	0.6 ± 0.3 N=27[g]	2.7 *** ± 0.3 N=26[g]
Butyl Benzyl Phthalate Intake (pnd 0 to 4) (mg/kg/day)[a]				
		66.61 ± 3.16 N=25[c]	378.75 ± 14.80 N=27[d]	1351.63 ± 87.10 N=27
Butyl Benzyl Phthalate Intake (pnd 4 to 7) (mg/kg/day)[a]				
		99.22 ± 4.63 N=27	520.83 ± 13.32 N=28	1638.60 ± 84.43 N=27

(continued)

Table 14. Summary and Statistical Analysis of F_0 Maternal Feed Consumption, Percent Food Efficiency and Butyl Benzyl Phthalate Intake During Lactation (page 3 of 3)

	Butyl Benzyl Phthalate (ppm in the feed)			
	0	750	3750	11250
Butyl Benzyl Phthalate Intake (pnd 7 to 14) (mg/kg/day)[a]				
		115.54 ± 4.07 N=27	618.14 ± 13.27 N=28	1850.07 ± 60.07 N=26[c]
Butyl Benzyl Phthalate Intake (pnd 14 to 21) (mg/kg/day)[a]				
		146.47 ± 5.23 N=26[f]	769.69 ± 20.41 N=28	2377.66 ± 80.32 N=27
Butyl Benzyl Phthalate Intake (pnd 0 to 21) (mg/kg/day)[a]				
		116.58 ± 4.09 N=24[g]	610.68 ± 14.17 N=27[g]	1905.21 ± 55.40 N=26[g]

[a]Reported as the mean ± S.E.M. pnd = postnatal day.
[b]Decrease in N is due to the entire litter of female 52 being dead or missing and presumed dead on or before postnatal day 0.
[c]Decrease in N is due to one or more females scratching the feed out of the feed jar and therefore an accurate feed weight could not be obtained.
[d]Decrease in N is due to the feed consumption value for one female being a statistical outlier and it was therefore removed.
[e]Decrease in N is due to the feed consumption value for one female being unrealistic (i.e. negative) and it was therefore excluded.
[f]Decrease in N is due to female 78 being found dead on postnatal day 17.
[g]Decrease in N is due to interim feed consumption value(s) for one or more females being missing and therefore the overall feed consumption value could not be calculated.
#Levene's test for homogeneity of variances was significant ($p<0.05$), therefore robust regression methods were used to test all treatment effects.
‡$p<0.05$; ANOVA Test.
‡‡‡$p<0.001$; ANOVA Test.
§$p<0.05$; Test for Linear Trend.
§§§$p<0.001$; Test for Linear Trend.
***$p<0.001$; Dunnett's Test.
†$p<0.05$; Wald Chi-square Test for overall treatment effect in robust linear regression model.
††$p<0.01$; Wald Chi-square Test for overall treatment effect in robust linear regression model.
†††$p<0.001$; Wald Chi-square Test for overall treatment effect in robust linear regression model.
ÞÞ$p<0.01$; Individual t-test for pairwise comparisons to control in robust linear regression model.
ÞÞÞ$p<0.001$; Individual t-test for pairwise comparisons to control in robust linear regression model.
Ÿ$p\leq 0.05$; Linear trend test in robust regression model.
ŸŸŸ$p<0.001$; Linear trend test in robust regression model.

Table 15. Summary of the F_0 Female Clinical Observations During Lactation (page 1 of 6)

A. Clinical Observations Summarized by Group

Observation	Butyl Benzyl Phthalate (ppm in the feed)			
	0	750	3750	11250
Alopecia		4	7	9
Chromodacryorrhea	1			1
Dead fetus palpated		1		
Ear(s): red			1	
Found dead		1		
Left rear leg swollen			1	
Limping on or not using right rear leg		1		
Mammary gland: swollen, caudal one on right side	1			
Piloerection		1		
Rust colored fur		2	3	1
Sore metatarsal joint			1	
Vagina: blood, discharge and or brown object slightly protruding	1	1		

B. Clinical Observations Summarized by Group and Day

Day[a]	Observation[b]	Butyl Benzyl Phthalate (ppm in the feed)			
		0	750	3750	11250
0	Alopecia: abdomen				1
	limb(s)		1	4	4
	multiple areas		3	2	3
	Ear: red, bilateral			1	
	Rust colored fur: multiple areas			1	
	shoulder(s)				1
1	Alopecia: abdomen				1
	limb(s)		1	4	3
	multiple areas		3	2	4
	Chromodacryorrhea: eye, left				1
	Ear: red, bilateral			1	
	Rust colored fur: multiple areas			1	
	shoulder(s)				1
	Vagina: blood at opening	1			
2	Alopecia: abdomen				1
	limb(s)		1	4	3
	multiple areas		3	2	4
	Ear: red, bilateral			1	
	Rust colored fur: multiple areas			1	
	shoulder(s)				1
3	Alopecia: abdomen				1
	limb(s)		1	5	3
	multiple areas		2	1	4
	multiple areas, growing in		1	1	
	Chromodacryorrhea: eye, left				1
	Ear: red, bilateral			1	
	Rust colored fur: multiple areas			1	
	shoulder(s)				1

(continued)

Table 15. Summary of the F_0 Female Clinical Observations During Lactation (page 2 of 6)

Day[a]	Observation[b]	Butyl Benzyl Phthalate (ppm in the feed) 0	750	3750	11250
4	Alopecia: abdomen				1
	limb(s)		1	5	4
	multiple areas		2	1	4
	multiple areas, growing in		1	1	
	Ear: red, bilateral			1	
	Left rear leg swollen, not bearing weight on it			1	
	Mammary gland: swollen, caudal one on right side	1			
	Rust colored fur: multiple areas			1	
	shoulder(s)				1
	Sore metatarsal joint			1	
	Vagina: brown discharge		1		
5	Alopecia: abdomen				1
	limb(s)		1	5	3
	limb(s), growing in				1
	multiple areas		2	1	3
	multiple areas, growing in			1	1
	Ear: red, bilateral			1	
	Left rear leg swollen			1	
	Mammary gland: swollen, caudal one on right side	1			
	Rust colored fur: multiple areas			1	
	shoulder(s)				1
	Vagina: slight discharge		1		
6	Alopecia: abdomen				1
	limb(s)		1	5	2
	limb(s), growing in				1
	multiple areas		2	1	4
	multiple areas, growing in			1	1
	Ear: red, bilateral			1	
	Left rear leg swollen, slightly bearing weight on it			1	
	Mammary gland: swollen, caudal one on right side	1			
	Rust colored fur: multiple areas			1	
	shoulder(s)				1
	Vagina: discharge		1		
7	Alopecia: abdomen				1
	limb(s)		1	5	2
	limb(s), growing in				1
	multiple areas		2		4
	multiple areas, growing in			2	
	Dead fetus palpated		1		
	Ear: red, bilateral			1	
	Left rear leg swollen, improving			1	
	Mammary gland: swollen, caudal one on right side	1			
	Not using right rear leg		1		
	Piloerection		1		
	Rust colored fur: limb(s)			1	
	multiple areas		2	1	
	shoulder(s)				1
	Vagina: discharge		1		

(continued)

Table 15. Summary of the F_0 Female Clinical Observations During Lactation (page 3 of 6)

Day[a]	Observation[b]	Butyl Benzyl Phthalate (ppm in the feed)			
		0	750	3750	11250
8	Alopecia: abdomen				1
	limb(s)		1	5	2
	limb(s), growing in				1
	multiple areas		2		4
	multiple areas, growing in			2	
	Chromodacryorrhea: eye, left				1
	Ear: red, bilateral			1	
	Left rear leg swollen			1	
	Limping on right rear leg		1		
	Mammary gland: swollen, caudal one on right side	1			
	Rust colored fur: limb(s)			1	
	multiple areas		2	1	
	shoulder(s)				1
	Vagina: discharge, improved		1		
9	Alopecia: abdomen				1
	limb(s)		1	5	2
	limb(s), growing in				1
	multiple areas		2		4
	multiple areas, growing in			1	
	Chromodacryorrhea: eye, left				1
	Ear: red, bilateral			1	
	Left rear leg swollen, almost gone			1	
	Limping on right rear leg		1		
	Mammary gland: swollen, caudal one on right side	1			
	Piloerection		1		
	Rust colored fur: limb(s)			1	
	multiple areas		2	1	
	shoulder(s)				1
	Vagina: discharge with brown object slightly protruding		1		
10	Alopecia: abdomen				1
	limb(s)		1	5	2
	multiple areas		2		4
	multiple areas, growing in			1	
	Chromodacryorrhea: eye, left, improving				1
	Ear: red, bilateral			1	
	Limping on right rear leg		1		
	Mammary gland: swollen, caudal one on right side	1			
	Piloerection		1		
	Rust colored fur: limb(s)			1	
	multiple areas		2	1	
	shoulder(s)				1
	Vagina: discharge, much improved		1		

(continued)

Table 15. Summary of the F_0 Female Clinical Observations During Lactation (page 4 of 6)

Day[a]	Observation[b]	Butyl Benzyl Phthalate (ppm in the feed) 0	750	3750	11250
11	Alopecia: abdomen				1
	limb(s)		1	5	2
	multiple areas		2		4
	multiple areas, growing in			1	
	Chromodacryorrhea: eye, left				1
	Ear: red, bilateral			1	
	Limping on right rear leg		1		
	Mammary gland: swollen, caudal one on right side	1			
	Piloerection		1		
	Rust colored fur: limb(s)			1	
	multiple areas		2	1	
	shoulder(s)				1
	Vagina: discharge		1		
12	Alopecia: abdomen				1
	limb(s)		1	5	2
	limb(s), grown in at all other areas			1	
	multiple areas		2		4
	Chromodacryorrhea: eye, left				1
	Ear: red, bilateral			1	
	Limping on right rear leg		1		
	Mammary gland: swollen, caudal one on right side	1			
	Piloerection		1		
	Rust colored fur: limb(s)			1	
	multiple areas		2	1	
	shoulder(s)				1
	Vagina: discharge, none		1		
13	Alopecia: abdomen				1
	limb(s)		1	6	2
	multiple areas		2		4
	Chromodacryorrhea: eye, left				1
	Ear: red, bilateral			1	
	Limping on right rear leg		1		
	Mammary gland: swollen, caudal one on right side	1			
	Piloerection		1		
	Rust colored fur: limb(s)			1	
	multiple areas		2	1	
	shoulder(s)				1
14	Alopecia: abdomen				1
	limb(s)		1	6	2
	multiple areas		2		4
	Chromodacryorrhea: eye, left				1
	eye, right	1			
	Ear: red, bilateral			1	
	Limping on right rear leg		1		
	Mammary gland: swollen, caudal one on right side	1			
	Piloerection		1		
	Rust colored fur: limb(s)			1	
	multiple areas		2	1	
	shoulder(s)				1

(continued)

Table 15. Summary of the F_0 Female Clinical Observations During Lactation (page 5 of 6)

Day[a]	Observation[b]	Butyl Benzyl Phthalate (ppm in the feed) 0	750	3750	11250
15	Alopecia: abdomen				1
	limb(s)		1	6	2
	multiple areas		2		4
	Chromodacryorrhea: eye, left				1
	Ear: red, bilateral			1	
	Limping on right rear leg		1		
	Mammary gland: swollen, caudal one on right side	1			
	Piloerection		1		
	Rust colored fur: limb(s)			1	
	multiple areas		2	1	
	shoulder(s)				1
16	Alopecia: abdomen				1
	limb(s)		1	6	2
	multiple areas		2		4
	Chromodacryorrhea: eye, left				1
	Ear: red, bilateral			1	
	Limping on right rear leg		1		
	Mammary gland: swollen, caudal one on right side	1			
	Piloerection		1		
	Rust colored fur: limb(s)			1	
	multiple areas		2	1	
	shoulder(s)				1
17	Alopecia: abdomen				1
	limb(s)		1	6	2
	multiple areas		2		4
	Ear: red, bilateral			1	
	Found dead		1		
	Mammary gland: swollen, caudal one on right side	1			
	Rust colored fur: limb(s)			2	
	multiple areas		2	1	
	shoulder(s)				1
18	Alopecia: abdomen				1
	limb(s)		1	6	2
	multiple areas		2		4
	Ear: red, bilateral			1	
	Mammary gland: swollen, caudal one on right side	1			
	Rust colored fur: limb(s)			2	
	multiple areas		2	1	
	shoulder(s)				1
19	Alopecia: abdomen				1
	limb(s)		1	6	2
	multiple areas		2		4
	Ear: red, bilateral			1	
	Mammary gland: swollen, caudal one on right side	1			
	Rust colored fur: limb(s)			2	
	multiple areas		2	1	
	shoulder(s)				1

(continued)

Table 15. Summary of the F_0 Female Clinical Observations During Lactation (page 6 of 6)

Day[a]	Observation[b]	Butyl Benzyl Phthalate (ppm in the feed)			
		0	750	3750	11250
20	Alopecia: abdomen				1
	limb(s)		1	6	2
	multiple areas		2		4
	Ear: red, bilateral			1	
	Mammary gland: swollen, caudal one on right side	1			
	Rust colored fur: limb(s)			2	
	multiple areas		2	1	
	shoulder(s)				1
21	Alopecia: abdomen				1
	limb(s)		1	6	2
	multiple areas		2		4
	Ear: red, bilateral			1	
	Mammary gland: swollen, caudal one on right side	1			
	Rust colored fur: limb(s)			2	
	multiple areas		2	1	
	shoulder(s)				1

[a]Lactational day.
[b]Clinical observations are tabulated once per day per animal.

Table 16. Summary and Statistical Analysis of F_0 Reproductive and Lactational Indexes for the F_1 Litters (page 1 of 3)

	Butyl Benzyl Phthalate (ppm in the feed)			
	0	750	3750	11250
No. Animals on Study				
Males	30	30	30	30
Females	30	30	30	30
No. Females Paired				
	30	30	30	30
No. Females that Mated				
	30	29	30	30
Mating Index (no. females that mated/no. females paired)				
	100.0	96.7	100.0	100.0
No. of Pregnant Females				
	27	28	28	28
Fertility Index (no. pregnant females/no. females that mated)				
	90.0	96.6	93.3	93.3
No. of Females with Live Litters (pnd 0)				
	26[a]	27[b]	28	27[c]
Gestational Index (no. females with live litters/no. females pregnant)				
	96.3	96.4	100.0	96.4
No. Males Paired				
	30	29[d]	30	30
No. Males that Mated				
	30	28	30	30
Mating Index (no. males that mated/no. males paired)				
	100.0	96.6	100.0	100.0
No. Males Siring Litters				
	27	27	28	28
Fertility Index (no. males siring litters/no. males that mated)				
	90.0	96.4	93.3	93.3
Pregnancy Index (no. pregnant females/no. males that mated)				
	90.0	100.0	93.3	93.3

(continued)

Table 16. Summary and Statistical Analysis of F_0 Reproductive and Lactational Indexes for the F_1 Litters (page 2 of 3)

	Butyl Benzyl Phthalate (ppm in the feed)			
	0	750	3750	11250
Precoital Interval (days)[e,f]				
	3.1 ± 0.6 N=30	2.5 ± 0.5 N=27	2.9 ± 0.4 N=27	3.1 ± 0.6 N=28
Gestational Length (days)[e,g]				
	22.2 ± 0.1 N=27	22.2 ± 0.1 N=26	22.0 ± 0.1 N=25	22.0 ± 0.1 N=25
No. of Live Litters:				
Postnatal Day 0	26	27	28	27
Postnatal Day 4	26	27	28	27
Postnatal Day 7	26	27	28	27
Postnatal Day 14	26	27	28	27
Postnatal Day 21	26	26[h]	28	27
No. Implantation Sites per Litter[b]				
	15.33 ± 0.58 N=27	13.61 ± 0.80 N=28	14.54 ± 0.57 N=28	13.79 ± 0.66 N=28
Percent Postimplantation Loss per Litter[b]				
	15.79 ± 4.52 N=27	17.65 ± 3.96 N=28	8.77 ± 1.99 N=28	14.18 ± 3.96 N=28
Number of Live Pups on Postnatal Day 0[b]				
	13.3 ± 0.9 N=27	12.2 ± 0.9 N=27	13.4 ± 0.6 N=28	13.1 ± 0.7 N=27
Number of Dead Pups on Postnatal Day 0[b]				
	0.6 ± 0.4 N=27	0.2 ± 0.1 N=27	0.1 ± 0.1 N=28	0.6 ± 0.3 N=27
Total Number of Pups on Postnatal Day 0[b]				
	13.9 ± 0.7 N=27	12.4 ± 0.9 N=27	13.5 ± 0.6 N=28	13.7 ± 0.6 N=27
Stillbirth Index (no. dead on pnd 0/total no. on pnd 0)[b]				
	5.5 ± 3.8 N=27	2.1 ± 1.0 N=27	1.0 ± 0.7 N=28	3.9 ± 2.0 N=27

(continued)

Table 16. Summary and Statistical Analysis of F_0 Reproductive and Lactational Indexes for the F_1 Litters (page 3 of 3)

	Butyl Benzyl Phthalate (ppm in the feed)			
	0	750	3750	11250
Live Birth Index (no. live on pnd 0/total no. on pnd 0)[b]				
	94.5 ± 3.8 N=27	97.9 ± 1.0 N=27	99.0 ± 0.7 N=28	96.1 ± 2.0 N=27
4 Day Survival Index (no. surviving 4 days/no. live on pnd 0)[b]				
	97.2 ± 1.3 N=26	96.3 ± 1.4 N=27	97.5 ± 1.0 N=28	92.6 ± 2.7 N=27
7 Day Survival Index (no. surviving 7 days/no. live on pnd 4)[b]				
	100.0 ± 0.0 N=26	99.6 ± 0.4 N=27	99.3 ± 0.5 N=28	99.3 ± 0.5 N=27
14 Day Survival Index (no. surviving 14 days/no. live on pnd 7)[b]				
	99.6 ± 0.4 N=26	99.3 ± 0.5 N=27	100.0 ± 0.0 N=28	98.8 ± 0.6 N=27
21 Day Survival Index (no. surviving 21 days/no. live on pnd 14)[b]				
	100.0 ± 0.0 N=26	99.6 ± 0.4 N=26	99.6 ± 0.4 N=28	99.2 ± 0.5 N=27
Lactational Index (no. surviving 21 days/no. live on pnd 4)[b]				
	99.6 ± 0.4 N=26	98.5 ± 0.9 N=26	98.9 ± 0.6 N=28	97.4 ± 1.0 N=27

[a]Female 52 had a litter of all dead pups.
[b]Female 218 had implantation sites only.
[c]Female 228 had implantation sites only.
[d]Male 63 was found dead on study day 40.
[e]Reported as the mean ± S.E.M.; pnd=postnatal day. All indexes are the average percent per litter.
[f]Precoital interval could only be calculated for those females for which sperm were detected in the vaginal smear.
[g]Gestational length could not be calculated for females that were pregnant, but for which sperm were never detected in the vaginal smear.
[h]Female 78 was found dead on postnatal day 17 and the surviving pups were euthanized.

Table 17. Summary and Statistical Analysis of the F_1 Litter Size, Pup Body Weights, Pup Anogenital Distance, Pup Nipple/Areolae Retention and Percent Male Pups during Lactation (page 1 of 5)

	Butyl Benzyl Phthalate (ppm in the feed)			
	0	750	3750	11250
No. of Live Litters:				
Postnatal Day 0	26	27	28	27
Postnatal Day 4	26	27	28	27
Postnatal Day 7	26	27	28	27
Postnatal Day 14	26	27	28	27
Postnatal Day 21	26	26[a]	28	27
Average Number of Live Pups per Litter (pnd 0)[b]	13.8 ± 0.7 N=26	12.2 ± 0.9 N=27	13.4 ± 0.6 N=28	13.1 ± 0.7 N=27
Average Number of Live Pups per Litter (pnd 4)[b]	13.5 ± 0.7 N=26	11.7 ± 0.8 N=27	13.0 ± 0.6 N=28	12.1 ± 0.7 N=27
Average Number of Live Pups per Litter (pnd 7)[b]	9.5 ± 0.3 N=26	8.9 ± 0.5 N=27	9.5 ± 0.2 N=28	9.3 ± 0.3 N=27
Average Number of Live Pups per Litter (pnd 14)[b]	9.5 ± 0.3 N=26	8.8 ± 0.5 N=27	9.5 ± 0.2 N=28	9.2 ± 0.3 N=27
Average Number of Live Pups per Litter (pnd 21)[b]	9.5 ± 0.3 N=26	9.0 ± 0.4 N=26	9.5 ± 0.2 N=28	9.1 ± 0.3 N=27
Average **Male** Pup Anogenital Distance (mm) per Litter (pnd 0)[b]	2.06 ‡‡‡ ± 0.03 §§§ N=26	2.01 ± 0.04 N=27	1.89 *** ± 0.02 N=28	1.71 *** ± 0.03 N=27
Average Adjusted **Male** Pup Anogenital Distance (mm) per Litter (pnd 0)[c]	2.04 ΩΩΩ ± 0.03 λλλ N=26	1.99 ± 0.03 N=27	1.88 φφφ ± 0.03 N=28	1.75 φφφ ± 0.03 N=27

(continued)

Table 17. Summary and Statistical Analysis of the F_1 Litter Size, Pup Body Weights, Pup Anogenital Distance, Pup Nipple/Areolae Retention and Percent Male Pups during Lactation (page 2 of 5)

	Butyl Benzyl Phthalate (ppm in the feed)			
	0	750	3750	11250
Average **Female** Pup Anogenital Distance (mm) per Litter (pnd 0)[b]				
	0.96 ± 0.02 N=26	0.97 ± 0.02 N=27	0.92 ± 0.02 N=28	0.92 ± 0.02 N=27
Average Adjusted **Female** Pup Anogenital Distance (mm) per Litter (pnd 0)[c]				
	0.96 ± 0.02 N=26	0.95 ± 0.02 N=27	0.92 ± 0.02 N=28	0.94 ± 0.02 N=27
Average Pup Body Weight (g) per Litter (pnd 0)[b]				
	6.55 ‡‡‡ ± 0.10 §§§ N=26	6.79 ± 0.13 N=27	6.55 ± 0.11 N=28	6.05 ** ± 0.11 N=27
Average **Male** Body Weight (g) per Litter (pnd 0)[b]				
	6.76 ‡‡‡ ± 0.10 §§§ N=26	6.93 ± 0.13 N=27	6.71 ± 0.11 N=28	6.15 ** ± 0.11 N=27
Average **Female** Body Weight (g) per Litter (pnd 0)[b]				
	6.35 ‡‡‡ ± 0.10 §§§ N=26	6.67 ± 0.16 N=27	6.41 ± 0.11 N=28	5.91 * ± 0.11 N=27
Average Pup Body Weight (g) per Litter (pnd 4)[b]				
	10.22 ‡‡‡ ± 0.22 §§§ N=26	10.86 ± 0.34 N=27	10.82 ± 0.21 N=28	8.71 *** ± 0.29 N=27
Average **Male** Body Weight (g) per Litter (pnd 4)[b]				
	10.50 ‡‡‡ ± 0.23 §§§ N=26	11.01 ± 0.33 N=27	11.02 ± 0.21 N=28	8.86 *** ± 0.33 N=27
Average **Female** Body Weight (g) per Litter (pnd 4)[b]				
#	10.01 ††† ± 0.21 ŸŸŸ N=25[d]	10.82 ± 0.38 N=27	10.59 ± 0.22 N=28	8.45 ÞÞÞ ± 0.25 N=27

(continued)

Table 17. Summary and Statistical Analysis of the F_1 Litter Size, Pup Body Weights, Pup Anogenital Distance, Pup Nipple/Areolae Retention and Percent Male Pups during Lactation (page 3 of 5)

	Butyl Benzyl Phthalate (ppm in the feed)			
	0	750	3750	11250
Average Pup Body Weight (g) per Litter (pnd 7)[b]				
#	15.97 ††† ± 0.34 ŸŸŸ N=26	16.79 ± 0.50 N=27	17.00 Þ ± 0.28 N=28	12.71 ÞÞÞ ± 0.54 N=27
Average **Male** Body Weight (g) per Litter (pnd 7)[b]				
#	16.41 ††† ± 0.34 ŸŸŸ N=26	16.90 ± 0.52 N=27	17.34 Þ ± 0.26 N=28	12.96 ÞÞÞ ± 0.58 N=27
Average **Female** Body Weight (g) per Litter (pnd 7)[b]				
#	15.64 ††† ± 0.37 ŸŸŸ N=25[d]	16.78 ± 0.55 N=27	16.64 Þ ± 0.30 N=28	12.33 ÞÞÞ ± 0.49 N=27
Average Pup Body Weight (g) per Litter (pnd 14)[b]				
	32.04 ‡‡‡ ± 0.49 §§§ N=26	33.30 ± 0.83 N=27	32.80 ± 0.47 N=28	25.46 *** ± 0.79 N=27
Average **Male** Body Weight (g) per Litter (pnd 14)[b]				
#	32.70 ††† ± 0.52 ŸŸŸ N=26	33.60 ± 0.83 N=27	33.40 ± 0.43 N=28	25.80 ÞÞÞ ± 0.92 N=27
Average **Female** Body Weight (g) per Litter (pnd 14)[b]				
#	31.51 ††† ± 0.53 ŸŸŸ N=25[d]	33.19 ± 0.95 N=27	32.21 ± 0.50 N=28	24.84 ÞÞÞ ± 0.74 N=27
Average Pup Body Weight (g) per Litter (pnd 21)[b]				
	49.32 ‡‡‡ ± 1.04 §§§ N=26	51.09 ± 1.34 N=26	49.55 ± 0.83 N=28	39.19 *** ± 1.02 N=27
Average **Male** Body Weight (g) per Litter (pnd 21)[b]				
	50.29 ‡‡‡ ± 1.13 §§§ N=26	51.78 ± 1.26 N=26	50.69 ± 0.77 N=28	40.06 *** ± 1.10 N=27
Average **Female** Body Weight (g) per Litter (pnd 21)[b]				
	48.18 ‡‡‡ ± 1.07 §§§ N=25[d]	50.57 ± 1.58 N=26	48.37 ± 0.89 N=28	37.98 *** ± 0.98 N=27

(continued)

Table 17. Summary and Statistical Analysis of the F_1 Litter Size, Pup Body Weights, Pup Anogenital Distance, Pup Nipple/Areolae Retention and Percent Male Pups during Lactation (page 4 of 5)

	Butyl Benzyl Phthalate (ppm in the feed)			
	0	750	3750	11250
Percent Male Pups per Litter (pnd 0)[b]				
	45.9 ± 2.7 N=26	51.5 ± 2.6 N=27	49.5 ± 2.6 N=28	50.8 ± 3.1 N=27
Percent Male Pups per Litter (pnd 4)[b]				
	47.1 ± 3.5 N=26	51.4 ± 2.8 N=27	49.8 ± 2.7 N=28	51.5 ± 3.3 N=27
Percent Male Pups per Litter (pnd 7)[b]				
	47.8 ± 3.0 N=26	51.6 ± 2.1 N=27	48.9 ± 1.7 N=28	50.9 ± 2.4 N=27
Percent Male Pups per Litter (pnd 14)[b]				
	48.0 ± 3.0 N=26	51.3 ± 2.1 N=27	48.9 ± 1.7 N=28	51.4 ± 2.5 N=27
Percent Male Pups per Litter (pnd 21)[b]				
	48.0 ± 3.0 N=26	51.0 ± 2.1 N=26	49.1 ± 1.7 N=28	51.9 ± 2.5 N=27
Average Number of Nipples per Male Pup[e]				
	0.00 ΓΓ ± 0.00 ββββ N=114	0.00 ± 0.00 N=117[f]	0.00 ± 0.00 N=132	0.72 ◊◊◊ ± 0.20 N=130
Percent Male Pups with One or More Nipples[e]				
	0.00 ΓΓ ββ	4.10	0.00	19.23 ◊◊◊
Average Number of Areolae per Male Pup[e]				
	0.07 ΓΓΓ ± 0.04 ββββ N=114	0.00 ± 0.00 N=117[f]	0.02 ± 0.02 N=132	1.29 ◊◊ ± 0.33 N=130
Percent Male Pups with One or More Areolae[e]				
	2.63 ΓΓΓ ββββ	4.10	0.76	32.31 ◊◊◊

(continued)

Table 17. Summary and Statistical Analysis of the F_1 Litter Size, Pup Body Weights, Pup Anogenital Distance, Pup Nipple/Areolae Retention and Percent Male Pups during Lactation (page 5 of 5)

[a]Female 78 was found dead on postnatal day 17 and the surviving pups were euthanized.
[b]Reported as the mean ± S.E.M.; pnd=postnatal day.
[c]Reported as the adjusted mean ± S.E.M. (body weight as covariate); pnd=postnatal day.
[d]Decrease in N is due to one litter having only male pups.
[e]Reported as the adjusted mean ± S.E.M. or percentage (adjusted for intralitter correlations).
[f]Data for one litter (female 200) was inadvertently not recorded.
#Levene's test for homogeneity of variances was significant ($p<0.05$), therefore robust regression methods were used to test all treatment effects.
‡‡‡$p<0.001$; ANOVA Test.
§§§$p<0.001$; Test for Linear Trend.
*$p<0.05$; Dunnett's Test.
**$p<0.01$; Dunnett's Test.
***$p<0.001$; Dunnett's Test.
ΩΩΩ$p<0.001$; Analysis of Covariance with body weight on postnatal day 0 as covariate.
λλλ$p<0.001$; Linear Trend Analysis of Covariance with body weight on postnatal day 0 as covariate.
φφφ$p<0.001$; Dunnett's Test with body weight on postnatal day 0 as covariate.
†††$p<0.001$; Wald Chi-square Test for overall treatment effect in robust regression model.
ŸŸŸ$p<0.001$; Linear trend test in robust regression model.
Þ$p<0.05$; Individual t-test for pairwise comparisons to control in robust regression model.
ÞÞÞ$p<0.001$; Individual t-test for pairwise comparisons to control in robust regression model.
ΓΓ$p<0.01$; Overall analysis of correlated data.
ΓΓΓ$p<0.001$; Overall analysis of correlated data.
ββ$p<0.01$; Test for Liner Trend on correlated data.
βββ$p<0.001$; Test for Liner Trend on correlated data.
◊◊$p<0.01$; Pairwise comparison of correlated data.
◊◊◊$p<0.001$; Pairwise comparison of correlated data.

Table 18. Summary of the F_1 Pup Clinical Observations on Postnatal Days 0 Through 21 (page 1 of 2)

Day[a]	Sex[b]	Clinical Observation	Butyl Benzyl Phthalate (ppm in the feed) 0	750	3750	11250
0	F	Abdomen: swollen		1		
		Cold and lethargic			1	
		Found dead	9	2	2	7
		Found dead in afternoon				1
		Hematoma: back				1
		nose			1	
		nose; no milk band	1			
		Pale	1			
		Tail: missing	1			
		tip black			1	
	M	Bite mark: left lower abdomen		1		
		Found dead	5	4	2	8
		Found dead in afternoon				4
		Found dead, cannibalized	2			
		Hematoma: head and nose; no milk band	1			
		nose	1	1	1	
		Tail: missing				1
		tip dark		1		
	U	Found dead, cannibalized	1			
1	F	Found dead		2	2	4
	M	Found dead			3	4
2	F	Found dead		1		2
	M	Found dead		1	1	
3	F	Found dead		4		1
	M	Found dead		2		
4	F	Abdomen: swollen but improved		1		
		Bite mark: head	1			
		Clubbed hind limbs and missing tail	1			
		Found dead	1			1
		Missing and presumed dead	4	3	3	3
		Tail: tip black	1			
	M	Found dead	1	1	1	2
		Found dead in afternoon			1	
		Hematoma: back				1
		Missing and presumed dead	1	1		4
		Small and yellow in color				1

(continued)

Table 18. Summary of the F_1 Pup Clinical Observations on Postnatal Days 0 Through 21 (page 2 of 2)

Day[a]	Sex[b]	Clinical Observation	Butyl Benzyl Phthalate (ppm in the feed) 0	750	3750	11250
7	F	Abdomen: swollen and not using hind legs properly		1		
		Bite mark: head, healing	1			
		Found dead, cannibalized			1	
		Missing and presumed dead				1
		No milk seen in stomach				1
	M	Found dead				1
		Missing and presumed dead		1		
9	M	Found dead				1
11	F	Abdomen: misshapened and tail hangs limp		1		
14	F	Euthanized moribund		1		
		Lethargic				1
		Missing and presumed dead	1	1		1
	M	Found dead		1		
		Missing and presumed dead				1
15	F	Found dead				1
17	F	Euthanized, dam found dead		1		
	M	Blood on neck and in mouth, raspy breathing, euthanized				1
		Euthanized, dam found dead		2		
19	F	Found dead			1	
21	M	Eye: right, missing		1		

[a]Postnatal day.
[b]F is female, M is male and U is could not be determined.

Table 19. Summary of the F_1 Pup Gross Necropsy Findings on Postnatal Days 0 Through 21 and Postnatal Day 4 Culled Pups Gross Necropsy Findings and Analysis (page 1 of 4)

PUP NECROPSY FINDINGS ON POSTNATAL DAYS 0 THROUGH 21:

Day[a]	Sex[b]	Necropsy Finding	Butyl Benzyl Phthalate (ppm in the feed) 0	750	3750	11250
0	F	Autolyzed, unable to evaluate	3			
		Ductus: closed; Air in lungs; No milk in stomach		1		5
		Ductus: closed; Air in lungs; Tail: chewed off; Abdominal organs autolyzed; Sexed externally				1
		Ductus: closed; Little milk in stomach			1	
		Ductus: open; Air in 2 lung lobes on right side only			1	
		Ductus: open; Air in lungs; No milk in stomach				1
		Ductus: open; No air in lungs; Abdominal organs autolyzed	1			
		Ductus: open; No air in lungs; Coverd with bedding; Placenta: attached		1		
		Ductus: open; No air in lungs; Hydroureter: bilateral	1			
		Ductus: open; No air in lungs; No milk in stomach	1			1
		Ductus: open; No air in lungs; No milk in stomach; Distended ureter: bilateral	1			
		Ductus: open; No air in lungs; No milk in stomach; Hydroureter: left; Placenta: attached	1			
		Edema; Bulge on head, no skull present; No apparent nose; Liver: pale; Abdominal organs autolyzed; Severely clubbed right hindlimb; Placenta: attached and pale	1			
	M	Cannibalized, unable to evaluate; Sexed externally	2			
		Ductus: closed; Air in lungs; Milk in stomach		1		1
		Ductus: closed; Air in lungs; No milk in stomach	1			7
		Ductus: closed; No air in lungs; Abdominal organs autolyzed	1			
		Ductus: open; No air in lungs			2	1
		Ductus: open; No air in lungs; No milk in stomach	1	2		2
		Ductus: open; No air in lungs; No milk in stomach; Heart: pale; Kidney: pale, bilateral		1		
		Ductus: open; Small amount of air in lungs				1
		Edema: Liver: pale; Abdominal and thoracic organs autolyzed	1			
		Edema; No air in lungs; Liver: pale, almost white; Autolyzed	1			
	U	Cannibalized, abdomen and right hindlimb	1			

(continued)

Table 19. Summary of the F_1 Pup Gross Necropsy Findings on Postnatal Days 0 Through 21 and Postnatal Day 4 Culled Pups Gross Necropsy Findings and Analysis (page 2 of 4)

PUP NECROPSY FINDINGS ON POSTNATAL DAYS 0 THROUGH 21:

Day[a]	Sex[b]	Necropsy Finding	Butyl Benzyl Phthalate (ppm in the feed) 0	750	3750	11250
1	F	Ductus: closed; Air in lungs; Abdominal organs autolyzed, unable to evaluate			1	
		Ductus: closed; Air in lungs; No milk in stomach				3
		Ductus: closed; Air in lungs; No milk in stomach; Heart: pale; Kidney: pale, bilateral		1		
		Ductus: closed; Milk in stomach		1		
		Ductus: closed; No milk in stomach			1	
		Ductus: open; Air in lungs; No milk in stomach				1
	M	Ductus: closed; Air in lungs; Abdominal organs autolyzed, unable to evaluate			1	
		Ductus: closed; Air in lungs; Little milk in stomach; Right auricular flap: slightly enlarged; Distended ureter: bilateral			1	
		Ductus: closed; Air in lungs; Milk in stomach				2
		Ductus: closed; Air in lungs; No milk in stomach			1	
		Ductus: closed; Air in lungs; No milk in stomach; Heart: pale				1
		Ductus: open; Air in lungs; No milk in stomach				1
2	F	Ductus: closed; Milk in stomach		1		2
	M	Ductus: closed; Air in lungs; Milk in stomach			1	
		Ductus: closed; Milk in stomach		1		
3	F	Ductus: closed; Air in lungs; Abdominal organs autolyzed, unable to evaluate		1		
		Ductus: closed; Milk in stomach		1		1
		Ductus: closed; No milk in stomach		2		
	M	Ductus: closed; Milk in stomach		1		
		Hydroureter: left; Distended ureter: right; Kidney: hydronephrosis, left; No milk in stomach		1		
4	F	Ductus: closed; Air in lungs; Milk in stomach	1			
		Ductus: closed; Air in lungs; No milk in stomach				1
	M	Autolyzed, unable to evaluate	1			
		Ductus: closed; Abdominal organs autolyzed, unable to evaluate		1	1	
		Ductus: closed; All other organs cannibalized, unable to evaluate				1
		Ductus: closed; Milk in stomach			1	
		Ductus: closed; No milk in stomach; Intestines: gas filled				1
7	F	Cannibalized, unable to evaluate			1	
	M	Ductus: closed; No milk in stomach				1
9	M	Ductus: closed; No milk in stomach				1

(continued)

Table 19. Summary of the F_1 Pup Gross Necropsy Findings on Postnatal Days 0 Through 21 and Postnatal Day 4 Culled Pups Gross Necropsy Findings and Analysis (page 3 of 4)

PUP NECROPSY FINDINGS ON POSTNATAL DAYS 0 THROUGH 21:

Day[a]	Sex[b]	Necropsy Finding	Butyl Benzyl Phthalate (ppm in the feed) 0	750	3750	11250
14	F	Euthanized moribund; Ductus: closed; No milk in stomach; Stomach: filled with air; Intestines: filled with air		1		
	M	Ductus: closed; Abdominal organs autolyzed, unable to evaluate		1		
15	F	Ductus: closed; No milk or food in stomach				1
17	M	Puncture wounds and blood at base of right ear and neck; Blood in mouth; Ductus: closed; Milk in stomach; Bite on neck punctured trachea				1
19	F	Ductus: closed; No milk in stomach			1	

PUP NECROPSY FINDINGS FOR POSTNATAL DAY 4 CULLED PUPS:

Day[a]	Sex[b]	Necropsy Finding	Butyl Benzyl Phthalate (ppm in the feed) 0	750	3750	11250
4	F	Distended ureter: bilateral	5	5	9	1
		Distended ureter: bilateral; Urinary bladder: full				2
		Distended ureter: left	1	4		
		Distended ureter: left; Hydroureter: right	1			
		Distended ureter: right	2	2	1	3
		Distended ureter: right; Hydroureter: left			3	
		Ductus: open; No milk in stomach				1
		Hydroureter: bilateral	1	5	3	
		Hydroureter: bilateral; Enlarged renal pelvis: left	1			
		Kidney: hydronephrosis, right				1
		No milk in stomach				1
	M	Distended ureter: bilateral	1	3	9	1
		Distended ureter: bilateral; No milk in stomach	1		1	
		Distended ureter: bilateral; Urinary bladder: full		1	2	
		Distended ureter: left	2	3	5	
		Distended ureter: left; Hydroureter: right; Kidney: hydronephrosis, right	1			
		Distended ureter: left; Kidney: hydronephrosis, bilateral	1			
		Distended ureter: right	2	1		
		Distended ureter: right; Hydroureter: left	1			
		Distended ureter: right; Hydroureter: left; Ductus: open				1
		Hydroureter: left	1	1	1	
		Kidney: hydronephrosis, bilateral			1	
		Testis: displaced in abdominal cavity just below kidney, bilateral				1
		Testis: displaced in abdominal cavity with the right testis just below the left kidney and the left testis just below the right testis				1

(continued)

Table 19. Summary of the F_1 Pup Gross Necropsy Findings on Postnatal Days 0 Through 21 and Postnatal Day 4 Culled Pups Gross Necropsy Findings and Analysis (page 4 of 4)

	Butyl Benzyl Phthalate (ppm in the feed)			
	0	750	3750	11250
No. **Male** Pups Examined	48	38	51	45
Number of PND 4 Culled **Male** Pups with One or More Urinary Tract Malformations[c]	4	1	2	1
Percent PND 4 Culled **Male** Pups with One or More Urinary Tract Malformations[c,d]	8.33 ± 3.47 N=48	2.63 ± 2.56 N=38	3.92 ± 2.73 N=51	2.22 ± 2.24 N=45
Number of PND 4 Culled **Male** Pups with One or More Reproductive Tract Malformations[e]	0	0	0	2
Percent PND 4 Culled **Male** Pups with One or More Reproductive Tract Malformations[d,e]	0.00 ± 0.00 N=48	0.00 ± 0.00 N=38	0.00 ± 0.00 N=51	4.44 ± 4.18 N=45
No. **Female** Pups Examined	55	37	45	30
Number of PND 4 Culled **Female** Pups with One or More Urinary Tract Malformations[c]	3	5	6	1
Percent PND 4 Culled **Female** Pups with One or More Urinary Tract Malformations[c,d]	5.45 ± 5.26 N=55	13.51 ± 9.11 N=37	13.33 ± 10.53 N=45	3.33 ± 2.94 N=30
Number of PND 4 Culled **Female** Pups with One or More Reproductive Tract Malformations[e]	0	0	0	0
Percent PND 4 Culled **Female** Pups with One or More Reproductive Tract Malformations[c,e]	0.00 ± 0.00 N=55	0.00 ± 0.00 N=37	0.00 ± 0.00 N=45	0.00 ± 0.00 N=30

[a]Postnatal day.
[b]F is female, M is male and U is unable to determine.
[c]Includes hydroureter and hydronephrosis.
[d]Reported as the percentage ± the standard error of the percentage (adjusted for intralitter correlation).
[e]Includes displacement of testes.

Table 20. Summary and Statistical Analysis of F_1 Pup Necropsy Weights and Selected Gross Necropsy Findings on Postnatal Day 21 (page 1 of 4)

	Butyl Benzyl Phthalate (ppm in the feed)			
	0	750	3750	11250
MALES	68	68	78	76[a]
Body Weight at Sacrifice (g)[b]	49.45 ΓΓΓ ± 1.24 βββ N=68	49.57 ± 1.14 N=68	50.00 ± 0.76 N=78	40.35 ♦♦♦ ± 1.03 N=76
Thymus Weight (g)[b]	0.2152 ΓΓΓ ±0.0071 βββ N=67[c]	0.2289 ±0.0089 N=68	0.2185 ±0.0074 N=78	0.1792 ♦♦♦ ±0.0072 N=74
Spleen Weight (g)[b]	0.2048 ΓΓΓ ±0.0089 βββ N=68	0.2060 ±0.0077 N=68	0.2018 ±0.0073 N=78	0.1461 ♦♦♦ ±0.0049 N=74
Brain Weight (g)[b]	1.4504 ΓΓΓ ±0.0105 βββ N=68	1.4769 ±0.0111 N=68	1.4900 ♦♦ ±0.0097 N=78	1.4065 ♦ ±0.0141 N=74
Paired Testes Weight (g)[b]	0.2223 ΓΓΓ ±0.0064 βββ N=68	0.2294 ±0.0058 N=68	0.2395 ♦ ±0.0034 N=78	0.1647 ♦♦♦ ±0.0058 N=75[d]
Paired Epididymides Weight (g)[b]	0.0566 ΓΓΓ ±0.0017 βββ N=68	0.0559 ±0.0019 N=68	0.0573 ±0.0015 N=78	0.0443 ♦♦♦ ±0.0015 N=63[e]
Relative Thymus Weight (% of sacrifice weight)[b]	0.4352 ±0.0131 N=67[c]	0.4601 ±0.0126 N=68	0.4362 ±0.0114 N=78	0.4427 ±0.0124 N=74
Relative Spleen Weight (% of sacrifice weight)[b]	0.4118 ΓΓΓ ±0.0114 βββ N=68	0.4146 ±0.0118 N=68	0.4007 ±0.0093 N=78	0.3632 ♦♦♦ ±0.0089 N=74

(continued)

Table 20. Summary and Statistical Analysis of F_1 Pup Necropsy Weights and Selected Gross Necropsy Findings on Postnatal Day 21 (page 2 of 4)

	Butyl Benzyl Phthalate (ppm in the feed)			
	0	750	3750	11250
Relative Brain Weight (% of sacrifice weight)[b]				
	2.9871 ΓΓΓ ±0.0607 βββ N=68	3.0242 ±0.0630 N=68	3.0055 ±0.0441 N=78	3.5509 ♦♦♦ ±0.0740 N=74
Relative Paired Testes Weight (% of sacrifice weight)[b]				
	0.4496 ΓΓΓ ±0.0062 βββ N=68	0.4642 ±0.0082 N=68	0.4805 ♦♦♦ ±0.0062 N=78	0.4037 ♦♦♦ ±0.0083 N=75[d]
Relative Paired Epididymides Weight (% of sacrifice weight)[b]				
	0.1153 ±0.0029 N=68	0.1130 ±0.0030 N=68	0.1150 ±0.0027 N=78	0.1112 ±0.0041 N=63[e]
No. **Male** Pups Examined	68	68	78	76
Number of PND 21 **Male** Pups with One or More Urinary Tract Malformations[f,g]				
	1	2	0	2
Percent PND 21 **Male** Pups with One or More Urinary Tract Malformations[f,g,h]				
	1.47 ± 1.44 N=68	2.94 ± 2.89 N=68	0.00 ± 0.00 N=78	2.63 ± 1.79 N=76
Number of PND 21 **Male** Pups with One or More Reproductive Tract Malformations[f,i]				
	0	0	0	25
Percent PND 21 **Male** Pups with One or More Reproductive Tract Malformations[f,h,i]				
	0.00 ΓΓΓ ± 0.00 βββ N=68	0.00 ± 0.00 N=68	0.00 ± 0.00 N=78	32.89 ♦♦♦ ± 7.67 N=76
FEMALES	73	68	81	67
Body Weight at Sacrifice (g)[b]				
	47.86 ΓΓΓ ± 1.10 βββ N=73	49.01 ± 1.55 N=68	47.72 ± 0.87 N=81	37.33 ♦♦♦ ± 1.15 N=67

(continued)

Table 20. Summary and Statistical Analysis of F_1 Pup Necropsy Weights and Selected Gross Necropsy Findings on Postnatal Day 21 (page 3 of 4)

	Butyl Benzyl Phthalate (ppm in the feed)			
	0	750	3750	11250
Thymus Weight (g)[b]				
	0.2318 ΓΓΓ ββββ	0.2369	0.2258	0.1797 ♦♦♦
	±0.0079	±0.0103	±0.0072	±0.0080
	N=73	N=68	N=81	N=67
Spleen Weight (g)[b]				
	0.1998 ΓΓΓ ββββ	0.2174	0.1909	0.1322 ♦♦♦
	±0.0086	±0.0124	±0.0058	±0.0050
	N=73	N=68	N=81	N=67
Brain Weight (g)[b]				
	1.4194 ΓΓΓ ββββ	1.4372	1.4361	1.3601 ♦♦
	±0.0115	±0.0133	±0.0095	±0.0186
	N=73	N=68	N=80[j]	N=67
Paired Ovaries Weight (g)[b]				
	0.0339 ΓΓΓ ββββ	0.0365	0.0346	0.0255 ♦♦♦
	±0.0010	±0.0015	±0.0008	±0.0013
	N=73	N=66[c]	N=81	N=65[c,f]
Uterus Weight (g)[b]				
	0.1081 ΓΓΓ ββββ	0.1059	0.1118	0.0855 ♦♦
	±0.0063	±0.0067	±0.0047	±0.0056
	N=73	N=68	N=81	N=66[f]
Relative Thymus Weight (% of sacrifice weight)[b]				
	0.4852	0.4827	0.4726	0.4769
	±0.0130	±0.0140	±0.0113	±0.0131
	N=73	N=68	N=81	N=67
Relative Spleen Weight (% of sacrifice weight)[b]				
	0.4143 ΓΓΓ ββββ	0.4383	0.3990	0.3541 ♦♦♦
	±0.0112	±0.0149	±0.0087	±0.0089
	N=73	N=68	N=81	N=67
Relative Brain Weight (% of sacrifice weight)[b]				
	3.0071 ΓΓΓ ββββ	2.9966	3.0444	3.7219 ♦♦♦
	±0.0557	±0.0843	±0.0464	±0.1018
	N=73	N=68	N=80[j]	N=67
Relative Paired Ovaries Weight (% of sacrifice weight)[b]				
	0.0710	0.0740	0.0727	0.0683
	±0.0017	±0.0017	±0.0016	±0.0025
	N=73	N=66[c]	N=81	N=65[c,f]

(continued)

Table 20. Summary and Statistical Analysis of F_1 Pup Necropsy Weights and Selected Gross Necropsy Findings on Postnatal Day 21 (page 4 of 4)

	Butyl Benzyl Phthalate (ppm in the feed)			
	0	750	3750	11250
Relative Uterus Weight (% of sacrifice weight)[b]				
	0.2236 ±0.0097 N=73	0.2154 ±0.0119 N=68	0.2335 ±0.0079 N=81	0.2310 ±0.0142 N=66[f]
No. **Female** Pups Examined	73	68	81	68
Number of PND 21 **Female** Pups with One or More Urinary Tract Malformations[g,h]				
	1	0	0	0
Percent PND 21 **Female** Pups with One or More Urinary Tract Malformations[g,h,i]				
	1.47 ± 1.44 N=73	0.00 ± 0.00 N=68	0.00 ± 0.00 N=81	0.00 ± 0.00 N=68
Number of PND 21 **Female** Pups with One or More Reproductive Tract Malformations[g]				
	0	0	0	0
Percent PND 21 **Female** Pups with One or More Reproductive Tract Malformations[g,i]				
	0.00 ± 0.00 N=73	0.00 ± 0.00 N=68	0.00 ± 0.00 N=81	0.00 ± 0.00 N=68

[a]Two additional male pups had body weight, paired testes weight and paired epididymides weight recorded and included because they had nipples.
[b]Reported as the mean ± S.E.M. (adjusted for intralitter correlation).
[c]Decrease in N is due to one or more weights being statistical outliers and therefore they were removed.
[d]Decrease in N is due to one male having only one testis.
[e]Decrease in N is due to one or more males having one or both epididymides missing or having parts of one or both epididymides missing.
[f]Decrease in N id due to one weight being unrealistic and therefore it was not included.
[g]See Table A-24 for a list of the gross necropsy findings on postnatal day 21.
[h]Includes hydronephrosis.
[i]Reported as the percentage ± the standard error of the percentage (adjusted for intralitter correlation).
[j]Includes missing epididymis (whole or part), epididymis reduced in size, missing testis, testis reduced in size and undescended testis.
rrr p<0.001; Overall analysis of correlated data.
βββ p<0.001; Test for Linear Trend on correlated data.
♦ p<0.05; Pairwise comparison of correlated data.
♦♦ p<0.01; Pairwise comparison of correlated data.
♦♦♦ p<0.001; Pairwise comparison of correlated data.

Table 21. Summary of the F_1 Pup Necropsy Findings on Postnatal Day 21 (page 1 of 1)

Sex[a]	Necropsy Finding	Butyl Benzyl Phthalate (ppm in the feed) 0	750	3750	11250
F	Kidney: hydronephrosis, left	1			
	Vertebrae: missing, sacral		1		
M	Epididymis: caput and corpus missing, bilateral				1
	caput and corpus missing, left				1
	caput and corpus missing, right				1
	cauda and corpus missing and caput reduced in size, bilateral				1
	cauda and corpus missing, left				3
	cauda missing, left				1
	cauda missing, right				2
	corpus missing, right				1
	corpus reduced in size, bilateral				2
	corpus reduced in size, right				1
	dark red and firm, bilateral				1
	missing, bilateral				2
	missing, left				3
	missing, right				2
	reduced in size, bilateral				2
	reduced in size, left				1
	reduced in size, right				1
	Eye: missing, right		1		
	Kidney: hydronephrosis, left				1
	hydronephrosis, right	1	2		1
	Nipples: 2				2
	3				6
	4				3
	5				2
	6				7
	Tail: missing				1
	Testis: dark red, left				1
	missing, left				1
	reduced in size, left				1
	undescended, bilateral				2
	undescended, left				3
	undescended, right				3

[a]F is female and M is male.

Table 22. Summary and Statistical Analysis of the F_0 Male Body Weights and Weight Changes During the Post Mating Holding Period (page 1 of 1)

	Butyl Benzyl Phthalate (ppm in the feed)			
	0	750	3750	11250
No. of Males on Study	30	30	30	30
Body Weight (sd 84) (g)[a]				
	557.9 ‡‡ ± 8.5 N=30	543.0 ± 6.5 N=29[b]	579.5 ± 9.3 N=30	543.4 ± 7.6 N=30
Body Weight (sd 91) (g)[a]				
	570.4 ‡‡ ± 8.5 N=30	556.0 ± 6.6 N=29	593.4 ± 9.7 N=30	556.1 ± 7.8 N=30
Body Weight (sd 98) (g)[a]				
	582.5 ‡‡ ± 9.0 N=30	570.3 ± 7.1 N=29	608.7 ± 10.1 N=30	570.9 ± 8.1 N=30
Body Weight (sd 105) (g)[a]				
	591.2 ‡‡ ± 9.4 N=30	577.7 ± 6.7 N=29	615.2 ± 10.3 N=30	577.4 ± 8.3 N=30
Body Weight Change (sd 84 to 91) (g)[a]				
	12.5 ± 0.9 N=30	13.1 ± 0.8 N=29	13.9 ± 1.1 N=30	12.7 ± 0.8 N=30
Body Weight Change (sd 91 to 98) (g)[a]				
	12.1 ± 0.8 N=30	14.2 ± 1.1 N=29	15.3 ± 1.1 N=30	14.8 ± 0.8 N=30
Body Weight Change (sd 98 to 105) (g)[a]				
	8.7 ± 1.1 N=30	7.4 ± 1.0 N=29	6.4 ± 1.1 N=30	6.4 ± 1.0 N=30

[a]Reported as the mean ± S.E.M.; sd=study day with study day 0 being the first day of exposure.
[b]Decrease in N is due to male 63 being found dead on study day 40.
‡‡$p<0.01$; ANOVA Test.

Table 23. Summary and Statistical Analysis of the F_0 Male Feed Consumption, Percent Food Efficiency and Butyl Benzyl Phthalate Intake During the Post Mating Holding Period (page 1 of 2)

	Butyl Benzyl Phthalate (ppm in the feed)			
	0	750	3750	11250
No. of Males on Study	30	30	30	30
Feed Consumption (sd 84 to 91) (g/day)[a]				
	27.8 ± 0.4 N=30	27.8 ± 0.3 N=29[b]	28.6 ± 0.5 N=29[c]	27.9 ± 0.4 N=30
Feed Consumption (sd 91 to 98) (g/day)[a]				
#	27.8 ± 0.4 N=30	27.6 ± 0.3 N=28[d]	28.6 ± 0.5 N=29[e]	27.7 ± 0.4 N=30
Feed Consumption (sd 98 to 105) (g/day)[a]				
	28.5 ± 0.4 N=30	27.3 ± 0.3 N=29	28.4 ± 0.5 N=30	28.0 ± 0.4 N=30
Feed Consumption (sd 84 to 91) (g/kg/day)[a]				
	49.4 ‡ ± 0.5 N=30	50.7 ± 0.6 N=29	48.9 ± 0.5 N=29[c]	51.0 ± 0.8 N=30
Feed Consumption (sd 91 to 98) (g/kg/day)[a]				
	48.3 ± 0.5 N=30	49.1 ± 0.5 N=28[d]	47.5 ± 0.5 N=29[e]	49.3 ± 0.7 N=30
Feed Consumption (sd 98 to 105) (g/kg/day)[a]				
	48.6 ‡‡ ± 0.4 N=30	47.7 ± 0.5 N=29	46.5 * ± 0.5 N=30	48.8 ± 0.7 N=30
Percent Food Efficiency (sd 84 to 91)[a]				
	6.4 ± 0.5 N=30	6.7 ± 0.4 N=29	6.9 ± 0.5 N=29[c]	6.5 ± 0.4 N=30
Percent Food Efficiency (sd 91 to 98)[a]				
	6.2 ± 0.4 N=30	7.4 ± 0.5 N=28[d]	7.7 ± 0.5 N=29[e]	7.7 ± 0.4 N=30

(continued)

Table 23. Summary and Statistical Analysis of the F_0 Male Feed Consumption, Percent Food Efficiency and Butyl Benzyl Phthalate Intake During the Post Mating Holding Period (page 2 of 2)

	Butyl Benzyl Phthalate (ppm in the feed)			
	0	750	3750	11250
Percent Food Efficiency (sd 98 to 105)[a]				
	4.3 ± 0.5 N=30	3.9 ± 0.5 N=29	3.2 ± 0.5 N=30	3.2 ± 0.6 N=30
Butyl Benzyl Phthalate Intake (sd 84 to 91) (mg/kg/day)[a]				
		38.01 ± 0.43 N=29	183.37 ± 1.73 N=29[c]	573.50 ± 9.12 N=30
Butyl Benzyl Phthalate Intake (sd 91 to 98) (mg/kg/day)[a]				
		36.85 ± 0.34 N=28[d]	178.10 ± 1.77 N=29[e]	554.36 ± 7.85 N=30
Butyl Benzyl Phthalate Intake (sd 98 to 105) (mg/kg/day)[a]				
		35.79 ± 0.37 N=29	174.39 ± 1.70 N=30	549.47 ± 7.37 N=30

[a]Reported as the mean ± S.E.M. sd = study day with study day 0 being the first day of exposure.
[b]Decrease in N is due to male 63 being found dead on study day 40.
[c]Decrease in N is due to the feed consumption value for one male being unrealistic (i.e. negative) and it was therefore excluded.
[d]Decrease in N is due to the feed weight for one male inadvertently not being recorded.
[e]Decrease in N is due to the feed consumption value for one male being a statistical outlier and it was therefore removed.
[#]Levene's test for homogeneity of variances was significant ($p<0.05$), therefore robust regression methods were used to test all treatment effects.
‡$p<0.05$; ANOVA Test.
‡‡$p<0.01$; ANOVA Test.
*$p<0.05$; Dunnett's Test.

Table 24. Summary of the F_0 Male Clinical Observations During the Post Mating Holding Period
(page 1 of 4)

A. Clinical Observations Summarized by Group

Observation	Butyl Benzyl Phthalate (ppm in the feed)			
	0	750	3750	11250
Alopecia	4	4	5	5
Found dead				1
Rust colored fur	1		1	2
Sore(s)				1

B. Clinical Observations Summarized by Group and Day

Day[a]	Observation[b]	Butyl Benzyl Phthalate (ppm in the feed)			
		0	750	3750	11250
85	Alopecia: face			1	
	limb(s)	3	2	2	3
	multiple areas	1			2
	neck, growing in			1	
	Rust colored fur: limb(s)	1		1	1
	Sore(s): neck				1
86	Alopecia: face			1	
	limb(s)	3	2	2	3
	multiple areas	1			2
	Rust colored fur: limb(s)	1		1	1
	Sore(s): neck				1
87	Alopecia: face			1	
	limb(s)	3	2	2	3
	multiple areas	1			2
	Rust colored fur: limb(s)	1		1	1
	Sore(s): neck				1
88	Alopecia: face			1	
	limb(s)	3	2	2	3
	multiple areas	1			2
	Rust colored fur: limb(s)	1		1	1
	Sore(s): neck				1
89	Alopecia: face			1	
	limb(s)	1	2	1	3
	limb(s), growing in	2		1	
	multiple areas	1			2
	Rust colored fur: limb(s)	1		1	1
	Sore(s): neck				1
90	Alopecia: face			1	
	limb(s)	1	2	1	3
	limb(s), growing in	1		1	
	multiple areas	1			2
	Rust colored fur: limb(s)	1		1	1
	Sore(s): neck				1

(continued)

Table 24. Summary of the F_0 Male Clinical Observations During the Post Mating Holding Period
(page 2 of 4)

Day[a]	Observation[b]	Butyl Benzyl Phthalate (ppm in the feed)			
		0	750	3750	11250
91	Alopecia: face			1	
	limb(s)	1	2	1	3
	limb(s), growing in			1	
	multiple areas	1			2
	Rust colored fur: limb(s)	1		1	1
	Sore(s): neck				1
92	Alopecia: face			1	
	limb(s)	1	2	1	3
	limb(s), growing in			1	
	multiple areas	1			2
	Rust colored fur: limb(s)	1		1	1
	Sore(s): neck				1
93	Alopecia: face			1	
	limb(s)	1	1	1	3
	limb(s), growing in		1	1	
	multiple areas	1			2
	Rust colored fur: limb(s)	1		1	1
	Sore(s): neck				1
94	Alopecia: face			1	
	limb(s)	1	2	1	3
	limb(s), growing in		1	1	
	multiple areas	1			2
	Rust colored fur: limb(s)	1		1	1
	Sore(s): neck				1
95	Alopecia: face			1	
	limb(s)	1	2	1	3
	limb(s), growing in		1	1	
	multiple areas	1			2
	Rust colored fur: limb(s)	1		1	1
	Sore(s): neck				1
96	Alopecia: face, growing in			1	
	limb(s)	1	2	1	3
	limb(s), growing in		1	1	
	multiple areas	1			2
	Rust colored fur: limb(s)	1		1	1
	Sore(s): neck				1
97	Alopecia: limb(s)	1	2	1	3
	limb(s), growing in		1	1	
	multiple areas				2
	multiple areas, growing in	1			
	Rust colored fur: limb(s)	1			
	limb(s), improved			1	
	limb(s), slight				1
	Sore(s): neck				1

(continued)

Table 24. Summary of the F_0 Male Clinical Observations During the Post Mating Holding Period
(page 3 of 4)

Day[a]	Observation[b]	Butyl Benzyl Phthalate (ppm in the feed) 0	750	3750	11250
98	Alopecia: limb(s)	1	2	2	3
	limb(s), growing in		1	1	
	multiple areas				2
	Rust colored fur: limb(s)	1			1
	Sore(s): neck				1
99	Alopecia: limb(s)	1	2	2	3
	limb(s), growing in		1		
	multiple areas				2
	Rust colored fur: limb(s)	1			1
	Sore(s): neck				1
100	Alopecia: limb(s)	1	3	2	3
	multiple areas				2
	Rust colored fur: limb(s)	1			1
	Sore(s): neck, treated with 0.2% nitrofurazone powder				1
101	Alopecia: limb(s)	1	3	2	3
	multiple areas				2
	Rust colored fur: limb(s)	1			1
	Sore(s): neck, treated with 0.2% nitrofurazone powder				1
102	Alopecia: limb(s)	1	3	2	3
	multiple areas				2
	Rust colored fur: limb(s)	1			1
	Sore(s): neck, treated with 0.2% nitrofurazone powder				1
103	Alopecia: limb(s)	1	3	2	2
	limb(s), growing in				1
	multiple areas				2
	Rust colored fur: limb(s)	1			1
	Sore(s): neck, treated with 0.2% nitrofurazone powder				1
104	Alopecia: limb(s)	1	3	1	2
	limb(s), growing in			1	1
	multiple areas				2
	Rust colored fur: limb(s)	1			
	limb(s), improved				1
	shoulder(s)				1
	Sore(s): neck, treated with 0.2% nitrofurazone powder				1
105	Alopecia: limb(s)	1	3	1	2
	multiple areas				2
	Rust colored fur: limb(s), much improved	1			
	shoulder(s)				1
	Sore(s): neck, treated with 0.2% nitrofurazone powder				1
106	Alopecia: limb(s)	1	3	1	2
	multiple areas				2
	Rust colored fur: shoulder(s)				1
	Sore(s): neck, treated with 0.2% nitrofurazone powder				1

(continued)

Table 24. Summary of the F_0 Male Clinical Observations During the Post Mating Holding Period
(page 4 of 4)

Day[a]	Observation[b]	Butyl Benzyl Phthalate (ppm in the feed)			
		0	750	3750	11250
107	Alopecia: limb(s)	1	3	1	2
	multiple areas				2
	Rust colored fur: shoulder(s)				1
	Sore(s): neck				1
108	Alopecia: limb(s)	1	3	1	2
	multiple areas				2
	Rust colored fur: shoulder(s)				1
	Sore(s): neck				1
109	Alopecia: limb(s)	1	3	1	2
	multiple areas				2
	Found dead				1
	Rust colored fur: shoulder(s)				1
	Sore(s): neck				1
110	Alopecia: limb(s)	1	3	1	2
	multiple areas				2
	Rust colored fur: shoulder(s)				1
	Sore(s): neck				1
111	Alopecia: limb(s)	1	3	1	1
	multiple areas				2
112	Alopecia: limb(s)		3	1	
	multiple areas				1
113	Alopecia: limb(s)		2		
	multiple areas				1

[a]Study day.
[b]Clinical observations are tabulated once per day per animal.

Table 25. Summary of the F_0 Female Body Weights and Weight Changes During the Post Mating Holding Period (page 1 of 2)

	Butyl Benzyl Phthalate (ppm in the feed)			
	0	750	3750	11250
No. of Females on Study	30	30	30	30
Body Weight (sd 84) (g)[a]				
	. ± . N=0	357.7 ± 19.8 N=3	356.9 ± 15.8 N=3	320.6 ± 6.6 N=2
Body Weight (sd 91) (g)[a]				
	. ± . N=0	363.4 ± 17.1 N=3	406.0 ± 20.0 N=3	392.3 ± 19.3 N=2
Body Weight (sd 98) (g)[a]				
	289.5 ± 15.9 N=3	353.9 ± 18.3 N=2	325.8 ± 24.7 N=2	291.6 ± 12.2 N=3
Body Weight (sd 105) (g)[a]				
	326.8 ± 19.4 N=3	361.8 ± 12.8 N=3	318.7 ± 16.2 N=2	290.1 ± 11.9 N=3
Body Weight (sd 112) (g)[a]				
	330.0 ± 18.7 N=3	364.1 ± 13.1 N=3	311.8 ± 13.0 N=2	291.9 ± 12.6 N=3
Body Weight Change (sd 84 to 91) (g)[a]				
	. ± . N=0	5.8 ± 14.2 N=3	49.1 ± 4.2 N=3	71.7 ± 12.7 N=2
Body Weight Change (sd 91 to 98) (g)[a]				
	. ± . N=0	-1.1 ± 7.5 N=2	. ± . N=0	. ± . N=0
Body Weight Change (sd 98 to 105) (g)[a]				
	4.7 ± 2.7 N=2	-0.8 ± 1.9 N=2	-7.2 ± 8.5 N=2	-1.5 ± 1.6 N=3

(continued)

Table 25. Summary of the F_0 Female Body Weights and Weight Changes During the Post Mating Holding Period (page 2 of 2)

	Butyl Benzyl Phthalate (ppm in the feed)			
	0	750	3750	11250
Body Weight Change (sd 105 to 112) (g)[a]				
	3.2 ± 4.0 N=3	2.2 ± 1.8 N=3	-6.9 ± 3.2 N=2	1.8 ± 1.9 N=3

[a]Reported as the mean ± S.E.M.; sd=study day with the first day of exposure being study day 0. Includes all females that were not found sperm and/or plug positive or females that did not have live litters or females whose entire litter died prior to postnatal day 21. Statistical analyses were not performed on these endpoints since not all females were represented.

Table 26. Summary of the F_0 Female Feed Consumption, Percent Food Efficiency and Butyl Benzyl Phthalate Intake During the Post Mating Holding Period (page 1 of 2)

	Butyl Benzyl Phthalate (ppm in the feed)			
	0	750	3750	11250
No. of Females on Study	30	30	30	30
Feed Consumption (sd 84 to 91) (g/day)[a]				
	. ± . N=0	23.0 ± 1.7 N=3	25.0 ± 0.3 N=3	21.0 ± 0.7 N=2
Feed Consumption (sd 91 to 98) (g/day)[a]				
	. ± . N=0	18.3 ± 0.5 N=2	. ± . N=0	. ± . N=0
Feed Consumption (sd 98 to 105) (g/day)[a]				
	21.0 ± 1.9 N=2	19.4 ± 0.7 N=2	19.3 ± 0.5 N=2	21.9 ± 4.1 N=3
Feed Consumption (sd 105 to 112) (g/day)[a]				
	20.4 ± 0.6 N=3	19.6 ± 0.6 N=3	19.0 ± 0.5 N=2	20.6 ± 3.3 N=3
Feed Consumption (sd 84 to 91) (g/kg/day)[a]				
	. ± . N=0	64.0 ± 5.4 N=3	66.0 ± 4.1 N=3	59.2 ± 4.2 N=2
Feed Consumption (sd 91 to 98) (g/kg/day)[a]				
	. ± . N=0	51.8 ± 4.8 N=2	. ± . N=0	. ± . N=0
Feed Consumption (sd 98 to 105) (g/kg/day)[a]				
	68.6 ± 3.6 N=2	54.8 ± 0.6 N=2	60.0 ± 2.3 N=2	75.2 ± 13.1 N=3
Feed Consumption (sd 105 to 112) (g/kg/day)[a]				
	62.5 ± 2.0 N=3	54.0 ± 1.8 N=3	60.3 ± 4.3 N=2	70.6 ± 10.1 N=3

(continued)

Table 26. Summary of the F_0 Female Feed Consumption, Percent Food Efficiency and Butyl Benzyl Phthalate Intake During the Post Mating Holding Period (page 2 of 2)

	Butyl Benzyl Phthalate (ppm in the feed)			
	0	750	3750	11250
Percent Food Efficiency (sd 84 to 91)[a]				
	. ± . N=0	2.4 ± 8.2 N=3	28.1 ± 2.8 N=3	49.0 ± 10.3 N=2
Percent Food Efficiency (sd 91 to 98)[a]				
	. ± . N=0	-1.0 ± 5.9 N=2	. ± . N=0	. ± . N=0
Percent Food Efficiency (sd 98 to 105)[a]				
	3.4 ± 2.1 N=2	-0.5 ± 1.4 N=2	-5.2 ± 6.2 N=2	-1.4 ± 1.2 N=3
Percent Food Efficiency (sd 105 to 112)[a]				
	2.2 ± 2.9 N=3	1.6 ± 1.3 N=3	-5.2 ± 2.5 N=2	1.5 ± 1.5 N=3
Butyl Benzyl Phthalate Intake (sd 84 to 91) (mg/kg/day)[a]				
		47.99 ± 4.08 N=3	247.32 ± 15.32 N=3	665.88 ± 46.99 N=2
Butyl Benzyl Phthalate Intake (sd 91 to 98) (mg/kg/day)[a]				
		38.88 ± 3.56 N=2	. ± . N=0	. ± . N=0
Butyl Benzyl Phthalate Intake (sd 98 to 105) (mg/kg/day)[a]				
		41.09 ± 0.46 N=2	224.82 ± 8.76 N=2	846.42 ± 147.09 N=3
Butyl Benzyl Phthalate Intake (sd 105 to 112) (mg/kg/day)[a]				
		40.49 ± 1.35 N=3	226.25 ± 16.06 N=2	794.44 ± 114.07 N=3

[a]Reported as the mean ± S.E.M.; sd=study day with the first day of exposure being study day 0. Includes all females that were not found sperm and/or plug positive or females that did not have live litters or females whose entire litter died prior to postnatal day 21. Statistical analyses were not performed on these endpoints since not all females were represented.

Table 27. Summary of the F_0 Female Clinical Observations During the Post Mating Holding Period
(page 1 of 2)

B. Clinical Observations Summarized by Group

Observation	Butyl Benzyl Phthalate (ppm in the feed)			
	0	750	3750	11250
Alopecia	1			1
Found dead	1			

B. Clinical Observations Summarized by Group and Day

Day[a]	Observation[b]	Butyl Benzyl Phthalate (ppm in the feed)			
		0	750	3750	11250
85	Alopecia: limb(s)	1			
86	Alopecia: limb(s)	1			
87	Alopecia: limb(s)	1			
88	Alopecia: limb(s)	1			
89	Alopecia: limb(s)	1			
90	Alopecia: limb(s)	1			
91	Alopecia: limb(s)	1			
92	Alopecia: limb(s)	1			
93	Alopecia: limb(s)	1			
94	Alopecia: limb(s)	1			
95	Alopecia: limb(s)	1			
96	Alopecia: chest				1
	limb(s)	1			
97	Alopecia: chest				1
	limb(s)	1			
98	Alopecia: chest				1
	limb(s)	1			
99	Alopecia: chest				1
	limb(s)	1			
100	Alopecia: chest				1
	limb(s)	1			
101	Alopecia: chest				1
	limb(s)	1			
102	Alopecia: chest, growing in				1
	limb(s)	1			
	Found dead	1			
103	Alopecia: limb(s)	1			

(continued)

Table 27. Summary of the F_0 Female Clinical Observations During the Post Mating Holding Period
(page 2 of 2)

Day[a]	Observation[b]	Butyl Benzyl Phthalate (ppm in the feed)			
		0	750	3750	11250
104	Alopecia: limb(s)	1			
105	Alopecia: limb(s)	1			
106	Alopecia: limb(s)	1			
107	Alopecia: limb(s)	1			
108	Alopecia: limb(s)	1			
109	Alopecia: limb(s)	1			
110	Alopecia: limb(s)	1			
111	Alopecia: limb(s)	1			
112	Alopecia: limb(s)	1			
113	Alopecia: limb(s)	1			

[a]Study day.
[b]Clinical observations are tabulated once per day per animal.

Table 28. Summary and Statistical Analysis of the F_0 Male Organ Weights, Relative Organ Weights, Sperm Evaluations and Selected Gross Necropsy Findings (page 1 of 5)

	Butyl Benzyl Phthalate (ppm in the feed)			
	0	750	3750	11250
No. of Males on Study	30	30	30	30
Sacrifice Body Weight (g)[a]				
	599.25 ‡ ± 9.46 N=30	583.79 ± 7.13 N=29[b]	621.42 ± 10.67 N=30	586.81 ± 8.78 N=29[c]
Brain Weight (g)[a]				
	2.1772 ± 0.0140 N=30	2.1836 ± 0.0159 N=29	2.2127 ± 0.0108 N=30	2.1860 ± 0.0149 N=29
Pituitary Weight (g)[a]				
	0.0169 ± 0.0003 N=30	0.0165 ± 0.0003 N=29	0.0168 ± 0.0003 N=30	0.0172 ± 0.0004 N=29
Thyroid Gland Weight (g)[a]				
	0.0315 ± 0.0010 N=30	0.0316 ± 0.0014 N=29	0.0321 ± 0.0010 N=30	0.0319 ± 0.0013 N=29
Liver Weight (g)[a]				
	23.1004 ‡‡‡ §§§ ± 0.4926 N=30	22.7739 ± 0.5554 N=29	24.8336 ± 0.4925 N=30	26.1971 *** ± 0.6292 N=29
Pancreas Weight (g)[a]				
	0.9094 ± 0.0212 N=30	0.8605 ± 0.0291 N=29	0.8493 ± 0.0311 N=29[d]	0.8899 ± 0.0322 N=29
Spleen Weight (g)[a]				
	0.8661 ± 0.0253 N=30	0.8189 ± 0.0207 N=28[d]	0.8945 ± 0.0215 N=30	0.8386 ± 0.0191 N=29
Paired Kidneys Weight (g)[a]				
	4.3476 ‡‡‡ §§§ ± 0.0666 N=30	4.2168 ± 0.0677 N=29	4.6549 ** ± 0.0657 N=30	4.6833 ** ± 0.0817 N=29
Paired Adrenal Glands Weight (g)[a]				
	0.0615 ± 0.0017 N=30	0.0607 ± 0.0017 N=29	0.0603 ± 0.0021 N=30	0.0607 ± 0.0016 N=29

(continued)

Table 28. Summary and Statistical Analysis of the F_0 Male Organ Weights, Relative Organ Weights, Sperm Evaluations and Selected Gross Necropsy Findings (page 2 of 5)

	Butyl Benzyl Phthalate (ppm in the feed)			
	0	750	3750	11250
Paired Testes Weight (g)[a]				
	3.4474 ± 0.0580 N=30	3.5550 ± 0.0512 N=29	3.6214 ± 0.0715 N=30	3.5704 ± 0.0645 N=29
Paired Epididymides Weight (g)[a]				
	1.4235 ± 0.0191 N=30	1.4243 ± 0.0177 N=29	1.4823 ± 0.0202 N=30	1.4240 ± 0.0179 N=29
Prostate Weight (g)[a]				
	0.8457 ± 0.0344 N=30	0.8335 ± 0.0421 N=29	0.8235 ± 0.0392 N=30	0.8210 ± 0.0387 N=29
Seminal Vesicles with Coagulating Gland Weight (g)[a]				
	2.3625 ‡ ± 0.0591 N=30	2.3130 ± 0.0559 N=29	2.5090 ± 0.0494 N=30	2.3031 ± 0.0629 N=29
Relative Brain Weight (% sacrifice weight)[a]				
	0.3653 ± 0.0048 N=30	0.3752 ± 0.0041 N=29	0.3587 ± 0.0055 N=30	0.3745 ± 0.0056 N=29
Relative Pituitary Weight (% sacrifice weight)[a]				
#	0.0028 † ± 0.0000 N=30	0.0028 ± 0.0001 N=29	0.0027 Þ ± 0.0000 N=30	0.0029 ± 0.0001 N=29
Relative Thyroid Gland Weight (% sacrifice weight)[a]				
	0.0053 ± 0.0002 N=30	0.0054 ± 0.0002 N=29	0.0052 ± 0.0002 N=30	0.0054 ± 0.0002 N=29
Relative Liver Weight (% sacrifice weight)[a]				
	3.8556 ‡‡‡ ± 0.0569 §§§ N=30	3.8981 ± 0.0761 N=29	4.0053 ± 0.0630 N=30	4.4597 *** ± 0.0744 N=29
Relative Pancreas Weight (% sacrifice weight)[a]				
	0.1525 ± 0.0039 N=30	0.1475 ± 0.0049 N=29	0.1374 ± 0.0045 N=29[d]	0.1528 ± 0.0064 N=29

(continued)

Table 28. Summary and Statistical Analysis of the F_0 Male Organ Weights, Relative Organ Weights, Sperm Evaluations and Selected Gross Necropsy Findings (page 3 of 5)

	Butyl Benzyl Phthalate (ppm in the feed)			
	0	750	3750	11250
Relative Spleen Weight (% sacrifice weight)[a]				
	0.1451 ± 0.0042 N=30	0.1411 ± 0.0039 N=28[d]	0.1450 ± 0.0041 N=30	0.1432 ± 0.0030 N=29
Relative Paired Kidneys Weight (% sacrifice weight)[a]				
	0.7277 ‡‡‡ ± 0.0104 §§§ N=30	0.7240 ± 0.0122 N=29	0.7523 ± 0.0102 N=30	0.7999 *** ± 0.0123 N=29
Relative Paired Adrenal Glands Weight (% sacrifice weight)[a]				
	0.0103 ± 0.0003 N=30	0.0104 ± 0.0003 N=29	0.0097 ± 0.0003 N=30	0.0104 ± 0.0002 N=29
Relative Paired Testes Weight (% sacrifice weight)[a]				
	0.5777 ± 0.0104 N=30	0.6111 ± 0.0110 N=29	0.5867 ± 0.0139 N=30	0.6114 ± 0.0129 N=29
Relative Paired Epididymides Weight (% sacrifice weight)[a]				
	0.2385 ± 0.0035 N=30	0.2446 ± 0.0034 N=29	0.2400 ± 0.0043 N=30	0.2441 ± 0.0046 N=29
Relative Prostate Weight (% sacrifice weight)[a]				
	0.1424 ± 0.0064 N=30	0.1435 ± 0.0075 N=29	0.1335 ± 0.0066 N=30	0.1423 ± 0.0081 N=29
Relative Seminal Vesicles with Coagulating Gland Weight (% sacrifice weight)[a]				
	0.3959 ± 0.0105 N=30	0.3979 ± 0.0109 N=29	0.4052 ± 0.0076 N=30	0.3955 ± 0.0128 N=29
Percent Motile Sperm[a]				
	77.6 ± 1.3 N=30	78.7 ± 1.0 N=29	77.5 ± 1.2 N=30	78.6 ± 1.4 N=29
Percent Progressively Motile Sperm[a]				
	64.6 ± 1.6 N=30	67.0 ± 1.8 N=29	64.0 ± 1.7 N=30	64.7 ± 2.0 N=29

(continued)

Table 28. Summary and Statistical Analysis of the F_0 Male Organ Weights, Relative Organ Weights, Sperm Evaluations and Selected Gross Necropsy Findings (page 4 of 5)

	Butyl Benzyl Phthalate (ppm in the feed)			
	0	750	3750	11250
Epididymal Sperm Concentration (10^6/g)[a]				
	946.71 ± 20.94 N=30	913.28 ± 24.81 N=29	948.18 ± 24.26 N=30	930.47 ± 21.23 N=29
Spermatid Head Concentration (10^6/g)[a]				
	104.23 ± 7.22 N=30	127.11 ± 5.51 N=29	129.47 ± 6.33 N=30	121.07 ± 8.70 N=29
Daily Sperm Production per Testis[a]				
	39.25 ‡ ± 3.06 N=30	49.25 * ± 2.22 N=29	50.90 * ± 2.71 N=30	47.25 ± 3.66 N=29
Efficiency of Daily Sperm Production[a]				
	22.61 ± 1.57 N=30	27.57 ± 1.20 N=29	28.09 ± 1.37 N=30	26.26 ± 1.89 N=29
Percent Abnormal Sperm[a]				
	2.12 ± 0.21 N=30	2.17 ± 0.27 N=29	2.33 ± 0.25 N=30	2.11 ± 0.26 N=29
Number of Males with One or More Urinary Tract Malformations[e,f]				
	0	0	0	0
Percent Males with One or More Urinary Tract Malformations[e,f,g]				
	0.00 ± 0.00 N=30	0.00 ± 0.00 N=30	0.00 ± 0.00 N=30	0.00 ± 0.00 N=30
Number of Males with One or More Reproductive Tract Malformations[e,h]				
	0	1	1	0
Percent Males with One or More Reproductive Tract Malformations[e,g,h]				
	0.00 ± 0.00 N=30	3.33 ± 3.33 N=30	3.33 ± 3.33 N=30	0.00 ± 0.00 N=30

(continued)

Table 28. Summary and Statistical Analysis of the F_0 Male Organ Weights, Relative Organ Weights, Sperm Evaluations and Selected Gross Necropsy Findings (page 5 of 5)

[a]Reported as the mean ± S.E.M.
[b]Decrease in N is due to male 63 being found dead on study day 40.
[c]Decrease in N is due to male 21 being found dead on study day 109.
[d]Decrease in N is due to one weight being a statistical outlier and therefore it was excluded.
[e]See Table A-33 for a list of the gross necropsy findings.
[f]Does not include enlarged kidney.
[g]Reported as the percentage ± the standard error of the percentage.
[h]Includes seminal vesicle(s) reduced in size.
[#]Levene's test for homogeneity of variances was significant ($p<0.05$), therefore robust regression methods were used to test all treatment effects.
[‡]$p<0.05$; ANOVA Test.
[‡‡‡]$p<0.001$; ANOVA Test.
[§§§]$p<0.001$; Test for Linear Trend.
[*]$p<0.05$; Dunnett's Test.
[**]$p<0.01$; Dunnett's Test.
[***]$p<0.001$; Dunnett's Test.
[†]$p<0.05$; Wald Chi-square Test for overall treatment effect in robust linear regression model.
[Þ]$p<0.05$; Individual t-test for pairwise comparisons to control in robust linear regression model.

Table 29. Summary of F_0 Male Gross Necropsy Findings and Microscopic Findings (page 1 of 2)

Gross Findings

Scheduled Sacrifice

Finding	Butyl Benzyl Phthalate (ppm in the feed) 0	750	3750	11250
Adrenal Gland: reduced in size, right		1		
Alopecia: limb(s)	1	3	1	2
multiple areas				2
Kidney: enlarged, bilateral				1
Rust colored fur: shoulder(s)				1
Seminal Vesicle: reduced in size, left			1	
reduced in size, right		1		
Sore(s): neck				1
Spleen: irregularly shaped			1	

Unscheduled Sacrifice

Finding	Butyl Benzyl Phthalate (ppm in the feed) 0	750	3750	11250
Adrenal Gland: enlarged, bilateral				1
Brain: friable		1		
Liver: enlarged				1
Lungs: diffuse small nodules				1
Spleen: enlarged				1

Microscopic Findings

Finding	Butyl Benzyl Phthalate (ppm in the feed) 0	750	3750	11250
ADRENAL GLAND				
Number Examined	0	1	0	1
Histiocytic Sarcoma				1
EPIDIDYMIS				
Number Examined	17	10	8	18
No Findings				
LIVER				
Number Examined	20	21	20	11
Cytologic Alteration, Hepatocyte				8
Histiocytic Sarcoma				1
Necrosis, Hepatocyte	1	1		1
LUNG				
Number Examined	0	0	0	1
Histiocytic Sarcoma				1

(continued)

Table 29. Summary of F_0 Male Gross Necropsy Findings and Microscopic Findings (page 2 of 2)

Microscopic Findings

Finding	Butyl Benzyl Phthalate (ppm in the feed)			
	0	750	3750	11250
PANCREAS				
Number Examined	10	0	0	11
Atrophy, Acinar Cell	1			2
Hyperplasia, Islet Cell	2			4
Inflammation, Chronic	4			2
PITUITARY GLAND				
Number Examined	10	1	0	11
Developmental Malformation, Pars Nervosa	1			1
Hyperplasia, Pars Distalis				1
PROSTATE GLAND				
Number Examined	17	10	8	18
Inflammation, Chronic	6	3	3	8
SEMINAL VESICLE				
Number Examined	17	10	9	18
No Findings				
SPLEEN				
Number Examined	0	0	0	1
Histiocytic Sarcoma				1
TESTIS (left)				
Number Examined	17	10	8	18
Atrophy, Seminiferous Tubule	1			1
Degeneration, Seminiferous Tubule		1		
THYROID				
Number Examined	10	1	0	11
Ultimobranchial Cyst	5			3

Table 30. Summary and Statistical Analysis of the F_0 Female Organ Weights, Relative Organ Weights, Ovarian Follicle Counts, Vaginal Cytology at Necropsy and Selected Gross Necropsy Findings (page 1 of 5)

	Butyl Benzyl Phthalate (ppm in the feed)			
	0	750	3750	11250
No. of Females on Study	30	30	30	30
Sacrifice Body Weight (g)[a]	342.21 ± 3.76 § N=29[b]	342.04 ± 5.17 N=29[c]	342.52 ± 3.71 N=30	330.08 ± 4.18 N=29[d]
Brain Weight (g)[a]	1.8948 ± 0.0192 N=29	1.9025 ± 0.0236 N=29	1.9194 ± 0.0199 N=30	1.9042 ± 0.0193 N=30
Pituitary Weight (g)[a]	0.0208 ± 0.0006 N=29	0.0202 ± 0.0005 N=29	0.0206 ± 0.0004 N=30	0.0198 ± 0.0005 N=29[e]
Thyroid Gland Weight (g)[a]	0.0213 ± 0.0009 § N=29	0.0228 ± 0.0011 N=29	0.0215 ± 0.0013 N=28[f,g]	0.0194 ± 0.0008 N=29[g]
Liver Weight (g)[a]	17.8712 ‡ ± 0.4995 §§ N=29	18.5246 ± 0.4939 N=29	19.4727 ± 0.5119 N=30	20.5107 ** ± 0.7749 N=30
Pancreas Weight (g)[a]	0.9081 ± 0.0319 N=29	0.8919 ± 0.0275 N=28[g]	0.8642 ± 0.0326 N=30	0.8749 ± 0.0305 N=30
Spleen Weight (g)[a]	0.5727 ± 0.0144 N=29	0.6052 ± 0.0180 N=28[g]	0.5985 ± 0.0135 N=30	0.5605 ± 0.0115 N=29[g]
Paired Kidneys Weight (g)[a]	2.9068 ‡ ± 0.0464 § N=29	2.9527 ± 0.0536 N=29	3.0896 * ± 0.0501 N=30	3.0681 ± 0.0567 N=30
Paired Adrenal Glands Weight (g)[a]	0.0876 ± 0.0023 N=29	0.0915 ± 0.0028 N=29	0.0879 ± 0.0027 N=30	0.0872 ± 0.0020 N=30

(continued)

Table 30. Summary and Statistical Analysis of the F_0 Female Organ Weights, Relative Organ Weights, Ovarian Follicle Counts, Vaginal Cytology at Necropsy and Selected Gross Necropsy Findings
(page 2 of 5)

	Butyl Benzyl Phthalate (ppm in the feed)			
	0	750	3750	11250
Paired Ovaries Weight (g)[a]				
#	0.1531 ††† ŸŸŸ ± 0.0059 N=28[h]	0.1653 ± 0.0043 N=29	0.1559 ± 0.0029 N=30	0.1329 ÞÞ ± 0.0050 N=30
Uterus Weight (g)[a]				
	0.5753 ‡‡‡ §§§ ± 0.0213 N=29	0.5682 ± 0.0199 N=29	0.5938 ± 0.0199 N=30	0.4588 *** ± 0.0191 N=30
Relative Brain Weight (% sacrifice weight)[a]				
	0.5549 ± 0.0064 § N=29	0.5589 ± 0.0093 N=29	0.5621 ± 0.0078 N=30	0.5800 ± 0.0091 N=29[d]
Relative Pituitary Weight (% sacrifice weight)[a]				
	0.0061 ± 0.0002 N=29	0.0059 ± 0.0002 N=29	0.0060 ± 0.0001 N=30	0.0060 ± 0.0002 N=29[d]
Relative Thyroid Gland Weight (% sacrifice weight)[a]				
	0.0062 ± 0.0003 N=29	0.0067 ± 0.0003 N=29	0.0064 ± 0.0004 N=28[f,g]	0.0059 ± 0.0002 N=28[d,g]
Relative Liver Weight (% sacrifice weight)[a]				
	5.2083 ‡‡‡ §§§ ± 0.1153 N=29	5.4377 ± 0.1487 N=29	5.6786 ± 0.1360 N=30	6.2166 *** ± 0.2008 N=29[d]
Relative Pancreas Weight (% sacrifice weight)[a]				
	0.2660 ± 0.0095 N=29	0.2618 ± 0.0072 N=28[g]	0.2530 ± 0.0100 N=30	0.2659 ± 0.0097 N=29[d]
Relative Spleen Weight (% sacrifice weight)[a]				
	0.1674 ± 0.0039 N=29	0.1774 ± 0.0053 N=28[g]	0.1750 ± 0.0040 N=30	0.1710 ± 0.0037 N=28[d,g]
Relative Paired Kidneys Weight (% sacrifice weight)[a]				
	0.8501 ‡‡‡ §§§ ± 0.0117 N=29	0.8669 ± 0.0169 N=29	0.9023 * ± 0.0121 N=30	0.9271 *** ± 0.0128 N=29[d]

(continued)

Table 30. Summary and Statistical Analysis of the F_0 Female Organ Weights, Relative Organ Weights, Ovarian Follicle Counts, Vaginal Cytology at Necropsy and Selected Gross Necropsy Findings
(page 3 of 5)

	Butyl Benzyl Phthalate (ppm in the feed)			
	0	750	3750	11250
Relative Paired Adrenal Glands Weight (% sacrifice weight)[a]				
	0.0256 ± 0.0006 N=29	0.0269 ± 0.0008 N=29	0.0256 ± 0.0007 N=30	0.0265 ± 0.0006 N=29[d]
Relative Paired Ovaries Weight (% sacrifice weight)[a]				
#	0.0449 ††† ŸŸŸ ± 0.0017 N=28[h]	0.0485 ± 0.0013 N=29	0.0456 ± 0.0009 N=30	0.0400 Þ ± 0.0016 N=29[d]
Relative Uterus Weight (% sacrifice weight)[a]				
	0.1692 ‡‡ §§§ ± 0.0071 N=29	0.1673 ± 0.0063 N=29	0.1744 ± 0.0064 N=30	0.1399 ** ± 0.0067 N=29[d]
Paired Ovarian Follicle Count[a,i]				
	281.1 ± 34.1 N=10			272.9 ± 35.1 N=10
VAGINAL CYTOLOGY EVALUATION AT NECROPSY[j]				
No. Females Evaluated	14[b,k]	17[c,k]	22[k]	17[k]
No. in Proestrus	3	6	3	2
% in Proestrus	21.43	40.00	14.29	12.50
No. in Estrus	4	3	5	1
% in Estrus	28.57	20.00	23.81	6.25
No. in Metestrus	7	6	10	12
% in Metestrus	50.00	40.00	47.62	75.00
No. in Diestrus	0	0	3	1
% in Diestrus	0.00	0.00	14.29	6.25
No. Stage Not Determined	0	2	1	1
No. No Cells Present[l]	0	0	0	0

(continued)

Table 30. Summary and Statistical Analysis of the F_0 Female Organ Weights, Relative Organ Weights, Ovarian Follicle Counts, Vaginal Cytology at Necropsy and Selected Gross Necropsy Findings (page 4 of 5)

	Butyl Benzyl Phthalate (ppm in the feed)			
	0	750	3750	11250
Number of Females with One or More Urinary Tract Malformations[m,n]				
	0	0	0	0
Percent Females with One or More Urinary Tract Malformations[m,n,o]				
	0.00 ± 0.00 N=30	0.00 ± 0.00 N=30	0.00 ± 0.00 N=30	0.00 ± 0.00 N=30
Number of Females with One or More Reproductive Tract Malformations[m,p]				
	0	0	0	0
Percent Females with One or More Reproductive Tract Malformations[m,o,p]				
	0.00 ± 0.00 N=30	0.00 ± 0.00 N=30	0.00 ± 0.00 N=30	0.00 ± 0.00 N=30

[a]Reported as the mean ± S.E.M.
[b]Decrease in N is due to female 52 being found dead on study day 103.
[c]Decrease in N is due to female 78 being found dead on postnatal day 17 (study day 110).
[d]Decrease in N is due to the sacrifice weight for one female inadvertently not being recorded.
[e]Decrease in N is due to the pituitary inadvertently not being saved for one female.
[f]Decrease in N is due to the thyroid for one female being weighed after it was fixed and therefore the weight was excluded since it was not a fresh weight.
[g]Decrease in N is due to the organ weight for one female being a statistical outlier and therefore it was excluded.
[h]Decrease in N is due to one ovary inadvertently being lost prior to weighing.
[i]Ovarian follicle counts were done for 10 control females and 10 females in the 11250 ppm Butyl Benzyl Phthalate dose group.
[j]For presentation and statistical analysis purposes those females in two stages were pooled in the following manner: proestrus/estrus and estrus/proestrus were considered proestrus; estrus/metestrus and metestrus/estrus were considered estrus; metestrus/diestrus and diestrus/metestrus were considered metestrus; and diestrus/proestrus and proestrus/diestrus were considered diestrus. The females for which the stage could not be determined or no cells were present were not included in the statistical analysis.
[k]Decrease in N is due to one or more females inadvertently not being smeared at the time of necropsy.
[l]These smears did not contain sloughed cells or the cells washed off during processing and therefore they could not be evaluated.
[m]See Table A-35 for a list of the gross necropsy findings.
[n]Does not include multiple foci on kidney.
[o]Reported as the percentage ± the standard error of the percentage.
[p]Does not include retained fetus in uterus or fluid filled uterus.
[#]Levene's test for homogeneity of variances was significant ($p<0.05$), therefore robust regression methods were used to test all treatment effects.

(continued)

Table 30. Summary and Statistical Analysis of the F_0 Female Organ Weights, Relative Organ Weights, Ovarian Follicle Counts, Vaginal Cytology at Necropsy and Selected Gross Necropsy Findings
(page 5 of 5)

‡p<0.05; ANOVA Test.
‡‡p<0.01; ANOVA Test.
‡‡‡p<0.001; ANOVA Test.
§p<0.05; Test for Linear Trend.
§§p<0.01; Test for Linear Trend.
§§§p<0.001; Test for Linear Trend.
*p<0.05; Dunnett's Test.
**p<0.01; Dunnett's Test.
***p<0.001; Dunnett's Test.
ŧŧŧp<0.001; Wald Chi-square Test for overall treatment effect in robust regression model.
ŸŸŸp<0.001; Linear trend test in robust regression model.
Þp<0.05; Individual t-test for pairwise comparisons to control in robust regression model.
ÞÞp<0.01; Individual t-test for pairwise comparisons to control in robust regression model.

Table 31. Summary of F_0 Female Gross Necropsy Findings and Microscopic Findings (page 1 of 2)

Gross Findings

Scheduled Sacrifice

Finding	Butyl Benzyl Phthalate (ppm in the feed) 0	750	3750	11250
3 x 2 x 1.5 cm firm tan subcutaneous mass in the lower abdomen	1			
Adrenal Gland: 1 mm red foci, left				1
Alopecia: abdomen				1
limb(s)	1	1	5	3
multiple areas		2		4
Chromodacryorrhea: eye, left				1
Ear: red, bilateral			1	
Eye: opaque from cage injury, left			1	
Rust colored fur: limb(s)			2	
multiple areas		2	1	
Spleen: irregularly shaped		1		
Uterus: fluid filled, both horns		1		

Unscheduled Sacrifice

Finding	Butyl Benzyl Phthalate (ppm in the feed) 0	750	3750	11250
Adrenal Gland: enlarged, bilateral		1		
Appears that female had not eaten since delivery of her litter 8 days earlier	1			
Feces: none	1			
Kidney: 1 mm white multiple foci on capsule, bilateral		1		
Small Intestines: no ingesta present	1			
Stomach: no food present	1			
Uterus: retained fetus, right horn		1		

Microscopic Findings

Finding	Butyl Benzyl Phthalate (ppm in the feed) 0	750	3750	11250
Adrenal Gland				
Number Examined	0	1	0	0
Necrosis, Hemorrhagic, Zona Fasciculata		1		
Cervix				
Number Examined	13	3	2	13
No Findings				
Kidneys				
Number Examined	0	1	0	0
Inflammation, Papilla, Cortex, Acute		1		

(continued)

Table 31. Summary of F_0 Female Gross Necropsy Findings and Microscopic Findings (page 2 of 2)

Microscopic Findings

Finding	Butyl Benzyl Phthalate (ppm in the feed) 0	750	3750	11250
Liver				
Number Examined	21	21	20	10
Cytologic Alteration, Hepatocyte	1		2	9
Leukemia, Mononuclear	1			
Necrosis, Hepatocyte	2			
Necrosis, Hepatocyte, Centrilobular		1		
Ovary				
Number Examined	13	4	2	13
Cyst	1	1	1	1
Oviduct				
Number Examined	13	4	2	13
No Findings				
Pancreas				
Number Examined	11	1	0	10
Inflammation, Chronic	1			
Pituitary Gland				
Number Examined	11	1	0	10
Developmental Malformation, Pars Nervosa	2			
Thyroid				
Number Examined	11	1	0	10
Ultimobranchial Cyst	2			2
Uterus				
Number Examined	13	4	2	13
Inflammation, Acute		1		
Vagina				
Number Examined	13	4	2	13
No Findings				

Table 32. Summary of the Fate of the F_1 Males and Females (page 1 of 1)

	Butyl Benzyl Phthalate (ppm in the feed)			
	0	750	3750	11250
MALES	30	30	30	30
Phase of Study				
Prebreed	0	0	1[a]	0
Mating	0	1[b]	1[c]	2[d]
Holding Period after Mating	0	0	0	0
Scheduled Sacrifice	30	29	28	28
FEMALES	30	30	30	30
Phase of Study				
Prebreed	0	0	0	0
Mating	0	0	0	0
Gestation	0	0	0	0
Lactation	0	0	0	0
Holding Period after Mating	0	0	0	0
Scheduled Sacrifice	30	30	30	30

[a]Male 1157 was euthanized moribund on study day 21 (study day 0 was the first day of the prebreed period and negative study days were during the holding period after weaning and prior to the start of the prebreed period).
[b]Male 1163 was found dead on study day 78.
[c]Male 1033 was found dead on study day 83.
[d]Male 1085 was euthanized moribund on study day 75 and male 1115 was euthanized moribund on study day 75.

Table 33. Summary and Statistical Analysis of the F_1 Female Vaginal Opening and the F_1 Male Preputial Separation Data (page 1 of 2)

	Butyl Benzyl Phthalate (ppm in the feed)			
	0	750	3750	11250
No. of Females Evaluated	30	30	30	30
Day of Vaginal Opening[a]				
	31.4 ‡‡‡ ± 0.3 §§§ N=30	32.0 ± 0.4 N=30	31.2 ± 0.3 N=30	34.1 *** ± 0.5 N=30
Body Weight (g) on Day of Acquisition[a]				
	108.25 ± 2.50 N=30	114.56 ± 3.41 N=30	109.10 ± 2.08 N=30	106.30 ± 2.72 N=30
Adjusted Day of Vaginal Opening[b]				
	31.5 ΩΩΩ ± 0.3 λλλ N=30	31.6 ± 0.3 N=30	31.2 ± 0.3 N=30	34.4 φφφ ± 0.3 N=30
No. of Males Evaluated	30	30	30	30
Day of Preputial Separation[a]				
#	40.9 ŧŧŧ ± 0.4 ŸŸŸ N=30	41.3 ± 0.4 N=30	40.6 ± 0.2 N=30	45.2 ÞÞÞ ± 0.4 N=30
Body Weight (g) on Day of Acquisition[a]				
#	208.17 ± 2.28 N=30	217.23 ± 4.15 N=30	212.83 ± 2.46 N=30	207.63 ± 4.00 N=30
Adjusted Day of Preputial Separation[b]				
	41.0 ŦŦŦ ± 0.3 ĦĦĦ N=30	41.1 ± 0.3 N=30	40.6 ± 0.2 N=30	45.4 θθθ ± 0.4 N=30

(continued)

Table 33. Summary and Statistical Analysis of the F_1 Female Vaginal Opening and the F_1 Male Preputial Separation Data (page 2 of 2)

[a]Reported as the mean ± S.E.M. with day being postnatal day.
[b]Reported as the adjusted mean (body weight as covariate) ± S.E.M.
[#]Levene's test for homogeneity of variances was significant ($p<0.05$), therefore robust regression methods were used to test all treatment effects.
‡‡‡$p<0.001$; ANOVA Test.
§§§$p<0.001$; Test for Linear Trend.
***$p<0.001$; Dunnett's Test.
ΩΩΩ$p<0.001$; Analysis of Covariance with body weight on day of acquisition as covariate.
λλλ$p<0.001$; Linear Trend Analysis of Covariance with body weight on day of acquisition as covariate.
φφφ$p<0.001$; Dunnett's Test with body weight on day of acquisition as covariate.
†††$p<0.001$; Wald Chi-square Test for overall treatment effect in robust regression model.
ÞÞÞ$p<0.001$; Individual t-test for pairwise comparisons to control in robust regression model.
ŸŸŸ$p<0.001$; Linear trend test in robust regression model.
₸₸₸$p<0.001$; Wald Chi-square Test for overall treatment effect in robust regression model with body weight on day of acquisition as a covariate.
θθθ$p<0.001$; Individual t-test for pairwise comparisons to control in robust regression model with body weight on day of acquisition as a covariate.
ĦĦĦ$p<0.001$; Linear trend test in robust regression model with body weight on day of acquisition as a covariate.

Table 34. Summary and Statistical Analysis of the F_1 Male Body Weights and Weight Changes During the Prebreed and Mating Periods (page 1 of 4)

	Butyl Benzyl Phthalate (ppm in the feed)			
	0	750	3750	11250
No. Males on Study	30	30	30	30
Body Weight (sd 0) (g)[a]	119.2 ‡‡‡ ± 5.3 §§§ N=30	127.7 ± 3.1 N=30	124.3 ± 3.3 N=30	95.3 *** ± 4.5 N=30
Body Weight (sd 7) (g)[a]	181.8 ‡‡‡ ± 6.4 §§§ N=30	194.7 ± 4.0 N=30	189.5 ± 4.0 N=30	149.5 *** ± 6.1 N=30
Body Weight (sd 14) (g)[a]	242.1 ‡‡‡ ± 6.6 §§§ N=30	256.7 ± 4.5 N=30	250.2 ± 4.7 N=30	205.3 *** ± 6.8 N=30
Body Weight (sd 21) (g)[a]	304.2 ‡‡‡ ± 6.9 §§§ N=30	319.1 ± 5.3 N=30	312.7 ± 6.5 N=30	263.7 *** ± 7.5 N=30
Body Weight (sd 28) (g)[a]	355.1 ‡‡‡ ± 6.6 §§§ N=30	369.7 ± 5.7 N=30	369.3 ± 5.4 N=29[b]	313.0 *** ± 7.6 N=30
Body Weight (sd 35) (g)[a]	398.9 ‡‡‡ ± 6.2 §§§ N=30	414.7 ± 6.2 N=30	417.8 ± 6.0 N=29	358.2 *** ± 8.0 N=30
Body Weight (sd 42) (g)[a]	435.8 ‡‡‡ ± 6.3 §§§ N=30	449.0 ± 6.6 N=30	452.9 ± 6.4 N=29	390.3 *** ± 8.3 N=30
Body Weight (sd 49) (g)[a]	464.0 ‡‡‡ ± 6.2 §§§ N=30	476.4 ± 7.0 N=30	483.0 ± 6.8 N=29	419.2 *** ± 8.7 N=30
Body Weight (sd 56) (g)[a]	490.0 ‡‡‡ ± 6.4 §§§ N=30	500.0 ± 7.3 N=30	509.6 ± 7.2 N=29	442.5 *** ± 9.0 N=30

(continued)

Table 34. Summary and Statistical Analysis of the F_1 Male Body Weights and Weight Changes During the Prebreed and Mating Periods (page 2 of 4)

	Butyl Benzyl Phthalate (ppm in the feed)			
	0	750	3750	11250
Body Weight (sd 63) (g)[a]	511.6 ‡‡‡ ± 6.7 §§§ N=30	519.7 ± 7.8 N=30	533.0 ± 7.5 N=29	463.2 *** ± 9.1 N=30
Body Weight (sd 70) (g)[a]	530.5 ‡‡‡ ± 7.1 §§§ N=30	537.4 ± 7.9 N=30	553.6 ± 7.8 N=29	481.9 *** ± 9.0 N=30
Body Weight (sd 77) (g)[a]	534.0 ‡‡‡ ± 6.9 §§§ N=30	540.0 ± 7.7 N=30	557.5 ± 7.3 N=29	484.8 *** ± 9.8 N=28[c]
Body Weight (sd 84) (g)[a]	552.4 ‡‡‡ ± 7.4 §§§ N=30	558.2 ± 8.5 N=29[d]	575.1 ± 8.0 N=28[e]	499.4 *** ± 10.2 N=28
Body Weight Change (sd 0 to 7) (g)[a]	62.6 ‡‡‡ ± 1.3 §§§ N=30	67.0 ± 1.1 N=30	65.1 ± 1.0 N=30	54.1 *** ± 1.7 N=30
Body Weight Change (sd 7 to 14) (g)[a]	60.3 ‡‡ ± 0.9 §§ N=30	62.0 ± 1.0 N=30	60.7 ± 1.7 N=30	55.8 * ± 1.4 N=30
Body Weight Change (sd 14 to 21) (g)[a]	62.1 ± 1.1 N=30	62.4 ± 1.2 N=30	62.5 ± 2.8 N=30	58.5 ± 1.4 N=30
Body Weight Change (sd 21 to 28) (g)[a]	50.9 ± 1.2 N=30	50.6 ± 1.1 N=30	52.3 ± 1.4 N=29[b]	49.2 ± 1.3 N=30
Body Weight Change (sd 28 to 35) (g)[a]	43.8 ± 1.7 N=30	45.0 ± 0.9 N=30	48.5 ± 1.3 N=29	45.3 ± 1.6 N=30

(continued)

Table 34. Summary and Statistical Analysis of the F_1 Male Body Weights and Weight Changes During the Prebreed and Mating Periods (page 3 of 4)

	Butyl Benzyl Phthalate (ppm in the feed)			
	0	750	3750	11250
Body Weight Change (sd 35 to 42) (g)[a]				
#	36.8 ± 1.7 Ÿ N=30	34.3 ± 0.9 N=30	35.2 ± 1.2 N=29	32.0 ± 1.1 N=30
Body Weight Change (sd 42 to 49) (g)[a]				
	28.2 ± 1.2 N=30	27.4 ± 0.8 N=30	30.1 ± 0.9 N=29	28.9 ± 0.9 N=30
Body Weight Change (sd 49 to 56) (g)[a]				
	26.1 ‡ ± 0.9 N=30	23.6 ± 0.8 N=30	26.6 ± 0.9 N=29	23.3 ± 0.9 N=30
Body Weight Change (sd 56 to 63) (g)[a]				
	21.6 ± 1.1 N=30	19.7 ± 0.9 N=30	23.4 ± 0.8 N=29	20.7 ± 1.1 N=30
Body Weight Change (sd 63 to 70) (g)[a]				
	18.9 ± 1.0 N=30	17.7 ± 0.9 N=30	20.6 ± 0.8 N=29	18.8 ± 1.0 N=30
Body Weight Change (sd 0 to 70) (g)[a]				
	411.3 ‡‡‡ ± 6.7 §§ N=30	409.7 ± 6.4 N=30	429.2 ± 7.0 N=29	386.6 * ± 7.1 N=30
Body Weight Change (sd 70 to 77) (g)[a]				
	3.6 ± 1.1 N=30	2.6 ± 1.4 N=30	3.9 ± 1.7 N=29	1.7 ± 1.6 N=28[c]
Body Weight Change (sd 77 to 84) (g)[a]				
#	18.3 ± 0.9 Ÿ N=30	19.0 ± 1.1 N=29[d]	17.9 ± 1.0 N=28[e]	14.6 ± 1.5 N=28

(continued)

Table 34. Summary and Statistical Analysis of the F_1 Male Body Weights and Weight Changes During the Prebreed and Mating Periods (page 4 of 4)

[a]Reported as the mean ± S.E.M.; sd=study day with study day 0 being the first day of the prebreed period.
[b]Decrease in N is due to male 1157 being euthanized moribund on study day 21.
[c]Decrease in N is due to males 1085 and 1115 being euthanized moribund on study day 75.
[d]Decrease in N is due to male 1163 being found dead on study day 78.
[e]Decrease in N is due to male 1033 being found dead on study day 83.
#Levene's test for homogeneity of variances was significant ($p<0.05$), therefore robust regression methods were used to test all treatment effects.
‡$p<0.05$; ANOVA Test.
‡‡$p<0.01$; ANOVA Test.
‡‡‡$p<0.001$; ANOVA Test.
§§$p<0.01$; Test for Linear Trend.
§§§$p<0.001$; Test for Linear Trend.
*$p<0.05$; Dunnett's Test.
***$p<0.001$; Dunnett's Test.
Ÿ$p<0.05$; Linear trend test in robust regression model.

Table 35. Summary and Statistical Analysis of the F_1 Male Feed Consumption, Percent Food Efficiency and Butyl Benzyl Phthalate Intake During the Prebreed and Mating Periods (page 1 of 6)

	Butyl Benzyl Phthalate (ppm in the feed)			
	0	750	3750	11250
No. Males on Study	30	30	30	30
Feed Consumption (sd 0 to 7) (g/day)[a]	20.1 ‡‡‡ ± 0.6 §§§ N=30	21.3 ± 0.4 N=30	21.0 ± 0.4 N=30	17.1 *** ± 0.6 N=30
Feed Consumption (sd 7 to 14) (g/day)[a]	23.7 ‡‡‡ ± 0.6 §§§ N=30	25.0 ± 0.4 N=30	24.4 ± 0.4 N=30	21.4 ** ± 0.6 N=30
Feed Consumption (sd 14 to 21) (g/day)[a]	26.9 ‡‡‡ ± 0.5 §§§ N=30	27.8 ± 0.4 N=30	27.3 ± 0.6 N=30	24.2 ** ± 0.5 N=30
Feed Consumption (sd 21 to 28) (g/day)[a]	28.9 ‡‡‡ ± 0.6 §§§ N=30	29.7 ± 0.5 N=30	29.5 ± 0.5 N=29[b]	26.5 ** ± 0.6 N=30
Feed Consumption (sd 28 to 35) (g/day)[a]	29.8 ‡‡‡ ± 0.6 §§§ N=30	30.2 ± 0.5 N=30	30.7 ± 0.5 N=29	27.7 * ± 0.6 N=30
Feed Consumption (sd 35 to 42) (g/day)[a]	30.0 ‡‡‡ ± 0.6 §§§ N=30	31.0 ± 0.5 N=30	30.7 ± 0.5 N=28[c]	28.1 * ± 0.6 N=30
Feed Consumption (sd 42 to 49) (g/day)[a]	29.6 ‡ ± 0.6 § N=30	30.4 ± 0.5 N=30	31.1 ± 0.5 N=29	28.5 ± 0.6 N=30
Feed Consumption (sd 49 to 56) (g/day)[a]	29.8 ‡‡ ± 0.5 §§ N=30	30.2 ± 0.5 N=30	31.0 ± 0.4 N=29	28.2 ± 0.7 N=30
Feed Consumption (sd 56 to 63) (g/day)[a]	29.5 ‡‡‡ ± 0.6 §§ N=30	30.0 ± 0.5 N=30	30.7 ± 0.4 N=29	27.6 * ± 0.7 N=30

(continued)

Table 35. Summary and Statistical Analysis of the F_1 Male Feed Consumption, Percent Food Efficiency and Butyl Benzyl Phthalate Intake During the Prebreed and Mating Periods (page 2 of 6)

	Butyl Benzyl Phthalate (ppm in the feed)			
	0	750	3750	11250
Feed Consumption (sd 63 to 70) (g/day)[a]	29.1 ‡‡ ± 0.5 §§ N=30	29.6 ± 0.4 N=30	30.5 ± 0.4 N=29	27.6 ± 0.7 N=29[d]
Feed Consumption (sd 0 to 70) (g/day)[a]	27.7 ‡‡‡ ± 0.5 §§§ N=30	28.5 ± 0.4 N=30	28.7 ± 0.4 N=28[e]	25.7 ** ± 0.6 N=29[e]
Feed Consumption (sd 77 to 84) (g/day)[a,f]	29.1 ± 0.5 N=28	29.5 ± 0.5 N=27[g]	30.2 ± 0.4 N=24[h]	28.2 ± 1.1 N=16[i]
Feed Consumption (sd 0 to 7) (g/kg/day)[a]	135.9 ‡‡ ± 2.2 §§ N=30	132.4 ± 1.2 N=30	134.7 ± 1.7 N=30	141.7 ± 2.4 N=30
Feed Consumption (sd 7 to 14) (g/kg/day)[a]	113.0 ‡‡‡ ± 1.8 §§§ N=30	111.3 ± 1.1 N=30	111.7 ± 1.9 N=30	122.4 ** ± 2.2 N=30
Feed Consumption (sd 14 to 21) (g/kg/day)[a]	99.5 ‡‡ ± 1.7 §§ N=30	96.8 ± 1.1 N=30	97.3 ± 1.4 N=30	104.4 ± 1.8 N=30
Feed Consumption (sd 21 to 28) (g/kg/day)[a]	88.0 ‡‡ ± 1.6 §§§ N=30	86.5 ± 1.1 N=30	86.2 ± 1.0 N=29[b]	92.6 * ± 1.2 N=30
Feed Consumption (sd 28 to 35) (g/kg/day)[a]	79.3 ‡‡ ± 1.3 §§ N=30	77.3 ± 1.1 N=30	78.0 ± 0.9 N=29	82.8 ± 1.2 N=30
Feed Consumption (sd 35 to 42) (g/kg/day)[a]	71.9 ‡ ± 1.2 §§ N=30	71.9 ± 1.2 N=30	70.7 ± 0.8 N=28[c]	75.3 ± 1.0 N=30

(continued)

Table 35. Summary and Statistical Analysis of the F_1 Male Feed Consumption, Percent Food Efficiency and Butyl Benzyl Phthalate Intake During the Prebreed and Mating Periods (page 3 of 6)

	Butyl Benzyl Phthalate (ppm in the feed)			
	0	750	3750	11250
Feed Consumption (sd 42 to 49) (g/kg/day)[a]	65.9 ‡‡ ± 1.2 §§§ N=30	65.8 ± 1.1 N=30	66.5 ± 0.9 N=29	70.8 ** ± 1.2 N=30
Feed Consumption (sd 49 to 56) (g/kg/day)[a]	62.6 ± 1.0 § N=30	62.0 ± 1.1 N=30	62.6 ± 0.8 N=29	65.6 ± 1.3 N=30
Feed Consumption (sd 56 to 63) (g/kg/day)[a]	58.9 ± 0.9 N=30	59.0 ± 1.0 N=30	59.1 ± 0.8 N=29	61.2 ± 1.1 N=30
Feed Consumption (sd 63 to 70) (g/kg/day)[a]	55.8 ± 0.8 § N=30	56.2 ± 0.8 N=30	56.3 ± 0.8 N=29	58.3 ± 1.1 N=29[d]
Feed Consumption (sd 0 to 70) (g/kg/day)[a]	75.8 ‡‡ ± 0.9 §§ N=30	75.5 ± 0.9 N=30	75.3 ± 0.7 N=28[e]	79.3 * ± 1.0 N=29[e]
Feed Consumption (sd 77 to 84) (g/kg/day)[a,f]	53.3 ± 0.7 N=28	53.9 ± 0.9 N=27[g]	53.6 ± 0.6 N=24[h]	56.0 ± 1.6 N=16[i]
Percent Food Efficiency (sd 0 to 7)[a] #	45.1 ± 0.9 N=30	45.1 ± 0.5 N=30	44.5 ± 0.5 N=30	45.5 ± 0.6 N=30
Percent Food Efficiency (sd 7 to 14)[a]	36.9 ± 0.9 N=30	35.4 ± 0.4 N=30	35.4 ± 0.8 N=30	37.6 ± 0.8 N=30
Percent Food Efficiency (sd 14 to 21)[a]	33.2 ± 0.6 N=30	32.1 ± 0.4 N=30	32.0 ± 1.4 N=30	34.6 ± 0.6 N=30

(continued)

Table 35. Summary and Statistical Analysis of the F_1 Male Feed Consumption, Percent Food Efficiency and Butyl Benzyl Phthalate Intake During the Prebreed and Mating Periods (page 4 of 6)

	Butyl Benzyl Phthalate (ppm in the feed)			
	0	750	3750	11250
Percent Food Efficiency (sd 21 to 28)[a]				
#	25.4 ± 0.7 Ÿ N=30	24.4 ± 0.5 N=30	25.3 ± 0.5 N=29[b]	26.8 ± 0.8 N=30
Percent Food Efficiency (sd 28 to 35)[a]				
#	21.2 † ± 0.9 Ÿ N=30	21.3 ± 0.4 N=30	22.6 ± 0.5 N=29	23.4 Þ ± 0.7 N=30
Percent Food Efficiency (sd 35 to 42)[a]				
	17.6 ± 0.7 N=30	15.9 ± 0.4 N=30	16.2 ± 0.5 N=28[c]	16.3 ± 0.5 N=30
Percent Food Efficiency (sd 42 to 49)[a]				
	13.7 ± 0.6 § N=30	12.9 ± 0.3 N=30	13.9 ± 0.4 N=29	14.6 ± 0.4 N=30
Percent Food Efficiency (sd 49 to 56)[a]				
	12.5 ± 0.4 N=30	11.1 ± 0.3 N=30	12.3 ± 0.4 N=29	11.8 ± 0.4 N=30
Percent Food Efficiency (sd 56 to 63)[a]				
	10.4 ‡ ± 0.4 N=30	9.3 ± 0.4 N=30	10.9 ± 0.4 N=29	10.7 ± 0.5 N=30
Percent Food Efficiency (sd 63 to 70)[a]				
	9.3 ± 0.5 N=30	8.6 ± 0.4 N=30	9.6 ± 0.3 N=29	10.2 ± 0.8 N=29[d]
Percent Food Efficiency (sd 0 to 70)[a]				
	21.3 ± 0.4 N=30	20.6 ± 0.2 N=30	21.3 ± 0.2 N=28[e]	21.7 ± 0.3 N=29[e]
Percent Food Efficiency (sd 77 to 84)[a,f]				
	9.3 ± 0.4 N=28	9.1 ± 0.5 N=27[g]	8.4 ± 0.5 N=24[h]	8.1 ± 0.8 N=16[i]

(continued)

Table 35. Summary and Statistical Analysis of the F_1 Male Feed Consumption, Percent Food Efficiency and Butyl Benzyl Phthalate Intake During the Prebreed and Mating Periods (page 5 of 6)

	Butyl Benzyl Phthalate (ppm in the feed)			
	0	750	3750	11250
Butyl Benzyl Phthalate Intake (sd 0 to 7) (mg/kg/day)[a]				
		99.32 ± 0.90 N=30	505.10 ± 6.52 N=30	1594.35 ± 26.47 N=30
Butyl Benzyl Phthalate Intake (sd 7 to 14) (mg/kg/day)[a]				
		83.49 ± 0.86 N=30	418.89 ± 7.15 N=30	1377.22 ± 25.10 N=30
Butyl Benzyl Phthalate Intake (sd 14 to 21) (mg/kg/day)[a]				
		72.60 ± 0.80 N=30	364.72 ± 5.24 N=30	1174.62 ± 19.94 N=30
Butyl Benzyl Phthalate Intake (sd 21 to 28) (mg/kg/day)[a]				
		64.85 ± 0.82 N=30	323.21 ± 3.90 N=29[b]	1041.87 ± 13.62 N=30
Butyl Benzyl Phthalate Intake (sd 28 to 35) (mg/kg/day)[a]				
		57.99 ± 0.86 N=30	292.37 ± 3.46 N=29	931.02 ± 13.85 N=30
Butyl Benzyl Phthalate Intake (sd 35 to 42) (mg/kg/day)[a]				
		53.92 ± 0.90 N=30	265.30 ± 2.96 N=28[c]	847.61 ± 11.35 N=30
Butyl Benzyl Phthalate Intake (sd 42 to 49) (mg/kg/day)[a]				
		49.37 ± 0.84 N=30	249.24 ± 3.40 N=29	796.30 ± 13.98 N=30
Butyl Benzyl Phthalate Intake (sd 49 to 56) (mg/kg/day)[a]				
		46.51 ± 0.83 N=30	234.83 ± 3.06 N=29	737.88 ± 14.72 N=30
Butyl Benzyl Phthalate Intake (sd 56 to 63) (mg/kg/day)[a]				
		44.29 ± 0.75 N=30	221.67 ± 2.97 N=29	688.03 ± 12.40 N=30

(continued)

Table 35. Summary and Statistical Analysis of the F_1 Male Feed Consumption, Percent Food Efficiency and Butyl Benzyl Phthalate Intake During the Prebreed and Mating Periods (page 6 of 6)

	Butyl Benzyl Phthalate (ppm in the feed)			
	0	750	3750	11250
Butyl Benzyl Phthalate Intake (sd 63 to 70) (mg/kg/day)[a]				
		42.14 ± 0.59 N=30	210.98 ± 2.92 N=29	655.70 ± 12.13 N=29[d]
Butyl Benzyl Phthalate Intake (sd 0 to 70) (mg/kg/day)[a]				
		56.64 ± 0.71 N=30	282.48 ± 2.79 N=28[e]	891.83 ± 11.33 N=29[e]
Butyl Benzyl Phthalate Intake (sd 77 to 84) (mg/kg/day)[a,f]				
		40.43 ± 0.65 N=27[g]	201.00 ± 2.17 N=24[h]	629.74 ± 17.61 N=16[i]

[a]Reported as the mean ± S.E.M.; sd=study day with study day 0 being the first day of the prebreed period.
[b]Decrease in N is due to male 1157 being euthanized moribund on study day 21.
[c]Decrease in N is due to the feed consumption value for one male being a statistical outlier and it was therefore removed.
[d]Decrease in N is due to the feed weight for one male being unrealistic (i.e. negative) and it was therefore excluded.
[e]Decrease in N is due to interim feed consumption value(s) for one or more males being missing and therefore the overall feed consumption value could not be calculated.
[f]Includes those males that had mated with a female. Statistical analyses were not performed on these endpoints since not all males were represented.
[g]Decrease in N is due to male 1163 being found dead on study day 78.
[h]Decrease in N is due to male 1033 being found dead on study day 83.
[i]Decrease in N is due to males 1085 and 1115 being euthanized moribund on study day 75.
[#]Levene's test for homogeneity of variances was significant ($p<0.05$), therefore robust regression methods were used to test all treatment effects.
[‡]$p<0.05$; ANOVA Test.
[‡‡]$p<0.01$; ANOVA Test.
[‡‡‡]$p<0.001$; ANOVA Test.
[§]$p<0.05$; Test for Linear Trend.
[§§]$p<0.01$; Test for Linear Trend.
[§§§]$p<0.001$; Test for Linear Trend.
[*]$p<0.05$; Dunnett's Test.
[**]$p<0.01$; Dunnett's Test.
[***]$p<0.001$; Dunnett's Test.
[†]$p<0.05$; Wald Chi-square Test for overall treatment effect in robust regression model.
[Þ]$p<0.05$; Individual t-test for pairwise comparisons to control in robust regression model.
[Ÿ]$p<0.05$; Linear trend test in robust regression model.

Table 36. Summary of the F_1 Male Clinical Observations During the Prebreed and Mating Periods
(page 1 of 7)

A. Clinical Observations Summarized by Group

Observation	Butyl Benzyl Phthalate (ppm in the feed)			
	0	750	3750	11250
Abnormal positioning of both rear legs and pain elicited upon percussion of anterior lumbar vertebral column, appears to be neurological problem, possibly traumatic in origin			1	
Alopecia	1	4	1	1
Chromodacryorrhea	1	1	1	2
Diarrhea				1
Ear(s): red or pale			1	1
Emaciated			1	
Euthanized moribund			1	2
Found dead		1	1	
Hypospadias				2
Lethargic			1	
Nipples present				4
Pale				1
Palpated abdomen and a large mass was felt in lower right quadrant				1
Paw: pale, all				1
Penis: blood coming from				2
Piloerection				1
Prone				1
Rough coat			1	
Rust colored fur	1			
Slightly dehydrated				1
Sore(s)			1	
Tail: pale				1
Thin fur		1		
Very small				4

B. Clinical Observations Summarized by Group and Day

Day[a]	Observation[b]	Butyl Benzyl Phthalate (ppm in the feed)			
		0	750	3750	11250
-12	Nipples present[c]				2
	Very small, given water dish				1
-11	Nipples present[c]				1
-10	Very small, given water dish				1
-8	Thin fur: abdomen		1		
-7	Alopecia: abdomen		1		
-6	Alopecia: abdomen		1		
	Slightly dehydrated, given water dish				1
-5	Alopecia: abdomen		1		
-4	Alopecia: abdomen		1		
-3	Alopecia: abdomen		1		

(continued)

Table 36. Summary of the F_1 Male Clinical Observations During the Prebreed and Mating Periods
(page 2 of 7)

Day[a]	Observation[b]	Butyl Benzyl Phthalate (ppm in the feed)			
		0	750	3750	11250
-2	Alopecia: abdomen		1		
-1	Alopecia: abdomen		1		
0	Alopecia: abdomen, growing in		1		
	Nipples present[c]				1
	Very small, given water dish				2
1	Alopecia: abdomen		1		
2	Alopecia: abdomen		1		
3	Alopecia: abdomen		1		
4	Alopecia: abdomen		1		
5	Alopecia: abdomen, grown in		1		
	Hypospadias found at preputial separation examination[c]				2
8	Chromodacryorrhea: eye, left		1		
9	Chromodacryorrhea: eye, left		1		
10	Chromodacryorrhea: eye, left		1		
11	Chromodacryorrhea: eye, left		1		
12	Chromodacryorrhea: eye, left		1		
13	Chromodacryorrhea: eye, left, mild		1		
14	Chromodacryorrhea: eye, left		1		
15	Chromodacryorrhea: eye, left		1		
16	Chromodacryorrhea: eye, left		1		
17	Chromodacryorrhea: eye, left		1		
18	Chromodacryorrhea: eye, left, gone		1		
	Emaciated			1	
	Rough coat			1	
19	Emaciated			1	
	Rough coat			1	
20	Chromodacryorrhea: eye, left		1		
	nose			1	
	Emaciated			1	
	Lethargic			1	
	Rough coat			1	

(continued)

Table 36. Summary of the F_1 Male Clinical Observations During the Prebreed and Mating Periods
(page 3 of 7)

Day[a]	Observation[b]	Butyl Benzyl Phthalate (ppm in the feed)			
		0	750	3750	11250
21	Abnormal positioning of both rear legs and pain elicited upon percussion of anterior lumbar vertebral column, appears to be neurological problem, possibly traumatic in origin			1	
	Chromodacryorrhea: eye, left		1		
	Emaciated			1	
	Euthanized moribund			1	
	Rough coat			1	
22	Chromodacryorrhea: eye, left		1		
23	Chromodacryorrhea: eye, left		1		
24	Chromodacryorrhea: eye, left		1		
25	Chromodacryorrhea: eye, left		1		
26	Chromodacryorrhea: eye, left, gone		1		
28	Alopecia: limb(s)			1	1
	Chromodacryorrhea: eye, left		1		
29	Alopecia: limb(s)			1	1
	Chromodacryorrhea: eye, left		1		
	eye, right				1
30	Alopecia: limb(s)			1	1
	Chromodacryorrhea: eye, left		1		
	eye, right, gone				1
31	Alopecia: limb(s)			1	1
	Chromodacryorrhea: eye, left		1		
32	Alopecia: limb(s)			1	1
	Chromodacryorrhea: eye, left		1		
33	Alopecia: limb(s)			1	1
	Chromodacryorrhea: eye, left		1		
34	Alopecia: limb(s)			1	1
	Chromodacryorrhea: eye, left		1		
35	Alopecia: limb(s)	1		1	1
	Chromodacryorrhea: eye, left		1		
36	Alopecia: limb(s)	1		1	1
	Chromodacryorrhea: eye, left		1		
	eye, right				1
37	Alopecia: limb(s)	1		1	1
	Chromodacryorrhea: eye, left, gone		1		
	eye, right				1
38	Alopecia: limb(s)	1		1	1
	Chromodacryorrhea: eye, right, gone				1

(continued)

Table 36. Summary of the F_1 Male Clinical Observations During the Prebreed and Mating Periods
(page 4 of 7)

Day[a]	Observation[b]	Butyl Benzyl Phthalate (ppm in the feed)			
		0	750	3750	11250
39	Alopecia: limb(s)	1		1	1
40	Alopecia: limb(s)	1		1	1
41	Alopecia: limb(s)	1		1	1
42	Alopecia: limb(s)	1		1	
	limb(s), improving				1
	Chromodacryorrhea: eye, left		1		
	eye, right				1
43	Alopecia: limb(s)	1		1	
	limb(s), growing in				1
	Chromodacryorrhea: eye, left		1		
	eye, right				1
44	Alopecia: limb(s)	1		1	
	Chromodacryorrhea: eye, left		1		
	eye, right, gone				1
45	Alopecia: limb(s)	1		1	
	Chromodacryorrhea: eye, left		1		
46	Alopecia: limb(s)	1		1	
	Chromodacryorrhea: eye, left		1		
47	Alopecia: limb(s)	1		1	
	Chromodacryorrhea: eye, left		1		
48	Alopecia: limb(s)	1		1	
	Chromodacryorrhea: eye, left		1		
49	Alopecia: limb(s)	1		1	
	Chromodacryorrhea: eye, left		1		
50	Alopecia: limb(s)	1		1	
	Chromodacryorrhea: eye, left		1		
51	Alopecia: limb(s)	1		1	
	Chromodacryorrhea: eye, left		1		
	Sore: back			1	
52	Alopecia: limb(s)	1		1	
	Chromodacryorrhea: eye, left		1		
	Sore: back			1	
53	Alopecia: limb(s)	1		1	
	Chromodacryorrhea: eye, left		1		
	eye, right				1
	Sore: back			1	
54	Alopecia: limb(s)	1		1	
	Chromodacryorrhea: eye, left		1		
	eye, right				1
	Sore: back, healing			1	

(continued)

Table 36. Summary of the F_1 Male Clinical Observations During the Prebreed and Mating Periods
(page 5 of 7)

Day[a]	Observation[b]	Butyl Benzyl Phthalate (ppm in the feed)			
		0	750	3750	11250
55	Alopecia: limb(s)	1		1	
	Chromodacryorrhea: eye, left		1		
	eye, right, gone				1
	Sore: back, healing			1	
56	Alopecia: limb(s)			1	
	limb(s), growing in	1			
	Chromodacryorrhea: eye, left		1		
	eye, right	1			
	Sore: back, healing			1	
57	Alopecia: limb(s)	1		1	
	Chromodacryorrhea: eye, left, gone		1		
	Sore: back, healed			1	
58	Alopecia: limb(s)	1		1	
59	Alopecia: limb(s)	1		1	
60	Alopecia: limb(s)	1		1	
61	Alopecia: limb(s)	1		1	
62	Alopecia: limb(s)	1		1	
63	Alopecia: limb(s)	1		1	
	multiple areas		1		
64	Alopecia: limb(s)	1		1	
	multiple areas		1		
65	Alopecia: limb(s)	1		1	
	multiple areas		1		
	Chromodacryorrhea: eye, left		1		
66	Alopecia: limb(s)	1		1	
	multiple areas		1		
67	Alopecia: limb(s)	1		1	
	multiple areas		1		
	Chromodacryorrhea: eye, left		1		
68	Alopecia: limb(s)	1		1	
	multiple areas		1		
	Chromodacryorrhea: eye, left		1		
69	Alopecia: limb(s)	1		1	
	multiple areas		1		
	Chromodacryorrhea: eye, left, improving		1		
70	Alopecia: limb(s)	1		1	
	multiple areas		1		
	Chromodacryorrhea: eye, left		1		

(continued)

Table 36. Summary of the F_1 Male Clinical Observations During the Prebreed and Mating Periods
(page 6 of 7)

Day[a]	Observation[b]	Butyl Benzyl Phthalate (ppm in the feed)			
		0	750	3750	11250
71	Alopecia: limb(s)	1		1	
	multiple areas		1		
	Chromodacryorrhea: eye, left		1		
72	Alopecia: limb(s)	1		1	
	multiple areas		1		
	Chromodacryorrhea: eye, left, improving		1		
73	Alopecia: limb(s)	1		1	
	multiple areas		1		
	Chromodacryorrhea: eye, left		1		
74	Alopecia: limb(s)	1		1	
	multiple areas		1		
	Chromodacryorrhea: eye, left		1		
75	Alopecia: limb(s)	1		1	
	multiple areas		1		
	Chromodacryorrhea: eye, bilateral				1
	eye, left		1		
	Diarrhea				1
	Ear: pale, bilateral				1
	Euthanized moribund				2
	Pale				1
	Palpated abdomen and a large mass was felt in lower right quadrant				1
	Paw: pale, all				1
	Penis: blood coming from				2
	Piloerection				1
	Prone				1
	Tail: pale				1
76	Alopecia: limb(s)	1		1	
	multiple areas		1		
	Chromodacryorrhea: eye, left, gone		1		
77	Alopecia: limb(s)	1		1	
	multiple areas		1		
	Ear: red, bilateral			1	
78	Alopecia: limb(s)	1		1	
	multiple areas		1		
	Ear: red, bilateral			1	
	Found Dead		1		
79	Alopecia: limb(s)	1		1	
	multiple areas		1		
	Chromodacryorrhea: eye, left		1		
	Ear: red, bilateral			1	

(continued)

Table 36. Summary of the F_1 Male Clinical Observations During the Prebreed and Mating Periods
(page 7 of 7)

Day[a]	Observation[b]	Butyl Benzyl Phthalate (ppm in the feed)			
		0	750	3750	11250
80	Alopecia: limb(s)	1		1	
	multiple areas		1		
	Chromodacryorrhea: eye, left		1		
	Ear: red, bilateral			1	
81	Alopecia: limb(s)	1		1	
	multiple areas		1		
	Ear: red, bilateral			1	
82	Alopecia: limb(s)	1		1	
	multiple areas		1		
	Ear: red, bilateral			1	
83	Alopecia: limb(s)	1	2	1	
	multiple areas		1		
	Ear: red, bilateral			1	
	Found Dead			1	
84	Alopecia: limb(s)	1	2	1	
	multiple areas		1		
	Ear: red, bilateral			1	
	Rust colored fur: multiple areas			1	

[a]Study day.
[b]Clinical observations are tabulated once per day per animal.
[c]No further notation was made unless a change occurred.

Table 37. Summary and Statistical Analysis of the F_1 Female Body Weights and Weight Changes During the Prebreed and Mating Periods (page 1 of 4)

	Butyl Benzyl Phthalate (ppm in the feed)			
	0	750	3750	11250
No. Females on Study	30	30	30	30
Body Weight (sd 0) (g)[a]				
#	104.7 ŧŧŧ ± 4.6 ŸŸŸ N=30	111.2 ± 2.4 N=30	107.6 ± 2.9 N=30	85.8 ÞÞÞ ± 3.2 N=30
Body Weight (sd 7) (g)[a]				
	147.1 ŧŧŧ ± 4.7 §§§ N=30	157.0 ± 2.7 N=30	152.6 ± 3.0 N=30	129.2 ** ± 4.0 N=30
Body Weight (sd 14) (g)[a]				
#	178.4 ŧŧŧ ± 4.8 ŸŸŸ N=30	187.3 ± 2.9 N=30	183.9 ± 3.1 N=30	162.4 ÞÞ ± 3.9 N=30
Body Weight (sd 21) (g)[a]				
	205.0 ŧŧŧ ± 4.8 §§§ N=30	213.2 ± 3.4 N=30	210.6 ± 3.3 N=30	187.4 ** ± 3.8 N=30
Body Weight (sd 28) (g)[a]				
	228.1 ŧŧŧ ± 4.9 §§§ N=30	235.0 ± 3.8 N=30	233.9 ± 3.6 N=30	209.8 ** ± 3.6 N=30
Body Weight (sd 35) (g)[a]				
	251.5 ŧŧŧ ± 5.0 §§§ N=30	255.9 ± 4.3 N=30	256.9 ± 3.9 N=30	227.9 *** ± 3.9 N=30
Body Weight (sd 42) (g)[a]				
	263.5 ŧŧŧ ± 4.7 §§§ N=30	268.6 ± 4.5 N=30	270.8 ± 4.1 N=30	243.6 ** ± 4.0 N=30
Body Weight (sd 49) (g)[a]				
	275.3 ŧŧŧ ± 4.6 §§§ N=30	282.4 ± 5.0 N=30	282.6 ± 4.1 N=30	254.7 ** ± 4.3 N=30
Body Weight (sd 56) (g)[a]				
	285.0 ŧŧŧ ± 4.8 §§§ N=30	289.2 ± 5.6 N=30	293.1 ± 4.3 N=30	263.8 ** ± 4.2 N=30

(continued)

Table 37. Summary and Statistical Analysis of the F_1 Female Body Weights and Weight Changes During the Prebreed and Mating Periods (page 2 of 4)

	Butyl Benzyl Phthalate (ppm in the feed)			
	0	750	3750	11250
Body Weight (sd 63) (g)[a]				
	294.7 ‡‡‡ ± 4.9 §§§ N=30	299.1 ± 5.8 N=30	305.4 ± 4.9 N=30	273.7 ** ± 4.2 N=30
Body Weight (sd 70) (g)[a]				
	302.8 ‡‡‡ ± 5.4 §§§ N=30	305.8 ± 6.3 N=30	311.4 ± 5.5 N=30	277.9 ** ± 4.4 N=30
Body Weight (sd 77) (g)[a,b]				
	342.8 ± 6.9 N=2	356.8 ± 59.1 N=2	327.8 ± 15.6 N=4	303.3 ± 6.4 N=12
Body Weight (sd 84) (g)[a,b]				
	361.2 ± 4.0 N=2	438.6 ± N=1	350.7 ± 15.5 N=4	307.6 ± 5.1 N=10
Body Weight Change (sd 0 to 7) (g)[a]				
	42.4 ± 1.0 N=30	45.8 ± 1.1 N=30	45.0 ± 1.0 N=30	43.3 ± 1.2 N=30
Body Weight Change (sd 7 to 14) (g)[a]				
	31.3 ± 1.1 N=30	30.3 ± 1.3 N=30	31.3 ± 1.1 N=30	33.2 ± 1.6 N=30
Body Weight Change (sd 14 to 21) (g)[a]				
	26.6 ± 1.1 N=30	25.9 ± 1.2 N=30	26.7 ± 0.8 N=30	25.0 ± 1.3 N=30
Body Weight Change (sd 21 to 28) (g)[a]				
	23.1 ± 1.1 N=30	21.8 ± 1.3 N=30	23.3 ± 0.9 N=30	22.4 ± 1.0 N=30
Body Weight Change (sd 28 to 35) (g)[a]				
	23.4 ‡‡ ± 1.5 §§ N=30	20.9 ± 1.2 N=30	23.0 ± 1.0 N=30	18.2 ** ± 1.0 N=30

(continued)

Table 37. Summary and Statistical Analysis of the F_1 Female Body Weights and Weight Changes During the Prebreed and Mating Periods (page 3 of 4)

	Butyl Benzyl Phthalate (ppm in the feed)			
	0	750	3750	11250
Body Weight Change (sd 35 to 42) (g)[a]				
#	12.0 ± 1.4 ŸŸ N=30	12.8 ± 1.1 N=30	13.9 ± 0.8 N=30	15.6 ± 0.8 N=30
Body Weight Change (sd 42 to 49) (g)[a]				
	11.8 ± 1.0 N=30	13.8 ± 0.9 N=30	11.8 ± 0.6 N=30	11.1 ± 1.0 N=30
Body Weight Change (sd 49 to 56) (g)[a]				
	9.7 ± 1.2 N=30	6.8 ± 1.1 N=30	10.5 ± 1.3 N=30	9.2 ± 0.8 N=30
Body Weight Change (sd 56 to 63) (g)[a]				
	9.7 ± 1.3 N=30	9.9 ± 0.9 N=30	12.3 ± 1.4 N=30	9.8 ± 1.3 N=30
Body Weight Change (sd 63 to 70) (g)[a]				
	8.1 ± 1.4 § N=30	6.7 ± 1.0 N=30	6.0 ± 1.3 N=30	4.3 ± 1.1 N=30
Body Weight Change (sd 0 to 70) (g)[a]				
	198.1 ± 5.3 N=30	194.6 ± 5.9 N=30	203.8 ± 5.4 N=30	192.1 ± 4.2 N=30
Body Weight Change (sd 70 to 77) (g)[a,b]				
	14.0 ± 2.7 N=2	6.7 ± 8.8 N=2	11.6 ± 3.7 N=4	14.0 ± 3.2 N=12
Body Weight Change (sd 77 to 84) (g)[a,b]				
	18.4 ± 10.9 N=2	22.7 ± . N=1	22.8 ± 0.2 N=4	11.4 ± 2.9 N=10

(continued)

Table 37. Summary and Statistical Analysis of the F_1 Female Body Weights and Weight Changes During the Prebreed and Mating Periods (page 4 of 4)

[a]Reported as the mean ± S.E.M.; sd=study day with study day 0 being the first day of the prebreed period.
[b]Includes all females that were not found sperm and/or plug positive. Statistical analyses were not performed on these endpoints since not all females were represented.
[#]Levene's test for homogeneity of variances was significant ($p<0.05$), therefore robust regression methods were used to test all treatment effects.
‡‡$p<0.01$; ANOVA Test.
‡‡‡$p<0.001$; ANOVA Test.
§$p<0.05$; Test for Linear Trend.
§§$p<0.01$; Test for Linear Trend.
§§§$p<0.001$; Test for Linear Trend.
**$p<0.01$; Dunnett's Test.
***$p<0.001$; Dunnett's Test.
†††$p<0.001$; Wald Chi-square Test for overall treatment effect in robust regression model.
ÞÞ$p<0.01$; Individual t-test for pairwise comparisons to control in robust regression model.
ÞÞÞ$p<0.001$; Individual t-test for pairwise comparisons to control in robust regression model.
ŸŸ$p<0.01$; Linear trend test in robust regression model.
ŸŸŸ$p<0.001$; Linear trend test in robust regression model.

Table 38. Summary and Statistical Analysis of the F_1 Female Feed Consumption, Percent Food Efficiency and Butyl Benzyl Phthalate Intake During the Prebreed Period (page 1 of 6)

	Butyl Benzyl Phthalate (ppm in the feed)			
	0	750	3750	11250
No. Females on Study	30	30	30	30
Feed Consumption (sd 0 to 7) (g/day)[a]				
	16.2 ‡‡‡ ± 0.4 §§ N=30	17.6 * ± 0.3 N=30	17.2 ± 0.3 N=30	15.3 ± 0.4 N=30
Feed Consumption (sd 7 to 14) (g/day)[a]				
	17.8 ‡‡ ± 0.4 § N=30	19.5 * ± 0.6 N=29[b]	18.2 ± 0.3 N=30	17.3 ± 0.4 N=30
Feed Consumption (sd 14 to 21) (g/day)[a]				
	19.9 ± 0.5 N=30	20.2 ± 0.5 N=28[b,c]	19.5 ± 0.4 N=30	19.1 ± 0.6 N=30
Feed Consumption (sd 21 to 28) (g/day)[a]				
	20.4 ± 0.5 N=30	21.2 ± 0.5 N=30	20.1 ± 0.4 N=30	19.9 ± 0.6 N=30
Feed Consumption (sd 28 to 35) (g/day)[a]				
	21.4 ± 0.5 N=30	22.5 ± 0.5 N=30	21.0 ± 0.4 N=30	21.1 ± 0.8 N=30
Feed Consumption (sd 35 to 42) (g/day)[a]				
	21.9 ± 0.6 N=30	22.2 ± 0.5 N=30	21.9 ± 0.6 N=30	21.3 ± 0.7 N=30
Feed Consumption (sd 42 to 49) (g/day)[a]				
	20.8 ± 0.5 N=30	21.8 ± 0.5 N=30	20.7 ± 0.4 N=29[b]	20.9 ± 0.8 N=29[c]
Feed Consumption (sd 49 to 56) (g/day)[a]				
	20.6 ± 0.6 N=30	21.0 ± 0.6 N=30	20.5 ± 0.5 N=30	20.8 ± 0.8 N=30
Feed Consumption (sd 56 to 63) (g/day)[a]				
#	19.9 ± 0.5 N=30	20.2 ± 0.4 N=30	20.8 ± 0.5 N=30	21.1 ± 0.8 N=30

(continued)

Table 38. Summary and Statistical Analysis of the F_1 Female Feed Consumption, Percent Food Efficiency and Butyl Benzyl Phthalate Intake During the Prebreed Period (page 2 of 6)

	Butyl Benzyl Phthalate (ppm in the feed)			
	0	750	3750	11250
Feed Consumption (sd 63 to 70) (g/day)[a]				
	20.0 ± 0.5 N=30	20.0 ± 0.4 N=30	20.1 ± 0.5 N=30	20.8 ± 0.7 N=30
Feed Consumption (sd 0 to 70) (g/day)[a]				
	19.9 ± 0.4 N=30	20.5 ± 0.4 N=27[d]	19.9 ± 0.3 N=29[d]	19.6 ± 0.4 N=29[d]
Feed Consumption (sd 0 to 7) (g/kg/day)[a]				
	130.7 ‡‡‡ ± 2.8 §§§ N=30	131.9 ± 1.9 N=30	133.1 ± 1.8 N=30	144.2 *** ± 2.2 N=30
Feed Consumption (sd 7 to 14) (g/kg/day)[a]				
	110.7 ‡‡ ± 2.2 §§ N=30	112.8 ± 2.8 N=29[b]	108.3 ± 1.1 N=30	120.2 ** ± 2.5 N=30
Feed Consumption (sd 14 to 21) (g/kg/day)[a]				
	104.6 ‡ ± 2.6 § N=30	101.2 ± 1.9 N=28[b,c]	99.3 ± 1.9 N=30	109.6 ± 3.3 N=30
Feed Consumption (sd 21 to 28) (g/kg/day)[a]				
	95.0 ‡ ± 2.4 § N=30	94.5 ± 1.6 N=30	90.7 ± 1.4 N=30	100.7 ± 3.0 N=30
Feed Consumption (sd 28 to 35) (g/kg/day)[a]				
	89.7 ‡ ± 2.3 § N=30	91.9 ± 1.7 N=30	85.7 ± 1.4 N=30	96.4 ± 3.5 N=30
Feed Consumption (sd 35 to 42) (g/kg/day)[a]				
	86.2 ± 3.3 N=30	84.8 ± 1.7 N=30	83.2 ± 2.1 N=30	90.6 ± 2.8 N=30
Feed Consumption (sd 42 to 49) (g/kg/day)[a]				
	77.4 ‡ ± 2.0 § N=30	79.2 ± 1.4 N=30	75.0 ± 1.1 N=29[b]	83.9 ± 3.0 N=29[c]

(continued)

Table 38. Summary and Statistical Analysis of the F_1 Female Feed Consumption, Percent Food Efficiency and Butyl Benzyl Phthalate Intake During the Prebreed Period (page 3 of 6)

	Butyl Benzyl Phthalate (ppm in the feed)			
	0	750	3750	11250
Feed Consumption (sd 49 to 56) (g/kg/day)[a]				
	73.8 ‡ ± 2.3 § N=30	73.5 ± 1.7 N=30	71.2 ± 1.4 N=30	80.3 ± 2.7 N=30
Feed Consumption (sd 56 to 63) (g/kg/day)[a]				
#	68.9 †† ± 1.7 ŸŸŸ N=30	69.0 ± 1.0 N=30	69.6 ± 1.6 N=30	78.6 ÞÞ ± 2.6 N=30
Feed Consumption (sd 63 to 70) (g/kg/day)[a]				
#	67.1 †† ± 1.3 ŸŸ N=30	66.4 ± 0.9 N=30	65.2 ± 1.3 N=30	75.6 ÞÞ ± 2.9 N=30
Feed Consumption (sd 0 to 70) (g/kg/day)[a]				
	86.7 ‡‡‡ ± 1.8 §§§ N=30	87.0 ± 1.2 N=27[d]	84.5 ± 1.2 N=29[d]	93.2 ** ± 1.6 N=29[d]
Percent Food Efficiency (sd 0 to 7)[a]				
	38.3 ‡ ± 1.3 § N=30	37.2 ± 0.8 N=30	37.6 ± 0.9 N=30	40.7 ± 0.7 N=30
Percent Food Efficiency (sd 7 to 14)[a]				
	25.3 ‡ ± 1.0 § N=30	22.6 ± 0.9 N=29[b]	24.7 ± 0.9 N=30	27.5 ± 1.3 N=30
Percent Food Efficiency (sd 14 to 21)[a]				
	19.3 ± 0.9 N=30	18.3 ± 0.7 N=28[b,c]	19.6 ± 0.6 N=30	19.1 ± 1.0 N=30
Percent Food Efficiency (sd 21 to 28)[a]				
	16.4 ± 0.9 N=30	14.6 ± 0.7 N=30	16.5 ± 0.5 N=30	16.2 ± 0.7 N=30
Percent Food Efficiency (sd 28 to 35)[a]				
	15.6 ‡‡ ± 0.9 § N=30	13.2 ± 0.7 N=30	15.7 ± 0.6 N=30	12.5 ** ± 0.7 N=30

(continued)

Table 38. Summary and Statistical Analysis of the F_1 Female Feed Consumption, Percent Food Efficiency and Butyl Benzyl Phthalate Intake During the Prebreed Period (page 4 of 6)

	Butyl Benzyl Phthalate (ppm in the feed)			
	0	750	3750	11250
Percent Food Efficiency (sd 35 to 42)[a]				
#	7.9 †† ± 0.9 ŸŸŸ N=30	8.2 ± 0.7 N=30	9.0 ± 0.5 N=30	10.7 ÞÞ ± 0.5 N=30
Percent Food Efficiency (sd 42 to 49)[a]				
	8.0 ± 0.6 N=30	9.0 ± 0.5 N=30	8.2 ± 0.5 N=29[b]	7.7 ± 0.7 N=29[c]
Percent Food Efficiency (sd 49 to 56)[a]				
	6.6 ‡ ± 0.8 N=30	4.4 ± 0.7 N=30	7.1 ± 0.8 N=30	6.3 ± 0.5 N=30
Percent Food Efficiency (sd 56 to 63)[a]				
	6.9 ± 0.9 N=30	6.9 ± 0.6 N=30	8.2 ± 0.9 N=30	6.7 ± 0.8 N=30
Percent Food Efficiency (sd 63 to 70)[a]				
	5.5 ± 1.0 § N=30	4.6 ± 0.7 N=30	3.9 ± 0.9 N=30	2.8 ± 0.7 N=30
Percent Food Efficiency (sd 0 to 70)[a]				
	14.3 ± 0.4 N=30	13.4 ± 0.3 N=27[d]	14.5 ± 0.2 N=29[d]	14.1 ± 0.3 N=29[d]
Butyl Benzyl Phthalate Intake (sd 0 to 7) (mg/kg/day)[a]				
		98.93 ± 1.43 N=30	499.11 ± 6.75 N=30	1622.04 ± 25.02 N=30
Butyl Benzyl Phthalate Intake (sd 7 to 14) (mg/kg/day)[a]				
		84.60 ± 2.12 N=29[b]	406.21 ± 4.28 N=30	1351.91 ± 28.20 N=30
Butyl Benzyl Phthalate Intake (sd 14 to 21) (mg/kg/day)[a]				
		75.87 ± 1.39 N=28[b,c]	372.35 ± 7.21 N=30	1232.93 ± 36.68 N=30

(continued)

Table 38. Summary and Statistical Analysis of the F_1 Female Feed Consumption, Percent Food Efficiency and Butyl Benzyl Phthalate Intake During the Prebreed Period (page 5 of 6)

	Butyl Benzyl Phthalate (ppm in the feed)			
	0	750	3750	11250
Butyl Benzyl Phthalate Intake (sd 21 to 28) (mg/kg/day)[a]				
		70.91 ± 1.22 N=30	340.11 ± 5.11 N=30	1132.59 ± 33.74 N=30
Butyl Benzyl Phthalate Intake (sd 28 to 35) (mg/kg/day)[a]				
		68.90 ± 1.29 N=30	321.22 ± 5.07 N=30	1084.52 ± 38.97 N=30
Butyl Benzyl Phthalate Intake (sd 35 to 42) (mg/kg/day)[a]				
		63.56 ± 1.27 N=30	311.94 ± 8.06 N=30	1018.84 ± 31.41 N=30
Butyl Benzyl Phthalate Intake (sd 42 to 49) (mg/kg/day)[a]				
		59.42 ± 1.02 N=30	281.32 ± 4.22 N=29[b]	943.72 ± 33.23 N=29[c]
Butyl Benzyl Phthalate Intake (sd 49 to 56) (mg/kg/day)[a]				
		55.11 ± 1.25 N=30	266.85 ± 5.37 N=30	903.29 ± 30.51 N=30
Butyl Benzyl Phthalate Intake (sd 56 to 63) (mg/kg/day)[a]				
		51.73 ± 0.73 N=30	261.15 ± 6.10 N=30	884.36 ± 29.03 N=30
Butyl Benzyl Phthalate Intake (sd 63 to 70) (mg/kg/day)[a]				
		49.83 ± 0.67 N=30	244.54 ± 4.98 N=30	851.05 ± 32.28 N=30
Butyl Benzyl Phthalate Intake (sd 0 to 70) (mg/kg/day)[a]				
		65.24 ± 0.92 N=27[d]	316.79 ± 4.33 N=29[d]	1048.15 ± 18.49 N=29[d]

(continued)

Table 38. Summary and Statistical Analysis of the F_1 Female Feed Consumption, Percent Food Efficiency and Butyl Benzyl Phthalate Intake During the Prebreed Period (page 6 of 6)

[a]Reported as the mean ± S.E.M.; sd=study day with study day 0 being the first day of the prebreed period.
[b]Decrease in N is due to the feed weight for one female inadvertently not being recorded.
[c]Decrease in N is due to one or more females scratching the feed out of the feed jar and therefore an accurate feed weight could not be obtained.
[d]Decrease in N is due to interim feed consumption value(s) for one or more males being missing and therefore the overall feed consumption value could not be calculated.
[#]Levene's test for homogeneity of variances was significant ($p<0.05$), therefore robust regression methods were used to test all treatment effects.
‡$p<0.05$; ANOVA Test.
‡‡$p<0.01$; ANOVA Test.
‡‡‡$p<0.001$; ANOVA Test.
§$p<0.05$; Test for Linear Trend.
§§$p<0.01$; Test for Linear Trend.
§§§$p<0.001$; Test for Linear Trend.
*$p<0.05$; Dunnett's Test.
**$p<0.01$; Dunnett's Test.
***$p<0.001$; Dunnett's Test.
††$p<0.01$; Wald Chi-square Test for overall treatment effect in robust regression model.
ÞÞ$p<0.01$; Individual t-test for pairwise comparisons to control in robust regression model.
ŸŸ$p<0.01$; Linear trend test in robust regression model.
ŸŸŸ$p<0.001$; Linear trend test in robust regression model.

Table 39. Summary of the F_1 Female Clinical Observations During the Prebreed and Mating Periods
(page 1 of 8)

A. Clinical Observations Summarized by Group

	Butyl Benzyl Phthalate (ppm in the feed)			
Observation	0	750	3750	11250
Alopecia	1		3	2
Chromodacryorrhea	1		4	
Ear(s): red		2		5
Face: swollen			1	
Rust colored fur				2
Scab(s)			1	
Sore(s)		1	1	
Teeth: malocclusion and/or trimmed			1	
Urethra: dried blood present			1	
Vagina: torn skin or abrasion above it		1		
Vaginal Smear: blood present				1
Very small, given water dish				3
Wound: shoulder(s)			1	

B. Clinical Observations Summarized by Group and Day

		Butyl Benzyl Phthalate (ppm in the feed)			
Day[a]	Observation[b]	0	750	3750	11250
-10	Very small, given water dish				1
0	Very small, given water dish				2
8	Chromodacryorrhea: nose			1	
	Face: swollen			1	
9	Chromodacryorrhea: eye, left			1	
	nose			1	
	Face: swollen, improving			1	
10	Chromodacryorrhea: eye, left			1	
11	Chromodacryorrhea: eye, left			1	
	Teeth: malocclusion, upper incisors[c]			1	
12	Chromodacryorrhea: eye, left			1	
	Teeth: trimmed, lower incisors			1	
13	Chromodacryorrhea: eye, left			1	
14	Chromodacryorrhea: eye, left			1	
	Wound: shoulder, left			1	
15	Chromodacryorrhea: eye, left			1	
	Scab(s): shoulder(s)			1	
16	Chromodacryorrhea: eye, left			1	
	Scab(s): shoulder(s)			1	
17	Chromodacryorrhea: eye, left			1	
	Scab(s): shoulder(s)			1	

(continued)

Table 39. Summary of the F_1 Female Clinical Observations During the Prebreed and Mating Periods
(page 2 of 8)

Day[a]	Observation[b]	Butyl Benzyl Phthalate (ppm in the feed) 0	750	3750	11250
18	Chromodacryorrhea: eye, left			1	
	Scab(s): shoulder(s), healing			1	
19	Chromodacryorrhea: eye, left			1	
	Scab(s): shoulder(s), healing			1	
	Sore(s): shoulder(s)		1		
	Teeth: trimmed, upper and lower incisors			1	
20	Chromodacryorrhea: eye, left			1	
	Scab(s): shoulder(s), healing			1	
	Sore(s): shoulder(s)		1		
21	Chromodacryorrhea: eye, left			1	
	Scab(s): shoulder(s), moist			1	
	Sore(s): shoulder(s)		1		
22	Chromodacryorrhea: eye, left			1	
	Sore(s): shoulder(s)		1	1	
23	Chromodacryorrhea: eye, left			1	
	Sore(s): shoulder(s)		1	1	
24	Chromodacryorrhea: eye, left			1	
	Sore(s): shoulder(s)		1	1	
25	Chromodacryorrhea: eye, left			1	
	Sore(s): shoulder(s)		1	1	
26	Chromodacryorrhea: eye, left			1	
	Sore(s): shoulder(s)		1	1	
	Teeth: trimmed, upper and lower incisors			1	
27	Chromodacryorrhea: eye, left			1	
	Sore(s): shoulder(s)		1	1	
28	Chromodacryorrhea: eye, left			1	
	Sore(s): shoulder(s)		1		
	shoulder(s), healing			1	
29	Chromodacryorrhea: eye, left			1	
	Sore(s): shoulder(s)		1		
	shoulder(s), healing			1	
30	Chromodacryorrhea: eye, left			1	
	Sore(s): shoulder(s)		1		
	shoulder(s), healing			1	
	Teeth: trimmed, upper and lower incisors			1	
31	Chromodacryorrhea: eye, left			1	
	Sore(s): shoulder(s)		1		
	shoulder(s), healing			1	

(continued)

Table 39. Summary of the F_1 Female Clinical Observations During the Prebreed and Mating Periods
(page 3 of 8)

Day[a]	Observation[b]	Butyl Benzyl Phthalate (ppm in the feed) 0	750	3750	11250
32	Chromodacryorrhea: eye, left			1	
	Sore(s): shoulder(s)		1		
	shoulder(s), healing			1	
33	Chromodacryorrhea: eye, left			1	
	Sore(s): shoulder(s)		1		
	shoulder(s), healing			1	
34	Chromodacryorrhea: eye, left			1	
	Sore(s): shoulder(s)		1		
	shoulder(s), healing			1	
35	Chromodacryorrhea: eye, left			1	
	Sore(s): shoulder(s)		1		
	shoulder(s), healing			1	
36	Chromodacryorrhea: eye, left			1	
	Scab(s): shoulder(s)			1	
	Sore(s): shoulder(s)		1		
37	Chromodacryorrhea: eye, left			1	
	Sore(s): shoulder(s)		1		
	shoulder(s), returned			1	
38	Chromodacryorrhea: eye, left			1	
	Sore(s): shoulder(s)		1	1	
39	Chromodacryorrhea: eye, left			1	
	Sore(s): shoulder(s)		1	1	
40	Chromodacryorrhea: eye, left			1	
	Sore(s): shoulder(s)		1	1	
41	Chromodacryorrhea: eye, left			1	
	Sore(s): shoulder(s)		1	1	
42	Alopecia: multiple areas			1	
	Chromodacryorrhea: eye, left			1	
	eye, right			1	
	Ear: red, bilateral				1
	Sore(s): shoulder(s)		1		
	shoulder(s), healing			1	
	Teeth: trimmed, lower incisors			1	
43	Alopecia: multiple areas			1	
	Chromodacryorrhea: eye, left			1	
	eye, right			1	
	Ear: red, bilateral				1
	Sore(s): shoulder(s)		1		
	shoulder(s), healing			1	

(continued)

Table 39. Summary of the F_1 Female Clinical Observations During the Prebreed and Mating Periods
(page 4 of 8)

Day[a]	Observation[b]	Butyl Benzyl Phthalate (ppm in the feed) 0	750	3750	11250
44	Alopecia: multiple areas			1	
	Chromodacryorrhea: eye, left			1	
	eye, right, gone			1	
	Ear: red, bilateral				1
	Sore(s): shoulder(s)		1		
45	Alopecia: multiple areas			1	
	Chromodacryorrhea: eye, left			1	
	Ear: red, bilateral				1
	Sore(s): shoulder(s)		1		
46	Alopecia: multiple areas			1	
	Chromodacryorrhea: eye, left			1	
	Ear: red, bilateral				1
	Sore(s): shoulder(s)		1		
47	Alopecia: multiple areas			1	
	Chromodacryorrhea: eye, left			1	
	Ear: red, bilateral				1
	Sore(s): shoulder(s)		1		
48	Alopecia: multiple areas			1	
	Chromodacryorrhea: eye, left			1	
	Ear: red, bilateral				1
	Sore(s): shoulder(s)		1		
49	Alopecia: multiple areas			1	
	Chromodacryorrhea: eye, left			1	
	eye, right			1	
	Ear: red, bilateral				1
	Sore(s): shoulder(s), healing		1		
	Teeth: trimmed, lower incisors			1	
50	Alopecia: multiple areas			1	
	Chromodacryorrhea: eye, left			1	
	eye, right			1	
	Ear: red, bilateral				1
	Sore(s): shoulder(s), healing		1		
51	Alopecia: multiple areas			1	
	Chromodacryorrhea: eye, left			1	
	eye, right			1	
	Ear: red, bilateral				1
	Sore(s): shoulder(s), healing		1		
52	Alopecia: abdomen	1			
	multiple areas			1	
	Chromodacryorrhea: eye, left			1	
	eye, right			1	
	Ear: red, bilateral				1

(continued)

Table 39. Summary of the F_1 Female Clinical Observations During the Prebreed and Mating Periods
(page 5 of 8)

Day[a]	Observation[b]	Butyl Benzyl Phthalate (ppm in the feed) 0	750	3750	11250
53	Alopecia: abdomen	1			
	multiple areas			1	
	Chromodacryorrhea: eye, left			1	
	eye, right			1	
	Ear: red, bilateral				1
54	Alopecia: abdomen	1			
	multiple areas			1	
	Chromodacryorrhea: eye, left			1	
	eye, right, improving			1	
	Ear: red, bilateral				2
55	Alopecia: abdomen	1			
	limb(s)			1	
	multiple areas			1	
	Chromodacryorrhea: eye, left			1	
	eye, right, improving			1	
	Ear: red, bilateral				2
56	Alopecia: abdomen	1			
	limb(s)			1	
	multiple areas			1	
	Chromodacryorrhea: eye, left			1	
	eye, right			1	
	Ear: red, bilateral				2
	Teeth: trimmed, lower incisors			1	
57	Alopecia: abdomen	1			
	limb(s)			1	
	multiple areas			1	
	Chromodacryorrhea: eye, left			1	
	eye, right			1	
	Ear: red, bilateral				2
58	Alopecia: abdomen, growing in	1			
	limb(s)			1	
	multiple areas			1	
	Chromodacryorrhea: eye, left			1	
	eye, right			1	
	Ear: red, bilateral				2
59	Alopecia: abdomen, growing in	1			
	limb(s)			1	
	multiple areas			1	
	Chromodacryorrhea: eye, left			1	
	eye, right			1	
	Ear: red, bilateral				2

(continued)

Table 39. Summary of the F_1 Female Clinical Observations During the Prebreed and Mating Periods
(page 6 of 8)

Day[a]	Observation[b]	Butyl Benzyl Phthalate (ppm in the feed) 0	750	3750	11250
60	Alopecia: abdomen, growing in	1			
	limb(s)			1	
	multiple areas			1	
	Chromodacryorrhea: eye, left			1	
	eye, right			1	
	Ear: red, bilateral				2
61	Alopecia: limb(s)			1	
	multiple areas			1	
	Chromodacryorrhea: eye, left			1	
	eye, right			1	
	Ear: red, bilateral				2
62	Alopecia: limb(s)			1	
	multiple areas			1	
	Chromodacryorrhea: eye, left			1	
	eye, right			1	
	Ear: red, bilateral				2
63	Alopecia: limb(s)			1	
	multiple areas			2	
	Chromodacryorrhea: eye, left			1	
	eye, right			1	
	Ear: red, bilateral		1		2
64	Alopecia: limb(s)			1	
	multiple areas			2	
	Chromodacryorrhea: eye, left			1	
	eye, right			1	
	Ear: red, bilateral		1		2
	Vaginal Smear: blood present				1
65	Alopecia: limb(s)			1	
	multiple areas			2	
	Chromodacryorrhea: eye, left			2	
	eye, right			1	
	Ear: red, bilateral		2		2
66	Alopecia: limb(s)			1	
	multiple areas			2	
	Chromodacryorrhea: eye, left			2	
	eye, right			1	
	Ear: red, bilateral		2		2
67	Alopecia: limb(s)			1	1
	multiple areas			2	
	Chromodacryorrhea: eye, left			2	
	eye, right			1	
	Ear: red, bilateral		2		2
	Rust colored fur: abdomen				1
	multiple areas				1

(continued)

Table 39. Summary of the F_1 Female Clinical Observations During the Prebreed and Mating Periods
(page 7 of 8)

Day[a]	Observation[b]	Butyl Benzyl Phthalate (ppm in the feed) 0	750	3750	11250
68	Alopecia: limb(s)			1	1
	multiple areas			2	
	Chromodacryorrhea: eye, left			2	
	eye, right	1		1	
	Ear: red, bilateral		2		2
	Rust colored fur: abdomen				1
	multiple areas				1
	Teeth: trimmed, lower incisors			1	
69	Alopecia: limb(s)			1	1
	multiple areas			2	
	Chromodacryorrhea: eye, left			1	
	eye, left, gone			1	
	eye, right	1		1	
	Ear: red, bilateral		2		2
	Rust colored fur: abdomen				1
	multiple areas				1
70	Alopecia: limb(s)			1	1
	multiple areas			2	
	Chromodacryorrhea: eye, left			1	
	eye, right	1		1	
	Ear: red, bilateral		2		3
	Rust colored fur: abdomen				1
	multiple areas				1
71	Alopecia: limb(s)			1	1
	multiple areas			2	
	Chromodacryorrhea: eye, left			1	
	eye, right	1			
	Ear: red, bilateral		2		3
	Rust colored fur: abdomen				1
	multiple areas				1
	Vagina: skin torn above it		1		
72	Alopecia: multiple areas			1	
	Chromodacryorrhea: eye, left			1	
	eye, right	1			
	Ear: red, bilateral				3
	Rust colored fur: abdomen				1
	Vagina: abrasion above it		1		
73	Ear: red, bilateral				2
	Rust colored fur: abdomen				1
	Vagina: abrasion above it		1		
74	Ear: red, bilateral				1
	Rust colored fur: abdomen				1
	Vagina: abrasion above it		1		

(continued)

Table 39. Summary of the F_1 Female Clinical Observations During the Prebreed and Mating Periods
(page 8 of 8)

Day[a]	Observation[b]	Butyl Benzyl Phthalate (ppm in the feed) 0	750	3750	11250
75	Ear: red, bilateral				2
	Rust colored fur: abdomen, gone				1
	Urethra: dried blood present			1	
	Vagina: abrasion above it, healing		1		
76	Ear: red, bilateral				2
	Vagina: abrasion above it, healing		1		
77	Ear: red, bilateral				3
	Vagina: abrasion above it, healing		1		
78	Alopecia: multiple areas				1
	Ear: red, bilateral				3
	Vagina: abrasion above it, healed		1		
79	Alopecia: abdomen	1			
	multiple areas				1
	Ear: red, bilateral				3
80	Alopecia: abdomen	1			
	multiple areas				1
	Ear: red, bilateral				3
81	Alopecia: abdomen	1			
	multiple areas				1
	Ear: red, bilateral				3
82	Alopecia: abdomen	1			
	multiple areas				1
	Ear: red, bilateral				3
83	Alopecia: abdomen	1			
	multiple areas				1
	Ear: red, bilateral				3
84	Alopecia: abdomen	1			
	multiple areas				1
	Ear: red, bilateral				3

[a]Study day.
[b]Clinical observations are tabulated once per day per animal.
[c]No further notation was made unless a change occurred.

Table 40. Summary and Statistical Analysis of the F_1 Female Vaginal Cytology Prior to Mating
(page 1 of 1)

	Butyl Benzyl Phthalate (ppm in the feed)			
	0	750	3750	11250
Number of Females Evaluated	30	30	30	30
Number of Females Cycling	30	29	29	30
Percent of Females Cycling	100.00	96.67	96.67	100.00
Number of Females with an Abnormal Cycle[a]	5	2	9	2
Percent of Females with an Abnormal Cycle[a]	16.67 £	6.90	31.03	6.67
Cycle Length (days)[a,b]	4.38 ‡ ± 0.10 N=30	4.27 ± 0.11 N=29	4.75 ± 0.15 N=29	4.55 ± 0.14 N=30

[a]Includes only those females that were cycling.
[b]Reported as the mean ± S.E.M.
£p<0.05; Chi-Square Test.
‡p<0.05; ANOVA Test.

Table 41. Summary and Statistical Analysis of the F_1 Female Body Weights and Weight Changes During Gestation (page 1 of 2)

	Butyl Benzyl Phthalate (ppm in the feed)			
	0	750	3750	11250
No. Sperm Positive, Pregnant Females	28	28	24	16
Body Weight (gd 0) (g)[a]				
	298.1 ‡ ± 5.6 § N=28	299.5 ± 5.3 N=28	308.4 ± 5.7 N=24	277.8 ± 7.4 N=16
Body Weight (gd 7) (g)[a]				
	326.0 ‡‡ ± 5.9 §§ N=28	332.5 ± 5.9 N=28	337.8 ± 5.8 N=24	301.4 * ± 7.0 N=16
Body Weight (gd 14) (g)[a]				
	357.7 ‡‡ ± 6.3 §§ N=28	362.6 ± 6.3 N=28	366.6 ± 6.7 N=24	325.9 ** ± 7.0 N=16
Body Weight (gd 20) (g)[a]				
	430.3 ‡‡‡ ± 7.5 §§§ N=28	437.7 ± 7.4 N=28	445.0 ± 8.2 N=24	386.0 *** ± 8.2 N=16
Body Weight Change (gd 0 to 7) (g)[a]				
#	27.9 ††† ± 1.5 ŸŸŸ N=28	33.0 Þ ± 1.8 N=28	29.4 ± 1.3 N=24	23.5 Þ ± 1.1 N=16
Body Weight Change (gd 7 to 14) (g)[a]				
	31.7 ‡‡ ± 1.2 §§§ N=28	30.1 ± 1.1 N=28	28.8 ± 1.5 N=24	24.5 ** ± 1.5 N=16
Body Weight Change (gd 14 to 20) (g)[a]				
	72.6 ‡‡‡ ± 1.9 §§ N=28	75.1 ± 2.3 N=28	78.4 ± 3.0 N=24	60.1 ** ± 3.9 N=16
Body Weight Change (gd 0 to 20) (g)[a]				
	132.1 ‡‡‡ ± 3.0 §§§ N=28	138.3 ± 3.7 N=28	136.6 ± 4.3 N=24	108.2 *** ± 4.8 N=16

(continued)

Table 41. Summary and Statistical Analysis of the F_1 Female Body Weights and Weight Changes During Gestation (page 2 of 2)

[a]Reported as the mean ± S.E.M. gd = gestational day.
[#]Levene's test for homogeneity of variances was significant ($p<0.05$), therefore robust regression methods were used to test all treatment effects.
‡$p<0.05$; ANOVA Test.
‡‡$p<0.01$; ANOVA Test.
‡‡‡$p<0.001$; ANOVA Test.
§$p<0.05$; Test for Linear Trend.
§§$p<0.01$; Test for Linear Trend.
§§§$p<0.001$; Test for Linear Trend.
*$p<0.05$; Dunnett's Test.
**$p<0.01$; Dunnett's Test.
***$p<0.001$; Dunnett's Test.
†††$p<0.001$; Wald Chi-square Test for overall treatment effect in robust regression model.
Þ$p\leq0.05$; Individual t-test for pairwise comparisons to control in robust regression model.
ŸŸŸ$p<0.001$; Linear trend test in robust regression model.

Table 42. Summary and Statistical Analysis of the F_1 Female Feed Consumption, Percent Food Efficiency and Butyl Benzyl Phthalate Intake During Gestation (page 1 of 3)

	Butyl Benzyl Phthalate (ppm in the feed)			
	0	750	3750	11250
No. Sperm Positive, Pregnant Females	28	28	24	16
Feed Consumption (gd 0 to 7) (g/day)[a]				
	21.5 ± 0.5 N=28	23.4 ± 0.5 N=28	22.5 ± 0.6 N=23[b]	23.2 ± 1.0 N=16
Feed Consumption (gd 7 to 14) (g/day)[a]				
	23.4 ± 0.6 N=28	24.5 ± 0.5 N=28	24.3 ± 0.7 N=24	23.4 ± 1.0 N=16
Feed Consumption (gd 14 to 20) (g/day)[a]				
#	24.2 ± 0.5 N=28	25.4 ± 0.5 N=28	25.5 ± 0.7 N=24	24.3 ± 1.2 N=16
Feed Consumption (gd 0 to 20) (g/day)[a]				
	23.0 ± 0.5 N=28	24.4 ± 0.4 N=28	24.1 ± 0.6 N=23[c]	23.6 ± 1.0 N=16
Feed Consumption (gd 0 to 7) (g/kg/day)[a]				
	69.1 ‡‡‡ ± 1.3 §§§ N=28	74.2 * ± 1.5 N=28	69.6 ± 1.4 N=23[b]	80.1 *** ± 2.7 N=16
Feed Consumption (gd 7 to 14) (g/kg/day)[a]				
	68.6 ± 1.3 § N=28	70.8 ± 1.1 N=28	69.3 ± 1.9 N=24	74.5 ± 2.4 N=16
Feed Consumption (gd 14 to 20) (g/kg/day)[a]				
#	61.6 ± 0.9 Ÿ N=28	63.8 ± 1.0 N=28	62.8 ± 1.0 N=24	68.0 ± 2.5 N=16
Feed Consumption (gd 0 to 20) (g/kg/day)[a]				
	65.3 ‡‡‡ ± 1.0 §§§ N=28	68.3 ± 1.0 N=28	66.0 ± 1.2 N=23[c]	73.0 *** ± 2.1 N=16

(continued)

Table 42. Summary and Statistical Analysis of the F_1 Female Feed Consumption, Percent Food Efficiency and Butyl Benzyl Phthalate Intake During Gestation (page 2 of 3)

	Butyl Benzyl Phthalate (ppm in the feed)			
	0	750	3750	11250
Percent Food Efficiency (gd 0 to 7)[a]				
	18.3 ‡‡ ± 0.8 §§ N=28	20.1 ± 0.9 N=28	18.7 ± 0.9 N=23[b]	15.0 * ± 1.0 N=16
Percent Food Efficiency (gd 7 to 14)[a]				
	19.4 ‡‡ ± 0.6 §§ N=28	17.5 ± 0.5 N=28	17.2 ± 0.9 N=24	15.4 ** ± 1.2 N=16
Percent Food Efficiency (gd 14 to 20)[a]				
#	50.2 ± 1.3 Ÿ N=28	49.3 ± 1.4 N=28	51.5 ± 1.8 N=24	42.6 ± 3.2 N=16
Percent Food Efficiency (gd 0 to 20)[a]				
#	28.8 †† ± 0.5 ŸŸŸ N=28	28.4 ± 0.6 N=28	28.7 ± 1.0 N=23[c]	23.6 ÞÞÞ ± 1.5 N=16
Butyl Benzyl Phthalate Intake (gd 0 to 7) (mg/kg/day)[a]				
		55.61 ± 1.11 N=28	260.91 ± 5.07 N=23[b]	901.17 ± 30.17 N=16
Butyl Benzyl Phthalate Intake (gd 7 to 14) (mg/kg/day)[a]				
		53.09 ± 0.83 N=28	259.74 ± 7.22 N=24	838.40 ± 27.30 N=16
Butyl Benzyl Phthalate Intake (gd 14 to 20) (mg/kg/day)[a]				
		47.83 ± 0.73 N=28	235.58 ± 3.67 N=24	765.24 ± 27.95 N=16
Butyl Benzyl Phthalate Intake (gd 0 to 20) (mg/kg/day)[a]				
		51.24 ± 0.78 N=28	247.36 ± 4.61 N=23[c]	821.13 ± 23.82 N=16

(continued)

Table 42. Summary and Statistical Analysis of the F_1 Female Feed Consumption, Percent Food Efficiency and Butyl Benzyl Phthalate Intake During Gestation (page 3 of 3)

[a]Reported as the mean ± S.E.M. gd = gestational day.
[b]Decrease in N is due to feed weight for one female inadvertently not being recorded.
[c]Decrease in N is due to interim feed consumption value(s) for one female being missing and therefore the overall feed consumption value could not be calculated.
[#]Levene's test for homogeneity of variances was significant ($p<0.05$), therefore robust regression methods were used to test all treatment effects.
‡‡$p<0.01$; ANOVA Test.
‡‡‡$p<0.001$; ANOVA Test.
§$p<0.05$; Test for Linear Trend.
§§$p<0.01$; Test for Linear Trend.
§§§$p<0.001$; Test for Linear Trend.
*$p<0.05$; Dunnett's Test.
**$p<0.01$; Dunnett's Test.
***$p<0.001$; Dunnett's Test.
††$p<0.01$; Wald Chi-square Test for overall treatment effect in robust regression model.
ÞÞÞ$p<0.001$; Individual t-test for pairwise comparisons to control in robust regression model.
Ÿ$p\leq0.05$; Linear trend test in robust regression model.
ŸŸŸ$p<0.001$; Linear trend test in robust regression model.

Table 43. Summary of the F_1 Female Clinical Observations During Gestation (page 1 of 5)

A. Clinical Observations Summarized by Group

Observation	Butyl Benzyl Phthalate (ppm in the feed)			
	0	750	3750	11250
Alopecia	1		5	3
Chromodacryorrhea	2	2	2	2
Ear(s): red		2		2
Rust colored fur			1	2
Scab(s)				1
Teeth: trimmed			1	

B. Clinical Observations Summarized by Group and Day

Day[a]	Observation[b]	Butyl Benzyl Phthalate (ppm in the feed)			
		0	750	3750	11250
0	Alopecia: limb(s)			1	
	multiple areas			2	
	Chromodacryorrhea: eye, left			1	
	eye, right	1		1	
	Ear: red, bilateral		2		2
	Rust colored fur: multiple areas				1
1	Alopecia: limb(s)			1	
	multiple areas			2	
	Chromodacryorrhea: eye, left			1	
	eye, right	1		1	
	Ear: red, bilateral		2		2
	Rust colored fur: multiple areas				1
2	Alopecia: limb(s)			1	
	multiple areas			2	
	Chromodacryorrhea: eye, left			1	
	eye, right	1		1	
	Ear: red, bilateral		2		2
	Rust colored fur: multiple areas				1
3	Alopecia: limb(s)			1	
	multiple areas			2	
	Chromodacryorrhea: eye, left			1	
	eye, right	1		1	
	Ear: red, bilateral		2		2
	Rust colored fur: multiple areas				1
4	Alopecia: limb(s)			1	
	multiple areas			2	
	Chromodacryorrhea: eye, left			1	
	eye, right	1		1	
	Ear: red, bilateral		2		2
	Rust colored fur: multiple areas				1

(continued)

Table 43. Summary of the F_1 Female Clinical Observations During Gestation (page 2 of 5)

Day[a]	Observation[b]	Butyl Benzyl Phthalate (ppm in the feed)			
		0	750	3750	11250
5	Alopecia: limb(s)			1	
	multiple areas			2	
	Chromodacryorrhea: eye, left			1	
	eye, right	1		1	
	eye, right, improving	1			
	Ear: red, bilateral		2		2
	Rust colored fur: multiple areas				1
	Teeth: trimmed, lower incisors			1	
6	Alopecia: limb(s)			1	
	multiple areas			2	
	Chromodacryorrhea: eye, left			1	
	eye, right	1		1	
	eye, right, improving	1			
	Ear: red, bilateral		2		2
	Rust colored fur: multiple areas				1
7	Alopecia: limb(s)			1	
	multiple areas			2	
	Chromodacryorrhea: eye, left			1	
	eye, right	2			
	eye, right, gone			1	
	Ear: red, bilateral		2		2
	Rust colored fur: multiple areas				1
8	Alopecia: limb(s)			1	1
	multiple areas			2	
	Chromodacryorrhea: eye, left			1	
	eye, right	2		1	
	Ear: red, bilateral		2		2
	Rust colored fur: multiple areas				1
9	Alopecia: limb(s)			1	1
	multiple areas			2	
	Chromodacryorrhea: eye, left		1	1	
	eye, right	1			
	eye, right, gone	1		1	
	Ear: red, bilateral		2		2
	Rust colored fur: multiple areas				1
10	Alopecia: limb(s)			1	1
	multiple areas			2	
	Chromodacryorrhea: eye, left		1	1	
	eye, right	2		1	1
	Ear: red, bilateral		2		2
	Rust colored fur: multiple areas				1

(continued)

Table 43. Summary of the F_1 Female Clinical Observations During Gestation (page 3 of 5)

Day[a]	Observation[b]	Butyl Benzyl Phthalate (ppm in the feed)			
		0	750	3750	11250
11	Alopecia: limb(s)			1	1
	multiple areas			2	
	Chromodacryorrhea: eye, left		1	1	
	eye, right	2			1
	eye, right, gone			1	
	Ear: red, bilateral		2		2
	Rust colored fur: multiple areas				1
12	Alopecia: limb(s)			1	1
	multiple areas			2	
	Chromodacryorrhea: eye, left		1	1	
	eye, right	1		1	1
	eye, right, improving	1			
	Ear: red, bilateral		2		2
	Rust colored fur: multiple areas				1
13	Alopecia: limb(s)			2	1
	multiple areas			2	
	Chromodacryorrhea: eye, left		1	1	
	eye, right	2			1
	eye, right, gone			1	
	Ear: red, bilateral		2		2
	Rust colored fur: multiple areas				1
	shoulder(s)			1	
	Scab(s): neck				1
14	Alopecia: limb(s)			2	1
	multiple areas			3	
	Chromodacryorrhea: eye, left		1	1	
	eye, right	2			
	eye, right, gone				1
	Ear: red, bilateral		2		2
	Rust colored fur: multiple areas				1
	shoulder(s)			1	
	Scab(s): neck				1
15	Alopecia: limb(s)			2	1
	multiple areas			3	
	Chromodacryorrhea: eye, left		1	1	
	eye, right	2			
	Ear: red, bilateral		2		2
	Rust colored fur: multiple areas				1
	shoulder(s)			1	
	Scab(s): neck				1

(continued)

Table 43. Summary of the F_1 Female Clinical Observations During Gestation (page 4 of 5)

Day[a]	Observation[b]	Butyl Benzyl Phthalate (ppm in the feed) 0	750	3750	11250
16	Alopecia: limb(s)			2	1
	multiple areas			3	
	neck				1
	Chromodacryorrhea: eye, left		1	1	
	eye, right	2		1	
	Ear: red, bilateral		2		2
	Rust colored fur: multiple areas				1
	shoulder(s)			1	1
	Scab(s): neck, gone				1
17	Alopecia: limb(s)			2	1
	multiple areas			3	
	neck				1
	Chromodacryorrhea: eye, left			1	
	eye, left, improving		1		
	eye, right	2		1	
	Ear: red, bilateral		2		2
	Rust colored fur: multiple areas				1
	shoulder(s)			1	1
18	Alopecia: limb(s)			2	1
	multiple areas			3	
	neck				1
	Chromodacryorrhea: eye, left			1	
	eye, left, improved		1		
	eye, right	2		1	
	Ear: red, bilateral		2		2
	Rust colored fur: multiple areas				1
	shoulder(s)			1	1
	Teeth: trimmed, lower incisors			1	
19	Alopecia: limb(s)			2	1
	multiple areas	1		3	
	neck				1
	Chromodacryorrhea: eye, left			1	1
	eye, left, gone		1		
	eye, right	1			
	eye, right, gone	1		1	
	Ear: red, bilateral		2		2
	Rust colored fur: multiple areas				1
	shoulder(s)			1	1
20	Alopecia: limb(s)			3	2
	multiple areas	1		2	
	neck				1
	Chromodacryorrhea: eye, left		1	1	1
	eye, right	1		1	
	Ear: red, bilateral		2		2
	Rust colored fur: multiple areas				1
	shoulder(s)			1	1

(continued)

Table 43. Summary of the F_1 Female Clinical Observations During Gestation (page 5 of 5)

Day[a]	Observation[b]	Butyl Benzyl Phthalate (ppm in the feed)			
		0	750	3750	11250
21	Alopecia: limb(s)			3	2
	multiple areas	1		2	
	neck				1
	Chromodacryorrhea: eye, left			1	1
	eye, left, gone		1		
	eye, right			1	
	Ear: red, bilateral		2		2
	Rust colored fur: multiple areas				1
	shoulder(s)			1	1
22	Alopecia: multiple areas	1			
	Ear: red, bilateral		1		

[a]Gestational day.
[b]Clinical observations are tabulated once per day per animal.

Table 44. Summary and Statistical Analysis of the F_1 Maternal Body Weights and Weight Changes During Lactation (page 1 of 2)

	Butyl Benzyl Phthalate (ppm in the feed)			
	0	750	3750	11250
No. Females with Live Litters	29	28	26	17
Body Weight (pnd 0) (g)[a]	334.0 ‡‡ ± 6.3 §§ N=29	340.2 ± 6.5 N=28	346.7 ± 6.3 N=26	307.3 * ± 6.8 N=17
Body Weight (pnd 4) (g)[a]	347.2 ‡‡‡ ± 5.5 §§§ N=29	350.5 ± 5.8 N=28	357.6 ± 5.3 N=26	316.3 ** ± 6.6 N=17
Body Weight (pnd 7) (g)[a]	357.6 ‡‡‡ ± 5.6 §§§ N=29	362.4 ± 5.5 N=28	368.8 ± 5.5 N=26	325.7 ** ± 6.7 N=17
Body Weight (pnd 14) (g)[a]	367.3 ‡‡‡ ± 5.6 §§§ N=29	369.9 ± 5.1 N=28	370.2 ± 5.4 N=26	336.9 ** ± 6.6 N=17
Body Weight (pnd 21) (g)[a]	339.7 ± 5.4 N=29	344.8 ± 4.2 N=28	352.9 ± 4.3 N=26	335.6 ± 5.2 N=17
Body Weight Change (pnd 0 to 4) (g)[a]	13.2 ± 2.4 N=29	10.3 ± 1.9 N=28	10.9 ± 2.4 N=26	9.1 ± 2.4 N=17
Body Weight Change (pnd 4 to 7) (g)[a]	10.4 ± 1.4 N=29	11.9 ± 1.2 N=28	11.2 ± 1.6 N=26	9.4 ± 2.3 N=17
Body Weight Change (pnd 7 to 14) (g)[a]	9.7 ± 2.7 N=29	7.5 ± 2.0 N=28	1.4 ± 2.8 N=26	11.1 ± 2.4 N=17
Body Weight Change (pnd 14 to 21) (g)[a]	-27.6 ‡‡‡ ± 3.6 §§§ N=29	-25.1 ± 3.3 N=28	-17.2 ± 3.7 N=26	-1.3 *** ± 3.7 N=17

(continued)

Table 44. Summary and Statistical Analysis of the F_1 Maternal Body Weights and Weight Changes During Lactation (page 2 of 2)

	Butyl Benzyl Phthalate (ppm in the feed)			
	0	750	3750	11250
Body Weight Change (pnd 0 to 21) (g)[a]				
	5.7 ‡‡ ± 4.6 §§§ N=29	4.6 ± 4.0 N=28	6.3 ± 3.9 N=26	28.3 ** ± 4.0 N=17

[a]Reported as the mean ± S.E.M. pnd = postnatal day.
‡‡p<0.01; ANOVA Test.
‡‡‡p<0.001; ANOVA Test.
§§p<0.01; Test for Linear Trend.
§§§p<0.001; Test for Linear Trend.
*p<0.05; Dunnett's Test.
**p<0.01; Dunnett's Test.
***p<0.001; Dunnett's Test.

Table 45. Summary and Statistical Analysis of F_1 Maternal Feed Consumption, Percent Food Efficiency and Butyl Benzyl Phthalate Intake During Lactation (page 1 of 3)

	Butyl Benzyl Phthalate (ppm in the feed)			
	0	750	3750	11250
No. Females with Live Litters	29	28	26	17
Feed Consumption (pnd 0 to 4) (g/day)[a]	32.6 ± 1.0 N=29	32.7 ± 1.2 N=27[b]	34.6 ± 1.1 N=26	31.9 ± 1.7 N=17
Feed Consumption (pnd 4 to 7) (g/day)[a]	49.4 ‡‡ ± 1.2 §§ N=29	48.8 ± 1.4 N=27[c]	50.8 ± 1.2 N=26	43.4 * ± 1.9 N=17
Feed Consumption (pnd 7 to 14) (g/day)[a] #	61.6 ± 1.0 N=29	60.4 ± 1.0 N=27[b]	62.4 ± 1.1 N=25[d]	56.4 ± 2.8 N=17
Feed Consumption (pnd 14 to 21) (g/day)[a] #	70.6 ± 1.0 N=29	70.5 ± 1.1 N=27[e]	71.6 ± 1.3 N=21[d]	69.5 ± 3.5 N=17
Feed Consumption (pnd 0 to 21) (g/day)[a]	57.3 ± 0.9 N=29	56.6 ± 1.1 N=24[f]	58.0 ± 1.0 N=20[f]	54.3 ± 2.5 N=17
Feed Consumption (pnd 0 to 4) (g/kg/day)[a]	96.4 ± 3.1 N=29	95.7 ± 3.9 N=27[b]	98.7 ± 3.3 N=26	102.5 ± 4.9 N=17
Feed Consumption (pnd 4 to 7) (g/kg/day)[a]	140.7 ± 3.3 N=29	136.7 ± 3.9 N=27[c]	140.4 ± 3.4 N=26	135.3 ± 5.8 N=17
Feed Consumption (pnd 7 to 14) (g/kg/day)[a] #	170.4 ± 2.7 N=29	165.7 ± 2.9 N=27[b]	169.7 ± 3.4 N=25[d]	170.6 ± 8.5 N=17
Feed Consumption (pnd 14 to 21) (g/kg/day)[a] #	200.7 ± 3.5 N=29	199.0 ± 3.5 N=27[e]	202.1 ± 5.0 N=21[d]	207.1 ± 11.1 N=17

(continued)

Table 45. Summary and Statistical Analysis of F_1 Maternal Feed Consumption, Percent Food Efficiency and Butyl Benzyl Phthalate Intake During Lactation (page 2 of 3)

	Butyl Benzyl Phthalate (ppm in the feed)			
	0	750	3750	11250
Feed Consumption (pnd 0 to 21) (g/kg/day)[a]				
#	164.9 ± 2.7 N=29	161.4 ± 3.6 N=24[f]	165.3 ± 3.7 N=20[f]	167.6 ± 7.9 N=17
Percent Food Efficiency (pnd 0 to 4)[a]				
	9.7 ± 1.7 N=29	6.9 ± 1.7 N=27[b]	7.5 ± 1.8 N=26	6.7 ± 1.7 N=17
Percent Food Efficiency (pnd 4 to 7)[a]				
	7.1 ± 1.0 N=29	8.2 ± 0.9 N=27[c]	7.1 ± 0.9 N=26	7.3 ± 1.5 N=17
Percent Food Efficiency (pnd 7 to 14)[a]				
	2.3 ± 0.7 N=29	1.9 ± 0.5 N=27[b]	0.6 ± 0.6 N=25[d]	3.0 ± 0.7 N=17
Percent Food Efficiency (pnd 14 to 21)[a]				
	-5.6 ‡‡‡ ± 0.7 §§§ N=29	-5.1 ± 0.7 N=27[e]	-3.6 ± 0.7 N=21[d]	0.0 *** ± 0.8 N=17
Percent Food Efficiency (pnd 0 to 21)[a]				
	0.5 ‡‡‡ ± 0.4 §§§ N=29	0.5 ± 0.4 N=24[f]	0.5 ± 0.4 N=20[f]	2.7 *** ± 0.4 N=17
Butyl Benzyl Phthalate Intake (pnd 0 to 4) (mg/kg/day)[a]				
		71.74 ± 2.90 N=27[b]	370.13 ± 12.30 N=26	1153.14 ± 55.54 N=17
Butyl Benzyl Phthalate Intake (pnd 4 to 7) (mg/kg/day)[a]				
		102.55 ± 2.89 N=27[c]	526.37 ± 12.62 N=26	1522.16 ± 64.85 N=17

(continued)

Table 45. Summary and Statistical Analysis of F_1 Maternal Feed Consumption, Percent Food Efficiency and Butyl Benzyl Phthalate Intake During Lactation (page 3 of 3)

	Butyl Benzyl Phthalate (ppm in the feed)			
	0	750	3750	11250
Butyl Benzyl Phthalate Intake (pnd 7 to 14) (mg/kg/day)[a]				
		124.27 ± 2.14 N=27[b]	636.28 ± 12.64 N=25[d]	1918.74 ± 95.10 N=17
Butyl Benzyl Phthalate Intake (pnd 14 to 21) (mg/kg/day)[a]				
		149.23 ± 2.65 N=27[e]	758.03 ± 18.87 N=21[d]	2330.28 ± 124.97 N=17
Butyl Benzyl Phthalate Intake (pnd 0 to 21) (mg/kg/day)[a]				
		121.02 ± 2.71 N=24[f]	620.04 ± 13.75 N=20[f]	1885.12 ± 88.96 N=17

[a]Reported as the mean ± S.E.M. pnd = postnatal day.
[b]Decrease in N is due to the feed jar not being weighed for one female.
[c]Decrease in N is due to the feed jar weight inadvertently not being recorded for one female.
[d]Decrease in N is due to the feed consumption value for one or more females being statistical outlier(s) and they were therefore removed.
[e]Decrease in N is due to one female scratching the feed out of the feed jar and therefore an accurate feed weight could not be obtained.
[f]Decrease in N is due to interim feed consumption value(s) for one or more females being missing and therefore the overall feed consumption value could not be calculated.
[#]Levene's test for homogeneity of variances was significant ($p<0.05$), therefore robust regression methods were used to test all treatment effects.
‡‡$p<0.01$; ANOVA Test.
‡‡‡$p<0.001$; ANOVA Test.
§§$p<0.01$; Test for Linear Trend.
§§§$p<0.001$; Test for Linear Trend.
*$p<0.05$; Dunnett's Test.
***$p<0.001$; Dunnett's Test.

Table 46. Summary of the F_1 Female Clinical Observations During Lactation (page 1 of 5)

A. Clinical Observations Summarized by Group

Observation	Butyl Benzyl Phthalate (ppm in the feed)			
	0	750	3750	11250
Alopecia	4		6	4
Bloody discharge		1		
Chromodacryorrhea	2	3	2	2
Ear(s): red		2	1	2
Piloerection			1	
Rust colored fur		2	1	3
Teeth: trimmed			1	

B. Clinical Observations Summarized by Group and Day

Day[a]	Observation[b]	Butyl Benzyl Phthalate (ppm in the feed)			
		0	750	3750	11250
0	Alopecia: abdomen	1			
	limb(s)			3	2
	multiple areas	1		2	
	neck				1
	Chromodacryorrhea: eye, left			1	1
	eye, right	1			
	Ear: red, bilateral		2		2
	Rust colored fur: multiple areas				1
	shoulder(s)			1	1
1	Alopecia: abdomen	1			
	limb(s)			3	2
	multiple areas	1		2	
	neck				1
	Bloody discharge		1		
	Chromodacryorrhea: eye, left			1	
	eye, left, improving				1
	eye, right			1	
	eye, right, improving	1			
	Ear: red, bilateral		2		2
	Rust colored fur: multiple areas				1
	shoulder(s)			1	1
2	Alopecia: abdomen	1			
	limb(s)			4	2
	multiple areas	1		2	
	neck				1
	Chromodacryorrhea: eye, left			1	
	eye, left, improving				1
	eye, right			1	
	eye, right, gone	1			
	Ear: red, bilateral		2		2
	Rust colored fur: multiple areas				1
	shoulder(s)			1	1

(continued)

Table 46. Summary of the F_1 Female Clinical Observations During Lactation (page 2 of 5)

Day[a]	Observation[b]	Butyl Benzyl Phthalate (ppm in the feed) 0	750	3750	11250
3	Alopecia: abdomen	1			
	limb(s)			4	2
	multiple areas	1		2	
	neck, growing in				1
	Chromodacryorrhea: eye, left			1	1
	eye, right			1	
	Ear: red, bilateral		2		2
	Rust colored fur: multiple areas				1
	shoulder(s)			1	1
4	Alopecia: abdomen	1			
	limb(s)			3	3
	limb(s), growing in			1	
	multiple areas	1		2	
	Chromodacryorrhea: eye, left		1	1	
	eye, left, improving				1
	eye, right	1		1	
	Ear: red, bilateral		2		2
	Rust colored fur: multiple areas				1
	shoulder(s)			1	1
	Teeth: trimmed, lower incisors			1	
5	Alopecia: abdomen	1			
	limb(s)			3	3
	limb(s), growing in			1	
	multiple areas	1		2	
	Chromodacryorrhea: eye, left		1	1	
	eye, left, gone				1
	eye, left, improving		1		
	eye, right	1		1	
	Ear: red, bilateral		2		2
	Rust colored fur: multiple areas				1
	shoulder(s)			1	
	shoulder(s), improving				1
6	Alopecia: abdomen	1			
	limb(s)			3	3
	limb(s), growing in			1	
	multiple areas	1		2	
	Chromodacryorrhea: eye, left			1	
	eye, left, gone		2		
	eye, right	1		1	
	Ear: red, bilateral		2		2
	Rust colored fur: multiple areas				1
	shoulder(s)			1	1

(continued)

Table 46. Summary of the F_1 Female Clinical Observations During Lactation (page 3 of 5)

Day[a]	Observation[b]	Butyl Benzyl Phthalate (ppm in the feed)			
		0	750	3750	11250
7	Alopecia: abdomen	1			
	limb(s)			3	3
	limb(s), growing in			1	
	multiple areas	1		2	
	Chromodacryorrhea: eye, left			1	
	eye, right			1	
	eye, right, improving	1			
	Ear: red, bilateral		2		2
	Rust colored fur: multiple areas				1
	shoulder(s)		1	1	1
8	Alopecia: abdomen	1			
	limb(s)			3	3
	limb(s), growing in			1	
	multiple areas	1		2	
	Chromodacryorrhea: eye, left			1	
	eye, right	1		1	
	Ear: red, bilateral		2		2
	Rust colored fur: multiple areas				1
	shoulder(s)		1	1	1
9	Alopecia: abdomen	1			
	limb(s)			3	3
	multiple areas	1		2	
	Chromodacryorrhea: eye, left			1	
	eye, right, gone			1	
	eye, right, improving	1			
	Ear: red, bilateral		2		2
	Rust colored fur: multiple areas				1
	shoulder(s)		1	1	
	shoulder(s), improving				1
10	Alopecia: abdomen	1			
	limb(s)			4	3
	multiple areas	1		2	
	Chromodacryorrhea: eye, left			1	
	eye, right	1		1	
	Ear: red, bilateral		2		2
	Rust colored fur: multiple areas				1
	shoulder(s)		1	1	1
11	Alopecia: abdomen	1			
	limb(s)			4	3
	multiple areas	1		2	
	Chromodacryorrhea: eye, left			1	
	eye, right	1		1	
	Ear: red, bilateral		2		2
	Rust colored fur: multiple areas				1
	shoulder(s)		1	1	1

(continued)

Table 46. Summary of the F_1 Female Clinical Observations During Lactation (page 4 of 5)

Day[a]	Observation[b]	Butyl Benzyl Phthalate (ppm in the feed) 0	750	3750	11250
12	Alopecia: abdomen	1			
	limb(s)	1		4	3
	multiple areas	1		2	
	Chromodacryorrhea: eye, left			1	
	eye, right	1		1	
	Ear: red, bilateral		2		2
	Rust colored fur: multiple areas				1
	shoulder(s)		1	1	
	shoulder(s), improving				1
13	Alopecia: abdomen	1			
	limb(s)			4	3
	multiple areas	2		2	
	Chromodacryorrhea: eye, left			1	
	eye, right	1		1	
	Ear: red, bilateral		2		2
	Rust colored fur: multiple areas				1
	shoulder(s)		1	1	1
14	Alopecia: abdomen	1			
	limb(s)	1		4	3
	multiple areas	2		2	
	Chromodacryorrhea: eye, left			1	
	eye, right	1			
	eye, right, gone			1	
	Ear: red, bilateral		2		2
	Piloerecton			1	
	Rust colored fur: multiple areas				1
	shoulder(s)		1	1	2
15	Alopecia: abdomen	1			
	limb(s)	1		3	3
	limb(s), growing in			1	
	multiple areas	2		2	
	Chromodacryorrhea: eye, left			1	
	eye, right	1		1	
	Ear: red, bilateral		2		2
	Rust colored fur: multiple areas				1
	shoulder(s)		1		2
	shoulder(s), improving			1	
	Teeth: trimmed, lower incisors			1	
16	Alopecia: abdomen	1			
	limb(s)	1		4	3
	multiple areas	2		2	
	Chromodacryorrhea: eye, left			1	
	eye, right			1	
	eye, right, improving	1			
	Ear: red, bilateral		2	1	2
	Rust colored fur: multiple areas				1
	shoulder(s)		1	1	1
	shoulder(s), improving				1

(continued)

Table 46. Summary of the F_1 Female Clinical Observations During Lactation (page 5 of 5)

Day[a]	Observation[b]	Butyl Benzyl Phthalate (ppm in the feed) 0	750	3750	11250
17	Alopecia: abdomen	1			
	limb(s)	1		4	3
	multiple areas	2		2	
	Chromodacryorrhea: eye, left			1	
	eye, right				1
	eye, right, gone			1	
	eye, right, improved	1			
	Ear: red, bilateral		2	1	2
	Rust colored fur: multiple areas				1
	shoulder(s)		1	1	1
18	Alopecia: limb(s)	1		4	3
	multiple areas	3		2	
	Chromodacryorrhea: eye, left			1	
	eye, right	1			1
	Ear: red, bilateral		2	1	2
	Rust colored fur: multiple areas				1
	shoulder(s)		1	1	1
19	Alopecia: limb(s)	1		4	3
	multiple areas	3		2	
	Chromodacryorrhea: eye, left		1	1	
	eye, right				1
	eye, right, improving	1			
	Ear: red, bilateral		2	1	2
	Rust colored fur: multiple areas		1		1
	shoulder(s)		1	1	1
20	Alopecia: limb(s)	1		4	3
	multiple areas	3		2	
	Chromodacryorrhea: eye, left		1	1	
	eye, right				1
	eye, right, gone	1			
	Ear: red, bilateral		2	1	2
	Rust colored fur: multiple areas		1		1
	shoulder(s)		1	1	
	shoulder(s), improving				1
21	Alopecia: limb(s)	1		4	3
	multiple areas	3		2	
	Chromodacryorrhea: eye, left		1	1	
	eye, right	1			
	eye, right, improved				1
	Ear: red, bilateral		2	1	2
	Rust colored fur: multiple areas		1		1
	shoulder(s)		1	1	

[a]Lactational day.
[b]Clinical observations are tabulated once per day per animal.

Table 47. Summary and Statistical Analysis of F_1 Reproductive and Lactational Indexes for the F_2 Litters (page 1 of 3)

	Butyl Benzyl Phthalate (ppm in the feed)			
	0	750	3750	11250
No. Animals on Study				
Males	30	30	30	30
Females	30	30	30	30
No. Females Paired				
	30	30	30	30
No. Females that Mated				
	29	29	28	21
Mating Index (no. females that mated/no. females paired)				
	96.7 ££ ΨΨ	96.7	93.3	70.0 Φ
No. of Pregnant Females				
	29	28	26	17
Fertility Index (no. pregnant females/no. females that mated)				
	100.0 £ ΨΨ	96.6	92.9	81.0 Φ
No. of Females with Live Litters (pnd 0)				
	29	28	26	17
Gestational Index (no. females with live litters/no. females pregnant)				
	100.0	100.0	100.0	100.0
No. Males Paired				
	30	30	29[a]	29[b]
No. Males that Mated				
	29	29	27	20
Mating Index (no. males that mated/no. males paired)				
	96.7 ££ ΨΨΨ	96.7	93.1	69.0 ΦΦ
No. Males Siring Litters				
	29	28	25	16
Fertility Index (no. males siring litters/no. males that mated)				
	100.0 £ ΨΨ	96.6	92.6	80.0 Φ
Pregnancy Index (no. pregnant females/no. males that mated)				
	100.0	96.6	96.3	85.0

(continued)

Table 47. Summary and Statistical Analysis of F_1 Reproductive and Lactational Indexes for the F_2 Litters (page 2 of 3)

	Butyl Benzyl Phthalate (ppm in the feed)			
	0	750	3750	11250
Precoital Interval (days)[c,d]				
	2.8 ± 0.2 N=28	2.7 ± 0.4 N=29	2.7 ± 0.3 N=26	2.9 ± 0.6 N=20
Gestational Length (days)[c,e]				
	22.0 ± 0.1 N=28	22.1 ± 0.1 N=28	22.0 ± 0.0 N=24	22.0 ± 0.1 N=16
No. of Live Litters:				
Postnatal Day 0	29	28	26	17
Postnatal Day 4	29	28	26	17
Postnatal Day 7	29	28	26	17
Postnatal Day 14	29	28	26	17
Postnatal Day 21	29	28	26	17
No. Implantation Sites per Litter[c]				
	15.86 ‡‡‡ ± 0.34 §§§ N=29	15.00 ± 0.56 N=28	15.23 ± 0.60 N=26	12.35 *** ± 0.83 N=17
Percent Postimplantation Loss per Litter[c]				
	10.02 ± 1.21 N=29	8.75 ± 1.90 N=28	6.67 ± 1.10 N=26	7.06 ± 2.36 N=17
Number of Live Pups on Postnatal Day 0[c]				
	14.2 ‡‡ ± 0.3 §§§ N=29	14.0 ± 0.5 N=28	14.2 ± 0.6 N=26	11.4 *** ± 0.7 N=17
Number of Dead Pups on Postnatal Day 0[c]				
	0.7 ± 0.2 N=29	0.5 ± 0.2 N=28	0.2 ± 0.1 N=26	0.2 ± 0.2 N=17
Total Number of Pups on Postnatal Day 0[c]				
	14.9 ‡‡ ± 0.4 §§§ N=29	14.5 ± 0.5 N=28	14.5 ± 0.6 N=26	11.6 *** ± 0.8 N=17
Stillbirth Index (no. dead on pnd 0/total no. on pnd 0)[c]				
	4.0 ± 1.1 N=29	3.4 ± 1.2 N=28	1.5 ± 0.5 N=26	1.5 ± 1.2 N=17

(continued)

Table 47. Summary and Statistical Analysis of F_1 Reproductive and Lactational Indexes for the F_2 Litters (page 3 of 3)

	Butyl Benzyl Phthalate (ppm in the feed)			
	0	750	3750	11250
Live Birth Index (no. live on pnd 0/total no. on pnd 0)[c]				
	96.0 ± 1.1 N=29	96.6 ± 1.2 N=28	98.5 ± 0.5 N=26	98.5 ± 1.2 N=17
4 Day Survival Index (no. surviving 4 days/no. live on pnd 0)[c]				
	98.3 ± 0.7 N=29	98.1 ± 0.9 N=28	96.9 ± 0.9 N=26	95.4 ± 2.0 N=17
7 Day Survival Index (no. surviving 7 days/no. live on pnd 4)[c]				
	99.3 ± 0.5 N=29	99.6 ± 0.4 N=28	100.0 ± 0.0 N=26	95.9 ± 4.1 N=17
14 Day Survival Index (no. surviving 14 days/no. live on pnd 7)[c]				
	99.7 ± 0.3 N=29	99.3 ± 0.5 N=28	100.0 ± 0.0 N=26	96.9 ± 2.2 N=17
21 Day Survival Index (no. surviving 21 days/no. live on pnd 14)[c]				
	100.0 ± 0.0 N=29	99.2 ± 0.5 N=28	100.0 ± 0.0 N=26	100.0 ± 0.0 N=17
Lactational Index (no. surviving 21 days/no. live on pnd 4)[c]				
	99.0 ± 0.6 N=29	98.2 ± 0.9 N=28	100.0 ± 0.0 N=26	94.1 ± 4.8 N=17

[a]Male 1157 was euthanized moribund study day 21.
[b]Male 1115 was euthanized moribund study day 75 after 4 days of pairing and had not mated with his female (female 1206). This male was not included as a male that was paired since he was not paired until he mated or until the 2 week mating period was over.
[c]Reported as the mean ± S.E.M.; pnd=postnatal day. All indexes are the average percent per litter.
[d]Precoital interval could only be calculated for those females for which sperm were detected in the vaginal smear.
[e]Gestational length could not be calculated for females that were pregnant, but for which sperm were never detected in the vaginal smear.
£p<0.05; Chi-Square Test.
££p<0.01; Chi-Square Test.
ΨΨp<0.01; Cochran-Armitage Test.
ΨΨΨp<0.001; Cochran-Armitage Test.
Φp<0.05; Fishers' Exact Test.
ΦΦp<0.01; Fishers' Exact Test.
‡‡p<0.01; ANOVA Test.
‡‡‡p<0.001; ANOVA Test.
§§§p<0.001; Test for Linear Trend.
***p<0.001; Dunnett's Test.

Table 48. Summary and Statistical Analysis of the F_2 Litter Size, Pup Body Weights, Pup Anogenital Distance, Pup Nipple/Areolae Retention and Percent Male Pups during Lactation (page 1 of 5)

	Butyl Benzyl Phthalate (ppm in the feed)			
	0	750	3750	11250
No. of Live Litters:				
Postnatal Day 0	29	28	26	17
Postnatal Day 4	29	28	26	17
Postnatal Day 7	29	28	26	17
Postnatal Day 14	29	28	26	17
Postnatal Day 21	29	28	26	17
Average Number of Live Pups per Litter (pnd 0)[a]				
	14.2 ‡‡ ± 0.3 §§§ N=29	14.0 ± 0.5 N=28	14.2 ± 0.6 N=26	11.4 *** ± 0.7 N=17
Average Number of Live Pups per Litter (pnd 4)[a]				
	14.0 ‡‡‡ ± 0.3 §§§ N=29	13.7 ± 0.5 N=28	13.8 ± 0.6 N=26	10.9 *** ± 0.7 N=17
Average Number of Live Pups per Litter (pnd 7)[a]				
#	9.9 ± 0.1 Ÿ N=29	9.8 ± 0.1 N=28	9.8 ± 0.1 N=26	8.8 ± 0.6 N=17
Average Number of Live Pups per LItter (pnd 14)[a]				
#	9.9 ± 0.1 Ÿ N=29	9.7 ± 0.1 N=28	9.8 ± 0.1 N=26	8.6 ± 0.6 N=17
Average Number of Live Pups per Litter (pnd 21)[a]				
#	9.9 ± 0.1 Ÿ N=29	9.6 ± 0.1 N=28	9.8 ± 0.1 N=26	8.6 ± 0.6 N=17
Average **Male** Pup Anogenital Distance (mm) per Litter (pnd 0)[a]				
	2.05 ‡‡‡ ± 0.01 §§§ N=29	2.05 ± 0.02 N=28	1.99 * ± 0.01 N=26	1.77 *** ± 0.03 N=17
Average Adjusted **Male** Pup Anogenital Distance (mm) per Litter (pnd 0)[b]				
	2.04 ΩΩΩ ± 0.01 λλλ N=29	2.05 ± 0.01 N=28	1.99 φ ± 0.02 N=26	1.79 φφφ ± 0.02 N=17

(continued)

Table 48. Summary and Statistical Analysis of the F_2 Litter Size, Pup Body Weights, Pup Anogenital Distance, Pup Nipple/Areolae Retention and Percent Male Pups during Lactation (page 2 of 5)

	Butyl Benzyl Phthalate (ppm in the feed)			
	0	750	3750	11250
Average **Female** Pup Anogenital Distance (mm) per Litter (pnd 0)[a]				
	0.98 ± 0.01 N=29	0.97 ± 0.01 N=28	0.96 ± 0.01 N=26	0.99 ± 0.02 N=17
Average Adjusted **Female** Pup Anogenital Distance (mm) per Litter (pnd 0)[b]				
	0.98 ± 0.01 N=29	0.97 ± 0.01 N=28	0.96 ± 0.01 N=26	0.99 ± 0.01 N=17
Average Pup Body Weight (g) per Litter (pnd 0)[a]				
	6.41 ± 0.10 § N=29	6.52 ± 0.12 N=28	6.34 ± 0.08 N=26	6.14 ± 0.10 N=17
Average **Male** Body Weight (g) per Litter (pnd 0)[a]				
	6.63 ± 0.11 § N=29	6.67 ± 0.12 N=28	6.50 ± 0.10 N=26	6.29 ± 0.12 N=17
Average **Female** Body Weight (g) per Litter (pnd 0)[a]				
	6.21 ± 0.10 § N=29	6.34 ± 0.11 N=28	6.20 ± 0.08 N=26	5.93 ± 0.09 N=17
Average Pup Body Weight (g) per Litter (pnd 4)[a]				
	10.44 ± 0.26 § N=29	10.63 ± 0.37 N=28	10.76 ± 0.31 N=26	9.51 ± 0.34 N=17
Average **Male** Body Weight (g) per Litter (pnd 4)[a]				
	10.68 ± 0.27 § N=29	10.78 ± 0.39 N=28	10.93 ± 0.31 N=26	9.66 ± 0.35 N=17
Average **Female** Body Weight (g) per Litter (pnd 4)[a]				
	10.19 ‡ ± 0.27 § N=29	10.41 ± 0.34 N=28	10.60 ± 0.30 N=26	9.24 ± 0.35 N=17

(continued)

Table 48. Summary and Statistical Analysis of the F_2 Litter Size, Pup Body Weights, Pup Anogenital Distance, Pup Nipple/Areolae Retention and Percent Male Pups during Lactation (page 3 of 5)

	Butyl Benzyl Phthalate (ppm in the feed)			
	0	750	3750	11250
Average Pup Body Weight (g) per Litter (pnd 7)[a]				
	16.91 ‡‡ ± 0.42 §§§ N=29	17.01 ± 0.53 N=28	17.30 ± 0.39 N=26	14.52 ** ± 0.62 N=17
Average **Male** Body Weight (g) per Litter (pnd 7)[a]				
	17.37 ‡‡ ± 0.42 §§§ N=29	17.16 ± 0.56 N=28	17.63 ± 0.40 N=26	14.76 ** ± 0.64 N=17
Average **Female** Body Weight (g) per Litter (pnd 7)[a]				
	16.49 ‡‡‡ ± 0.43 §§§ N=29	16.79 ± 0.48 N=28	16.97 ± 0.39 N=26	14.10 ** ± 0.66 N=17
Average Pup Body Weight (g) per Litter (pnd 14)[a]				
	33.87 ‡‡‡ ± 0.64 §§§ N=29	34.04 ± 0.74 N=28	34.46 ± 0.61 N=26	29.53 *** ± 0.97 N=17
Average **Male** Body Weight (g) per Litter (pnd 14)[a]				
	34.64 ‡‡‡ ± 0.64 §§§ N=29	34.14 ± 0.82 N=28	34.90 ± 0.65 N=26	30.16 *** ± 0.90 N=17
Average **Female** Body Weight (g) per Litter (pnd 14)[a]				
	33.23 ‡‡‡ ± 0.66 §§§ N=29	33.85 ± 0.65 N=28	33.98 ± 0.58 N=26	28.59 *** ± 1.03 N=17
Average Pup Body Weight (g) per Litter (pnd 21)[a]				
	50.01 ‡‡‡ ± 0.79 §§§ N=29	51.48 ± 1.13 N=28	52.07 ± 0.93 N=26	44.63 ** ± 1.36 N=17
Average **Male** Body Weight (g) per Litter (pnd 21)[a]				
	51.62 ‡‡‡ ± 0.83 §§§ N=29	52.41 ± 1.27 N=28	53.05 ± 0.97 N=26	45.63 ** ± 1.38 N=17
Average **Female** Body Weight (g) per Litter (pnd 21)[a]				
	48.51 ‡‡‡ ± 0.80 §§§ N=29	50.40 ± 0.97 N=28	50.98 ± 0.91 N=26	43.11 ** ± 1.21 N=17

(continued)

Table 48. Summary and Statistical Analysis of the F_2 Litter Size, Pup Body Weights, Pup Anogenital Distance, Pup Nipple/Areolae Retention and Percent Male Pups during Lactation (page 4 of 5)

	Butyl Benzyl Phthalate (ppm in the feed)			
	0	750	3750	11250
Percent Male Pups per Litter (pnd 0)[a]				
	49.7 ± 2.6 N=29	52.9 ± 2.4 N=28	49.8 ± 2.2 N=26	53.9 ± 4.6 N=17
Percent Male Pups per Litter (pnd 4)[a]				
	49.7 ± 2.6 N=29	52.7 ± 2.4 N=28	49.7 ± 2.0 N=26	55.5 ± 4.4 N=17
Percent Male Pups per Litter (pnd 7)[a]				
#	49.1 ± 1.6 N=29	50.6 ± 1.4 N=28	50.7 ± 1.2 N=26	56.4 ± 3.9 N=17
Percent Male Pups per Litter (pnd 14)[a]				
#	49.3 ± 1.6 N=29	51.0 ± 1.4 N=28	50.7 ± 1.2 N=26	54.8 ± 3.9 N=17
Percent Male Pups per Litter (pnd 21)[a]				
#	49.3 ± 1.6 N=29	51.0 ± 1.4 N=28	50.7 ± 1.2 N=26	54.8 ± 3.9 N=17
Average Number of Nipples per Male Pup[c]				
	0.00 ΓΓ ± 0.00 β N=141	0.00 ± 0.00 N=138	0.00 ± 0.00 N=129	0.51 ◊ ± 0.24 N=79
Percent Male Pups with One or More Nipples[c]				
	0.00 β	0.00	0.00	16.46
Average Number of Areolae per Male Pup[c]				
	0.05 ΓΓΓ ± 0.03 βββ N=141	0.12 ± 0.04 N=138	0.19 ± 0.08 N=129	3.14 ◊◊◊ ± 0.50 N=79
Percent Male Pups with One or More Areolae[c]				
	2.13 ΓΓΓ βββ	5.07	5.43	72.15 ◊◊◊

(continued)

Table 48. Summary and Statistical Analysis of the F_2 Litter Size, Pup Body Weights, Pup Anogenital Distance, Pup Nipple/Areolae Retention and Percent Male Pups during Lactation (page 5 of 5)

[a]Reported as the mean ± S.E.M.; pnd=postnatal day.
[b]Reported as the adjusted mean ± S.E.M. (body weight as covariate); pnd=postnatal day.
[c]Reported as the adjusted mean ± S.E.M. or percentage (adjusted for intralitter correlations).
#Levene's test for homogeneity of variances was significant ($p<0.05$), therefore robust regression methods were used to test all treatment effects.
‡$p<0.05$; ANOVA Test.
‡‡$p<0.01$; ANOVA Test.
‡‡‡$p<0.001$; ANOVA Test.
§$p<0.05$; Test for Linear Trend.
§§§$p<0.001$; Test for Linear Trend.
*$p<0.05$; Dunnett's Test.
**$p<0.01$; Dunnett's Test.
***$p<0.001$; Dunnett's Test.
ΩΩΩ$p<0.001$; Analysis of Covariance with body weight on postnatal day 0 as covariate.
λλλ$p<0.001$; Linear Trend Analysis of Covariance with body weight on postnatal day 0 as covariate.
φ$p<0.05$; Dunnett's Test with body weight on postnatal day 0 as covariate.
φφφ$p<0.001$; Dunnett's Test with body weight on postnatal day 0 as covariate.
Ÿ$p<0.05$; Linear trend test in robust regression model.
ГГ$p<0.01$; Overall analysis of correlated data.
ГГГ$p<0.001$; Overall analysis of correlated data.
β$p<0.05$; Test for Liner Trend on correlated data.
βββ$p<0.001$; Test for Liner Trend on correlated data.
◊$p<0.05$; Pairwise comparison of correlated data.
◊◊◊$p<0.001$; Pairwise comparison of correlated data.

Table 49. Summary of the F_2 Pup Clinical Observations on Postnatal Days 0 Through 21 (page 1 of 2)

Day[a]	Sex[b]	Clinical Observation	Butyl Benzyl Phthalate (ppm in the feed)			
			0	750	3750	11250
0	F	Anogenital area: blood present	1			
		Bite mark: anogenital area		1		
		face			1	
		jaw, lower				1
		leg, right rear		1		
		Found dead	14	2	3	1
		Hematoma: face			1	
		foot, right rear		2		
		head, neck and shoulder(s)		1		
		shoulder(s), dorsal area			1	
		Pale				1
		Pale; Bleeding at umbilical area	1			
		Tail: bent			1	
	M	Bite mark: leg, right rear	1			
		Found dead	4	11	3	3
		Hematoma: anogenital area		1		
		face and neck	1			
		jaw, lower	1			
		nose		1		
		Jaw: skin bitten off, lower				1
		Nose and back: dark area present		1		
		Tail and rear end: purple			1	
		distal third purple			1	
		tip bitten off			1	
	U	Found dead, cannibalized	2	1		
1	F	Found dead			1	3
		No milk band		1		
	M	Cyanotic		1		
		Found dead	1		2	
2	F	Euthanized moribund			1	
		Found dead		1		2
	M	Found dead		1		2
3	F	Found dead		1		
		Very small; No milk band	1			
	M	Cyanotic				2

(continued)

Table 49. Summary of the F_2 Pup Clinical Observations on Postnatal Days 0 Through 21 (page 2 of 2)

Day[a]	Sex[b]	Clinical Observation	Butyl Benzyl Phthalate (ppm in the feed) 0	750	3750	11250
4	F	Bite mark: face	1			
		face, healed			1	
		head, left side	1			
		Cyanotic				2
		Euthanized moribund				2
		Hematoma: shoulder(s)				1
		Missing and presumed dead	5	1	3	1
		No milk band		2		
		Tail: distal half dark purplish black		1		
	M	Cyanotic				2
		Euthanized moribund				1
		Found dead				1
		Jaw: healing, lower				1
		Missing and presumed dead	2	4	5	
		No milk band		3		
5	M	Found dead				1
7	F	Missing and presumed dead	1	1		1
	M	Found dead	1			
		Missing and presumed dead				2
		No milk band				1
		White area at area where umbilical cord was		1		
12	F	Umbilical hernia				1
	M	Very small and thin; Bite mark: face		1		
13	F	Found dead		1		
14	F	Missing and presumed dead	1	1		
	M	Missing and presumed dead				3
16	F	Bite mark: face				1
	M	Found dead		1		
17	F	Found dead, cannibalized		1		
21	F	Face: alopecia				1
	M	Eye: microphthalmia, right	1			
		opaque and small, left			1	

[a]Postnatal day.
[b]F is female, M is male and U is could not be determined.

Table 50. Summary of the F_2 Pup Gross Necropsy Findings on Postnatal Days 0 Through 21 and Postnatal Day 4 Culled Pups Gross Necropsy Findings and Analysis (page 1 of 3)

PUP NECROPSY FINDINGS ON POSTNATAL DAYS 0 THROUGH 21:

Day[a]	Sex[b]	Necropsy Finding	Butyl Benzyl Phthalate (ppm in the feed) 0	750	3750	11250
0	F	Ductus closed; Air in lungs; No milk in stomach		1		
		Ductus open; Air in lungs; Milk in stomach			1	
		Ductus open; Air in lungs; No milk in stomach	1		1	
		Ductus open; No air in lungs			1	
		Ductus open; No air in lungs; Abdominal organs autolyzed	1			
		Ductus open; No air in lungs; Diaphragm: right side thin and missing on left side; Body: purple				1
		Ductus open; No air in lungs; No milk in stomach	6	1		
		Ductus open; No air in lungs; No milk in stomach; Distended ureter: left	1			
		Ductus open; No air in lungs; No milk in stomach; Hydroureter: bilateral	1			
		Ductus open; No air in lungs; No milk in stomach; Hydroureter: left	1			
		Ductus open; No air in lungs; No milk in stomach; Placenta still attached	2			
		Ductus open; Some air in lungs; No milk in stomach; Placenta still attached	1			
	M	Ductus closed; Air in lungs; No milk in stomach		1		
		Ductus open; Air in lungs; No milk in stomach		1		
		Ductus open; Air in lungs; No milk in stomach; Distended ureter: right	1			
		Ductus open; No air in lungs; Abdominal organs autolyzed		3	2	
		Ductus open; No air in lungs; No milk in stomach	3	6	1	3
	U	Cannibalized, unable to evaluate		1		
		Cannibalized, unable to evaluate; Head and lower limbs only present	1			
1	F	Air in lungs			1	
		No milk in stomach				3
	M	Air in lungs; No milk in stomach			1	
		Ductus open; Air in lungs	1			
		No milk in stomach			1	
2	F	Ductus open; Air in lungs; Abdominal organs autolyzed				1
		Ductus open; Air in lungs; No milk in stomach		1		1
		Euthanized moribund; Distended ureter: bilateral; Kidney: petechial hemorrhages, bilateral			1	
	M	Air in lungs				1
		Air in lungs; Abdominal organs autolyzed		1		
		Ductus open; Air in lungs				1

(continued)

Table 50. Summary of the F_2 Pup Gross Necropsy Findings on Postnatal Days 0 Through 21 and Postnatal Day 4 Culled Pups Gross Necropsy Findings and Analysis (page 2 of 3)

PUP NECROPSY FINDINGS ON POSTNATAL DAYS 0 THROUGH 21:

Day[a]	Sex[b]	Necropsy Finding	Butyl Benzyl Phthalate (ppm in the feed) 0	750	3750	11250
3	F	No milk in stomach		1		
4	F	Ductus open; Lungs: dark red; Milk in stomach				1
		Euthanized moribund; Lungs: dark red; Stomach: distended with air and no milk present				1
	M	Euthanized moribund; No milk in stomach				1
		No findings				1
5	M	No milk in stomach				1
7	M	No milk in stomach	1			
13	F	No milk in stomach; Liver: half-normal size		1		
16	M	Feet and ears yellowish; No milk in stomach; Liver: several yellow areas; Kidney: dark in color, bilateral		1		
17	F	Cannibalized, unable to evaluate		1		

PUP NECROPSY FINDINGS FOR POSTNATAL DAY 4 CULLED PUPS:

Day[a]	Sex[b]	Necropsy Finding	Butyl Benzyl Phthalate (ppm in the feed) 0	750	3750	11250
4	F	Distended ureter: bilateral	3	2	3	2
		Distended ureter: left	1	3	1	
		Distended ureter: right		1		3
		Hydroureter: bilateral	5	7	13	1
		Hydroureter: left	2		2	
		Kidney: hydronephrosis, left			1	
		Urinary bladder: 2mm diameter area of thinning wall	1			
	M	Distended ureter: bilateral	7	3	7	1
		Distended ureter: left	4	4	2	1
		Distended ureter: right	3			
		Distended ureter: right; Hydroureter: left				
		Hydroureter: bilateral			2	1
		Hydroureter: left	1	1		
		Hydroureter: left; Distended ureter: right; Urinary bladder: full			1	
		Hydroureter: left; Urinary bladder: full			1	
		Hypospadias				1

(continued)

Table 50. Summary of the F_2 Pup Gross Necropsy Findings on Postnatal Days 0 Through 21 and Postnatal Day 4 Culled Pups Gross Necropsy Findings and Analysis (page 3 of 3)

	Butyl Benzyl Phthalate (ppm in the feed)			
	0	750	3750	11250
No. **Male** Pups Examined	61	61	47	14
Number of PND 4 Culled **Male** Pups with One or More Urinary Tract Malformations[c]	1	1	4	1
Percent PND 4 Culled **Male** Pups with One or More Urinary Tract Malformations[c,d]	1.64 ± 1.64 N=61	1.64 ± 1.68 N=61	8.51 ± 5.78 N=47	7.14 ± 7.30 N=14
Number of PND 4 Culled **Male** Pups with One or More Reproductive Tract Malformations[e]	0	0	0	1
Percent PND 4 Culled **Male** Pups with One or More Reproductive Tract Malformations[d,e]	0.00 ± 0.00 N=61	0.00 ± 0.00 N=61	0.00 ± 0.00 N=47	7.14 ± 7.30 N=14
No. **Female** Pups Examined	55	47	56	15
Number of PND 4 Culled **Female** Pups with One or More Urinary Tract Malformations[c]	8	7	16	1
Percent PND 4 Culled **Female** Pups with One or More Urinary Tract Malformations[c,d]	14.55 ± 5.71 N=55	14.89 ± 6.80 N=47	28.57 ± 7.42 N=56	6.67 ± 6.33 N=15
Number of PND 4 Culled **Female** Pups with One or More Reproductive Tract Malformations[e]	0	0	0	0
Percent PND 4 Culled **Female** Pups with One or More Reproductive Tract Malformations[d,e]	0.00 ± 0.00 N=55	0.00 ± 0.00 N=47	0.00 ± 0.00 N=56	0.00 ± 0.00 N=15

[a]Postnatal day.
[b]F is female, M is male and U is unable to determine.
[c]Includes hydroureter and thinning wall of urinary bladder.
[d]Reported as the percentage ± the standard error of the percentage (adjusted for intralitter correlation).
[e]Includes hypospadias.

Table 51. Summary and Statistical Analysis of F_2 Pup Necropsy Weights and Selected Gross Necropsy Findings on Postnatal Day 21 (page 1 of 4)

	Butyl Benzyl Phthalate (ppm in the feed)			
	0	750	3750	11250
MALES	86	83	78	54[a]
Body Weight at Sacrifice (g)[b]	51.78 ΓΓΓ ± 0.90 βββ N=86	51.91 ± 1.30 N=83	52.65 ± 0.97 N=78	45.89 ♦♦♦ ± 1.20 N=54
Thymus Weight (g)[b]	0.2360 Γ ±0.0075 ββ N=86	0.2300 ±0.0082 N=83	0.2238 ±0.0080 N=78	0.2042 ♦♦ ±0.0082 N=49
Spleen Weight (g)[b]	0.2106 ΓΓΓ ±0.0067 βββ N=86	0.2197 ±0.0084 N=83	0.2139 ±0.0065 N=78	0.1549 ♦♦♦ ±0.0084 N=49
Brain Weight (g)[b]	1.4854 ±0.0115 N=86	1.4652 ±0.0141 N=83	1.4952 ±0.0118 N=77[c]	1.4616 ±0.0140 N=49
Paired Testes Weight (g)[b]	0.2432 ΓΓΓ ±0.0081 βββ N=86	0.2397 ±0.0077 N=82[d]	0.2405 ±0.0054 N=78	0.1949 ♦♦♦ ±0.0050 N=54
Paired Epididymides Weight (g)[b]	0.0570 ±0.0022 β N=86	0.0533 ±0.0022 N=82[e]	0.0531 ±0.0020 N=78	0.0503 ±0.0018 N=40[e,f]
Relative Thymus Weight (% of sacrifice weight)[b]	0.4541 ±0.0089 N=86	0.4426 ±0.0116 N=83	0.4249 ±0.0124 N=78	0.4440 ±0.0134 N=49
Relative Spleen Weight (% of sacrifice weight)[b]	0.4056 ΓΓΓ ±0.0087 βββ N=86	0.4228 ±0.0107 N=83	0.4056 ±0.0090 N=78	0.3335 ♦♦♦ ±0.0107 N=49

(continued)

Table 51. Summary and Statistical Analysis of F_2 Pup Necropsy Weights and Selected Gross Necropsy Findings on Postnatal Day 21 (page 2 of 4)

	Butyl Benzyl Phthalate (ppm in the feed)			
	0	750	3750	11250
Relative Brain Weight (% of sacrifice weight)[b]				
	2.8958 ΓΓΓ ±0.0400 βββ N=86	2.8764 ±0.0599 N=83	2.8668 ±0.0482 N=77[c]	3.2229 ♦♦♦ ±0.0748 N=49
Relative Paired Testes Weight (% of sacrifice weight)[b]				
	0.4675 ΓΓ ±0.0105 ββ N=86	0.4604 ±0.0078 N=82[d]	0.4579 ±0.0082 N=78	0.4266 ♦♦ ±0.0086 N=54
Relative Paired Epididymides Weight (% of sacrifice weight)[b]				
	0.1097 ±0.0034 N=86	0.1028 ±0.0034 N=82[e]	0.1016 ±0.0038 N=78	0.1082 ±0.0036 N=40[e,f]
No. **Male** Pups Examined	86	83	78	54
Number of PND 21 **Male** Pups with One or More Urinary Tract Malformations[g,h]				
	1	1	7	1
Percent PND 21 **Male** Pups with One or More Urinary Tract Malformations[g,h,i]				
	1.16 ± 1.15 N=86	1.20 ± 1.19 N=83	8.97 ± 3.89 N=78	1.85 ± 1.82 N=54
Number of PND 21 **Male** Pups with One or More Reproductive Tract Malformations[g,j]				
	0	1	0	13
Percent PND 21 **Male** Pups with One or More Reproductive Tract Malformations[g,i,j]				
	0.00 ΓΓ ± 0.00 ββ N=86	1.20 ± 1.19 N=83	0.00 ± 0.00 N=78	24.07 ♦♦ ± 8.04 N=54
FEMALES	87	83	77	43
Body Weight at Sacrifice (g)[b]				
	48.45 ΓΓΓ ± 0.87 βββ N=87	49.67 ± 0.87 N=83	49.96 ± 0.90 N=77	42.99 ♦♦♦ ± 1.08 N=43

(continued)

Table 51. Summary and Statistical Analysis of F_2 Pup Necropsy Weights and Selected Gross Necropsy Findings on Postnatal Day 21 (page 3 of 4)

	Butyl Benzyl Phthalate (ppm in the feed)			
	0	750	3750	11250
Thymus Weight (g)[b]				
	0.2394 Γ ±0.0076 βββ N=87	0.2323 ±0.0060 N=83	0.2261 ±0.0073 N=77	0.2036 ♦♦ ±0.0077 N=43
Spleen Weight (g)[b]				
	0.1966 ΓΓΓ ±0.0062 βββ N=87	0.2125 ±0.0056 N=83	0.2041 ±0.0054 N=77	0.1457 ♦♦♦ ±0.0067 N=43
Brain Weight (g)[b]				
	1.4304 ±0.0133 N=87	1.4305 ±0.0104 N=83	1.4493 ±0.0117 N=77	1.4093 ±0.0178 N=43
Paired Ovaries Weight (g)[b]				
	0.0324 ΓΓ ±0.0013 βββ N=87	0.0323 ±0.0012 N=83	0.0305 ±0.0010 N=77	0.0263 ♦♦ ±0.0012 N=43
Uterus Weight (g)[b]				
	0.0796 ΓΓ ±0.0037 N=87	0.0843 ±0.0047 N=82[c]	0.0929 ♦ ±0.0045 N=76[c]	0.0687 ±0.0048 N=42[c]
Relative Thymus Weight (% of sacrifice weight)[b]				
	0.4926 ±0.0103 N=87	0.4677 ±0.0091 N=83	0.4523 ±0.0110 N=77	0.4723 ±0.0115 N=43
Relative Spleen Weight (% of sacrifice weight)[b]				
	0.4042 ΓΓΓ ±0.0085 βββ N=87	0.4270 ♦ ±0.0075 N=83	0.4082 ±0.0075 N=77	0.3363 ♦♦♦ ±0.0095 N=43
Relative Brain Weight (% of sacrifice weight)[b]				
	2.9779 ΓΓΓ ±0.0442 βββ N=87	2.9086 ±0.0460 N=83	2.9252 ±0.0447 N=77	3.3172 ♦♦♦ ±0.0772 N=43
Relative Paired Ovaries Weight (% of sacrifice weight)[b]				
	0.0665 ±0.0022 N=87	0.0649 ±0.0019 N=83	0.0615 ±0.0024 N=77	0.0614 ±0.0027 N=43

(continued)

Table 51. Summary and Statistical Analysis of F_2 Pup Necropsy Weights and Selected Gross Necropsy Findings on Postnatal Day 21 (page 4 of 4)

	Butyl Benzyl Phthalate (ppm in the feed)			
	0	750	3750	11250
Relative Uterus Weight (% of sacrifice weight)[b]				
	0.1637 ±0.0066 N=87	0.1701 ±0.0093 N=82[c]	0.1861 ±0.0081 N=76[c]	0.1610 ±0.0104 N=42[c]
No. **Female** Pups Examined	87	83	77	43
Number of PND 21 **Female** Pups with One or More Urinary Tract Malformations[g,h]				
	1	1	1	0
Percent PND 21 **Female** Pups with One or More Urinary Tract Malformations[g,h,i]				
	1.15 ± 1.14 N=87	1.20 ± 1.19 N=83	1.30 ± 1.28 N=77	0.00 ± 0.00 N=43
Number of PND 21 **Female** Pups with One or More Reproductive Tract Malformations[g]				
	0	0	0	0
Percent PND 21 **Female** Pups with One or More Reproductive Tract Malformations[g,i]				
	0.00 ± 0.00 N=87	0.00 ± 0.00 N=83	0.00 ± 0.00 N=77	0.00 ± 0.00 N=43

[a]Five additional male pups had body weight, paired testes weight and paired epididymides weight recorded and included because they had nipples.
[b]Reported as the mean ± S.E.M. (adjusted for intralitter correlation).
[c]Decrease in N is due to one weight being a statistical outlier and therefore it was removed.
[d]Decrease in N is due to one male having only one testis.
[e]Decrease in N is due to one or more males having one or both epididymides missing or having parts of one or both epididymides missing.
[f]Decrease in N is due to the epididymides from one male being lost during necropsy prior to weighing.
[g]See Table A-61 for a list of the gross necropsy findings on postnatal day 21.
[h]Includes hydronephrosis.
[i]Reported as the percentage ± the standard error of the percentage (adjusted for intralitter correlation).
[j]Includes missing epididymis (whole or part), missing testis, missing seminal vesicle and seminal vesicle reduced in size.
Γ $p<0.05$; Overall analysis of correlated data.
ΓΓ $p<0.01$; Overall analysis of correlated data.
ΓΓΓ $p<0.001$; Overall analysis of correlated data.
β $p<0.05$; Test for Linear Trend on correlated data.
ββ $p<0.01$; Test for Linear Trend on correlated data.
βββ $p<0.001$; Test for Linear Trend on correlated data.
♦ $p<0.05$; Pairwise comparison of correlated data.
♦♦ $p<0.01$; Pairwise comparison of correlated data.
♦♦♦ $p<0.001$; Pairwise comparison of correlated data.

Table 52. Summary of the F_2 Pup Necropsy Findings on Postnatal Day 21 (page 1 of 1)

Sex[a]	Necropsy Finding	Butyl Benzyl Phthalate (ppm in the feed)			
		0	750	3750	11250
F	Kidney: hydronephrosis, bilateral		1		
	hydronephrosis, left			1	
	hydronephrosis, right	1			
	Tail: kinked			1	
	Thymus: multiple red foci			1	
	Umbilical hernia only involving fat				1
M	Epididymis: caput and corpus missing, bilateral				2
	caput and corpus missing, left				1
	caput and corpus missing, right				4
	caput missing, left				2
	missing, bilateral				4
	missing, left				4
	missing, right		1		3
	Eye: eyelid not completely open, left			1	
	reduced in size and blood present in eyeball, right	1			
	Kidney: hydronephrosis, right	1	1	7	1
	Nipples: 1				4
	2				3
	4				2
	6				4
	Seminal Vesicles: missing, bilateral				2
	missing, left				1
	reduced in size				2
	Skull: 8 mm by 1 mm deep depression on right side, brain underneath flat in appearance	1			
	Tail: red				1
	tip red				1
	Testis: missing, right		1		

[a]F is female and M is male.

Table 53. Summary and Statistical Analysis of the F_1 Male Body Weights and Weight Changes During the Post Mating Holding Period (page 1 of 1)

	Butyl Benzyl Phthalate (ppm in the feed)			
	0	750	3750	11250
No. of Males on Study	30	30	30	30
Body Weight (sd 84) (g)[a]	552.4 ‡‡‡ ± 7.4 §§§ N=30	558.2 ± 8.5 N=29[b]	575.1 ± 8.0 N=28[c]	499.4 *** ± 10.2 N=28[d]
Body Weight (sd 91) (g)[a]	568.4 ‡‡‡ ± 7.6 §§§ N=30	576.3 ± 9.2 N=29	592.5 ± 8.3 N=28	513.8 *** ± 10.3 N=28
Body Weight (sd 98) (g)[a]	585.9 ‡‡‡ ± 8.1 §§§ N=30	591.5 ± 9.4 N=29	612.0 ± 8.5 N=28	533.4 *** ± 10.8 N=28
Body Weight (sd 105) (g)[a]	597.6 ‡‡‡ ± 8.5 §§§ N=30	602.8 ± 9.7 N=29	620.3 ± 8.9 N=28	543.6 *** ± 11.0 N=28
Body Weight Change (sd 84 to 91) (g)[a]	16.0 ± 1.1 N=30	18.1 ± 1.0 N=29[b]	17.4 ± 1.3 N=28[c]	14.5 ± 1.0 N=28[d]
Body Weight Change (sd 91 to 98) (g)[a]	17.6 ‡ ± 1.5 § N=30	15.2 ± 0.8 N=29	19.5 ± 1.0 N=28	19.6 ± 1.2 N=28
Body Weight Change (sd 98 to 105) (g)[a]	11.6 ± 1.1 N=30	11.3 ± 0.9 N=29	8.3 ± 1.4 N=28	10.2 ± 1.1 N=28

[a]Reported as the mean ± S.E.M.; sd=study day with study day 0 being the first day of the prebreed period.
[b]Decrease in N is due to male 1163 being found dead on study day 78.
[c]Decrease in N is due to male 1033 being found dead on study day 83 and male 1157 being euthanized moribund on study day 21.
[d]Decrease in N is due to males 1085 and 1115 being euthanized moribund on study day 75.
‡p<0.05; ANOVA Test.
‡‡‡p<0.001; ANOVA Test.
§p<0.05; Test for Linear Trend.
§§§p<0.001; Test for Linear Trend.
***p<0.001; Dunnett's Test.

Table 54. Summary and Statistical Analysis of the F_1 Male Feed Consumption, Percent Food Efficiency and Butyl Benzyl Phthalate Intake During the Post Mating Holding Period (page 1 of 2)

	Butyl Benzyl Phthalate (ppm in the feed)			
	0	750	3750	11250
No. of Males on Study	30	30	30	30
Feed Consumption (sd 84 to 91) (g/day)[a]				
	28.7 ‡ ± 0.5 § N=30	29.7 ± 0.5 N=29[b]	29.8 ± 0.4 N=28[c]	27.8 ± 0.7 N=28[d]
Feed Consumption (sd 91 to 98) (g/day)[a]				
	28.2 ‡‡ ± 0.5 N=30	28.7 ± 0.5 N=29	30.0 ± 0.4 N=28	27.1 ± 0.7 N=28
Feed Consumption (sd 98 to 105) (g/day)[a]				
	28.0 ± 0.5 N=30	28.4 ± 0.4 N=29	29.0 ± 0.4 N=28	27.3 ± 0.6 N=28
Feed Consumption (sd 84 to 91) (g/kg/day)[a]				
	51.1 ‡‡‡ ± 0.6 §§§ N=30	52.5 ± 0.7 N=29[b]	51.1 ± 0.6 N=28[c]	55.0 *** ± 0.9 N=28[d]
Feed Consumption (sd 91 to 98) (g/kg/day)[a]				
	48.9 ‡ ± 0.7 §§ N=30	49.3 ± 0.7 N=29	49.9 ± 0.5 N=28	51.8 * ± 0.9 N=28
Feed Consumption (sd 98 to 105) (g/kg/day)[a]				
	47.4 ‡‡‡ ± 0.5 §§§ N=30	47.8 ± 0.6 N=29	47.2 ± 0.5 N=28	50.7 *** ± 0.8 N=28
Percent Food Efficiency (sd 84 to 91)[a]				
	8.0 ± 0.5 N=30	8.6 ± 0.4 N=29[b]	8.3 ± 0.6 N=28[c]	7.6 ± 0.5 N=28[d]
Percent Food Efficiency (sd 91 to 98)[a]				
	8.7 ‡‡ ± 0.7 §§ N=30	7.6 ± 0.4 N=29	9.3 ± 0.5 N=28	10.3 ± 0.5 N=28

(continued)

Table 54. Summary and Statistical Analysis of the F_1 Male Feed Consumption, Percent Food Efficiency and Butyl Benzyl Phthalate Intake During the Post Mating Holding Period (page 2 of 2)

	Butyl Benzyl Phthalate (ppm in the feed)			
	0	750	3750	11250
Percent Food Efficiency (sd 98 to 105)[a]				
	5.9 ± 0.5 N=30	5.6 ± 0.5 N=29	4.0 ± 0.7 N=28	5.3 ± 0.5 N=28
Butyl Benzyl Phthalate Intake (sd 84 to 91) (mg/kg/day)[a]				
		39.40 ± 0.53 N=29[b]	191.66 ± 2.12 N=28[c]	618.45 ± 10.06 N=28[d]
Butyl Benzyl Phthalate Intake (sd 91 to 98) (mg/kg/day)[a]				
		36.95 ± 0.56 N=29	187.17 ± 1.76 N=28	582.64 ± 10.47 N=28
Butyl Benzyl Phthalate Intake (sd 98 to 105) (mg/kg/day)[a]				
		35.83 ± 0.46 N=29	176.93 ± 1.73 N=28	570.38 ± 9.48 N=28

[a]Reported as the mean ± S.E.M.; sd=study day with study day 0 being the first day of the prebreed period.
[b]Decrease in N is due to male 1163 being found dead on study day 78.
[c]Decrease in N is due to male 1033 being found dead on study day 83 and male 1157 being euthanized moribund on study day 21.
[d]Decrease in N is due to males 1085 and 1115 being euthanized moribund on study day 75.
‡p<0.05; ANOVA Test.
‡‡p<0.01; ANOVA Test.
‡‡‡p<0.001; ANOVA Test.
§p<0.05; Test for Linear Trend.
§§p<0.01; Test for Linear Trend.
§§§p<0.001; Test for Linear Trend.
*p<0.05; Dunnett's Test.
***p<0.001; Dunnett's Test.

Table 55. Summary of the F_1 Male Clinical Observations During the Post Mating Holding Period
(page 1 of 4)

A. Clinical Observations Summarized by Group

Observation	Butyl Benzyl Phthalate (ppm in the feed)			
	0	750	3750	11250
Alopecia	1	3	1	1
Chromodacryorrhea		2	2	
Ear(s): red			1	
Rust colored fur	1	1	1	

B. Clinical Observations Summarized by Group and Day

Day[a]	Observation[b]	Butyl Benzyl Phthalate (ppm in the feed)			
		0	750	3750	11250
85	Alopecia: limb(s)	1	2	1	
	multiple areas		1		
	Chromodacryorrhea: nose		1	1	
	Ear: red, bilateral			1	
	Rust colored fur: multiple areas	1			
86	Alopecia: limb(s)	1	2	1	
	multiple areas		1		
	Chromodacryorrhea: eye, left		1		
	nose, gone		1	1	
	Ear: red, bilateral			1	
	Rust colored fur: multiple areas	1			
87	Alopecia: limb(s)	1	2	1	
	multiple areas		1		
	Chromodacryorrhea: eye, left		1		
	Ear: red, bilateral			1	
	Rust colored fur: multiple areas	1			
88	Alopecia: limb(s)	1	2	1	
	multiple areas		1		
	Chromodacryorrhea: eye, left, gone		1		
	Ear: red, bilateral			1	
	Rust colored fur: multiple areas	1			
89	Alopecia: limb(s)	1	2	1	1
	multiple areas		1		
	Chromodacryorrhea: eye, left		1		
	Ear: red, bilateral			1	
	Rust colored fur: multiple areas	1			
90	Alopecia: limb(s)	1	2	1	1
	multiple areas		1		
	Ear: red, bilateral			1	
	Rust colored fur: multiple areas	1			
91	Alopecia: limb(s)	1	2	1	1
	multiple areas		1		
	Ear: red, bilateral			1	
	Rust colored fur: multiple areas	1			

(continued)

Table 55. Summary of the F_1 Male Clinical Observations During the Post Mating Holding Period
(page 2 of 4)

Day[a]	Observation[b]	Butyl Benzyl Phthalate (ppm in the feed)			
		0	750	3750	11250
92	Alopecia: limb(s)	1	2	1	1
	multiple areas		1		
	Ear: red, bilateral			1	
	Rust colored fur: multiple areas	1			
93	Alopecia: limb(s)	1	2	1	1
	multiple areas		1		
	Ear: red, bilateral			1	
	Rust colored fur: multiple areas	1			
94	Alopecia: limb(s)	1	2	1	1
	multiple areas		1		
	Chromodacryorrhea: eye, left		1		
	Ear: red, bilateral			1	
	Rust colored fur: multiple areas	1			
95	Alopecia: limb(s)	1	2	1	1
	multiple areas		1		
	Chromodacryorrhea: eye, left		1		
	Ear: red, bilateral			1	
	Rust colored fur: multiple areas	1			
96	Alopecia: limb(s)	1	2	1	1
	multiple areas		1		
	Ear: red, bilateral			1	
97	Alopecia: limb(s)	1	1	1	1
	limb(s), growing in		1		
	multiple areas		1		
	Ear: red, bilateral			1	
	Rust colored fur: multiple areas	1			
98	Alopecia: limb(s)	1	1	1	1
	limb(s), grown in		1		
	multiple areas		1		
	Chromodacryorrhea: eye, left		1		
	Ear: red, bilateral			1	
	Rust colored fur: multiple areas	1			
99	Alopecia: limb(s)	1	1	1	1
	multiple areas		1		
	Ear: red, bilateral			1	
	Rust colored fur: multiple areas	1			
100	Alopecia: limb(s)	1		1	1
	limb(s), growing in		1		
	multiple areas		1		
	Ear: red, bilateral			1	
	Rust colored fur: multiple areas	1			

(continued)

Table 55. Summary of the F_1 Male Clinical Observations During the Post Mating Holding Period
(page 3 of 4)

Day[a]	Observation[b]	Butyl Benzyl Phthalate (ppm in the feed)			
		0	750	3750	11250
101	Alopecia: limb(s)	1		1	1
	limb(s), growing in		1		
	multiple areas		1		
	Chromodacryorrhea: eye, left		1		
	Ear: red, bilateral			1	
	Rust colored fur: multiple areas	1			
102	Alopecia: limb(s)	1		1	1
	limb(s), growing in		1		
	multiple areas		1		
	Chromodacryorrhea: eye, left, gone		1		
	Ear: red, bilateral			1	
	Rust colored fur: multiple areas	1			
103	Alopecia: limb(s)	1	1	1	1
	multiple areas		1		
	Ear: red, bilateral			1	
	Rust colored fur: multiple areas	1			
104	Alopecia: limb(s)	1	1	1	1
	multiple areas		1		
	Ear: red, bilateral			1	
	Rust colored fur: multiple areas	1			
105	Alopecia: limb(s)		1	1	1
	limb(s), growing in	1			
	multiple areas		1		
	Ear: red, bilateral			1	
	Rust colored fur: multiple areas	1			
	side(s)			1	
106	Alopecia: limb(s)		1	1	1
	multiple areas		1		
	Chromodacryorrhea: eye, left		1		
	Ear: red, bilateral			1	
	Rust colored fur: multiple areas	1			
	shoulder(s)		1		
	side(s)			1	
107	Alopecia: limb(s)		1	1	1
	multiple areas		1		
	Chromodacryorrhea: eye, left		1		
	Ear: red, bilateral			1	
	Rust colored fur: multiple areas	1			
	shoulder(s)		1		
	side(s)			1	

(continued)

Table 55. Summary of the F_1 Male Clinical Observations During the Post Mating Holding Period
(page 4 of 4)

Day[a]	Observation[b]	Butyl Benzyl Phthalate (ppm in the feed)			
		0	750	3750	11250
108	Alopecia: limb(s)		1	1	1
	multiple areas		1		
	Chromodacryorrhea: eye, left		1		
	Ear: red, bilateral			1	
	Rust colored fur: multiple areas	1			
	shoulder(s)		1		
	side(s)			1	
109	Alopecia: limb(s)		1	1	1
	multiple areas		1		
	Chromodacryorrhea: eye, left		1		
	Ear: red, bilateral			1	
	Rust colored fur: multiple areas	1			
	shoulder(s)		1		
	side(s)			1	
110	Alopecia: limb(s)		1	1	1
	multiple areas		1		
	Chromodacryorrhea: eye, left		1		
	eye, right			1	
	Ear: red, bilateral			1	
	Rust colored fur: multiple areas	1			
	shoulder(s)		1		
	side(s)			1	
111	Alopecia: limb(s)		1		
	Chromodacryorrhea: eye, left		1		
	eye, right			1	
	Ear: red, bilateral			1	
	Rust colored fur: multiple areas	1			
	shoulder(s)		1		
112	Alopecia: limb(s)		1		
	Chromodacryorrhea: eye, right			1	
	Ear: red, bilateral			1	
	Rust colored fur: multiple areas	1			
	shoulder(s)		1		
113	Alopecia: limb(s)		1		
	Ear: red, bilateral			1	
	Rust colored fur: shoulder(s)		1		

[a]Study day.
[b]Clinical observations are tabulated once per day per animal.

Table 56. Summary of the F_1 Female Body Weights and Weight Changes During the Post Mating Holding Period (page 1 of 2)

	Butyl Benzyl Phthalate (ppm in the feed)			
	0	750	3750	11250
No. of Females on Study	30	30	30	30
Body Weight (sd 84) (g)[a]				
	361.2 ± 4.0 N=2	438.6 ± . N=1	350.7 ± 15.5 N=4	307.6 ± 5.1 N=10
Body Weight (sd 91) (g)[a]				
	400.6 ± 35.4 N=2	435.1 ± . N=1	371.8 ± 22.9 N=4	317.4 ± 11.1 N=10
Body Weight (sd 98) (g)[a]				
	359.1 ± . N=1	373.7 ± 70.5 N=2	355.1 ± 14.2 N=4	302.2 ± 5.5 N=13
Body Weight (sd 105) (g)[a]				
	389.6 ± . N=1	385.3 ± 66.2 N=2	365.1 ± 13.7 N=4	310.0 ± 5.3 N=13
Body Weight (sd 112) (g)[a]				
	385.6 ± . N=1	391.1 ± 68.9 N=2	366.7 ± 16.4 N=4	310.9 ± 5.2 N=13
Body Weight Change (sd 84 to 91) (g)[a]				
	39.4 ± 31.4 N=2	-3.5 ± . N=1	21.2 ± 22.2 N=4	9.8 ± 9.8 N=10
Body Weight Change (sd 91 to 98) (g)[a]				
	-6.0 ± . N=1	9.1 ± . N=1	3.5 ± 4.6 N=2	-1.1 ± 3.1 N=9
Body Weight Change (sd 98 to 105) (g)[a]				
	30.5 ± . N=1	11.5 ± 4.2 N=2	9.9 ± 1.8 N=4	7.8 ± 1.8 N=13

(continued)

Table 56. Summary of the F_1 Female Body Weights and Weight Changes During the Post Mating Holding Period (page 2 of 2)

	Butyl Benzyl Phthalate (ppm in the feed)			
	0	750	3750	11250
Body Weight Change (sd 105 to 112) (g)[a]				
	-4.0 ± . N=1	5.9 ± 2.6 N=2	1.6 ± 4.9 N=4	1.0 ± 1.6 N=13

[a]Reported as the mean ± S.E.M.; sd=study day with study day 0 being the first day of the prebreed period. Includes all females that were not found sperm and/or plug positive or females that did not have live litters or females whose entire litter died prior to postnatal day 21. Statistical analyses were not performed on these endpoints since not all females were represented.

Table 57. Summary of the F_1 Female Feed Consumption, Percent Food Efficiency and Butyl Benzyl Phthalate Intake During the Post Mating Holding Period (page 1 of 2)

	Butyl Benzyl Phthalate (ppm in the feed)			
	0	750	3750	11250
No. of Females on Study	30	30	30	30
Feed Consumption (sd 84 to 91) (g/day)[a]	24.1 ± 2.6 N=2	25.7 ± . N=1	20.7 ± 1.0 N=4	23.1 ± 1.3 N=10
Feed Consumption (sd 91 to 98) (g/day)[a]	21.0 ± . N=1	24.0 ± . N=1	19.9 ± 1.0 N=2	19.9 ± 0.9 N=8[b]
Feed Consumption (sd 98 to 105) (g/day)[a]	21.6 ± . N=1	23.1 ± 1.9 N=2	21.9 ± 0.5 N=4	21.9 ± 1.2 N=13
Feed Consumption (sd 105 to 112) (g/day)[a]	22.2 ± . N=1	23.4 ± 3.2 N=2	21.6 ± 0.9 N=4	20.4 ± 1.0 N=13
Feed Consumption (sd 84 to 91) (g/kg/day)[a]	63.0 ± 3.7 N=2	58.8 ± . N=1	57.2 ± 0.7 N=4	74.1 ± 4.4 N=10
Feed Consumption (sd 91 to 98) (g/kg/day)[a]	57.9 ± . N=1	54.5 ± . N=1	58.3 ± 2.9 N=2	65.1 ± 2.4 N=8[b]
Feed Consumption (sd 98 to 105) (g/kg/day)[a]	57.8 ± . N=1	61.9 ± 6.2 N=2	60.9 ± 1.2 N=4	71.5 ± 3.6 N=13
Feed Consumption (sd 105 to 112) (g/kg/day)[a]	57.2 ± . N=1	60.7 ± 2.2 N=2	59.1 ± 1.5 N=4	65.7 ± 2.6 N=13

(continued)

Table 57. Summary of the F_1 Female Feed Consumption, Percent Food Efficiency and Butyl Benzyl Phthalate Intake During the Post Mating Holding Period (page 2 of 2)

	Butyl Benzyl Phthalate (ppm in the feed)			
	0	750	3750	11250
Percent Food Efficiency (sd 84 to 91)[a]				
	21.6 ± 16.3 N=2	-1.9 ± . N=1	13.3 ± 15.1 N=4	5.3 ± 5.5 N=10
Percent Food Efficiency (sd 91 to 98)[a]				
	-4.1 ± . N=1	5.4 ± . N=1	2.7 ± 3.4 N=2	-1.2 ± 2.4 N=8[b]
Percent Food Efficiency (sd 98 to 105)[a]				
	20.1 ± . N=1	7.4 ± 3.2 N=2	6.6 ± 1.3 N=4	4.7 ± 1.2 N=13
Percent Food Efficiency (sd 105 to 112)[a]				
	-2.6 ± . N=1	3.4 ± 1.1 N=2	0.7 ± 3.2 N=4	0.5 ± 1.1 N=13
Butyl Benzyl Phthalate Intake (sd 84 to 91) (mg/kg/day)[a]				
		44.10 ± . N=1	214.59 ± 2.61 N=4	833.16 ± 49.28 N=10
Butyl Benzyl Phthalate Intake (sd 91 to 98) (mg/kg/day)[a]				
		40.87 ± . N=1	218.53 ± 10.93 N=2	732.43 ± 26.60 N=8[b]
Butyl Benzyl Phthalate Intake (sd 98 to 105) (mg/kg/day)[a]				
		46.42 ± 4.65 N=2	228.37 ± 4.49 N=4	803.92 ± 41.04 N=13
Butyl Benzyl Phthalate Intake (sd 105 to 112) (mg/kg/day)[a]				
		45.56 ± 1.66 N=2	221.63 ± 5.79 N=4	738.80 ± 29.12 N=13

[a]Reported as the mean ± S.E.M.; sd=study day with study day 0 being the first day of the prebreed period. Includes all females that were not found sperm and/or plug positive or females that did not have live litters or females whose entire litter died prior to postnatal day 21. Statistical analyses were not performed on these endpoints since not all females were represented.

[b]Decrease in N is due to one female scratching the feed out of the feed jar and therefore an accurate feed weight could not be obtained.

Table 58. Summary of the F_1 Female Clinical Observations During the Post Mating Holding Period
(page 1 of 3)

A. Clinical Observations Summarized by Group

Observation	Butyl Benzyl Phthalate (ppm in the feed)			
	0	750	3750	11250
Alopecia	1			1
Chromodacryorrhea				1
Ear(s): red				3
Rust colored fur				2

B. Clinical Observations Summarized by Group and Day

Day[a]	Observation[b]	Butyl Benzyl Phthalate (ppm in the feed)			
		0	750	3750	11250
85	Alopecia: abdomen	1			
	multiple areas				1
	Ear: red, bilateral				3
86	Alopecia: abdomen	1			
	multiple areas				1
	Ear: red, bilateral				3
87	Alopecia: abdomen	1			
	multiple areas				1
	Ear: red, bilateral				3
88	Alopecia: abdomen	1			
	multiple areas				1
	Ear: red, bilateral				3
89	Alopecia: abdomen	1			
	multiple areas				1
	Ear: red, bilateral				3
90	Alopecia: abdomen	1			
	multiple areas				1
	Ear: red, bilateral				3
91	Alopecia: abdomen	1			
	multiple areas				1
	Ear: red, bilateral				3
92	Alopecia: abdomen, growing in	1			
	multiple areas				1
	Ear: red, bilateral				3
93	Alopecia: abdomen, growing in	1			
	multiple areas				1
	Ear: red, bilateral				3
94	Alopecia: multiple areas				1
	Ear: red, bilateral				3

(continued)

Table 58. Summary of the F_1 Female Clinical Observations During the Post Mating Holding Period
(page 2 of 3)

Day[a]	Observation[b]	Butyl Benzyl Phthalate (ppm in the feed) 0	750	3750	11250
95	Alopecia: multiple areas				1
	Ear: red, bilateral				3
96	Alopecia: multiple areas				1
	Ear: red, bilateral				3
97	Alopecia: multiple areas				1
	Ear: red, bilateral				3
98	Alopecia: multiple areas				1
	Ear: red, bilateral				3
	Rust colored fur: abdomen				1
99	Alopecia: multiple areas				1
	Ear: red, bilateral				3
	Rust colored fur: abdomen				1
100	Alopecia: multiple areas				1
	Ear: red, bilateral				3
	Rust colored fur: abdomen				1
101	Alopecia: multiple areas				1
	Ear: red, bilateral				3
	Rust colored fur: abdomen				1
102	Alopecia: multiple areas				1
	Ear: red, bilateral				3
	Rust colored fur: abdomen				1
103	Alopecia: multiple areas				1
	Ear: red, bilateral				3
	Rust colored fur: abdomen				1
104	Alopecia: multiple areas				1
	Ear: red, bilateral				3
	Rust colored fur: abdomen				1
	side(s)				1
105	Alopecia: multiple areas				1
	Chromodacryorrhea: eye, right				1
	Ear: red, bilateral				3
	Rust colored fur: abdomen				1
	side(s)				1
106	Alopecia: multiple areas				1
	Chromodacryorrhea: eye, right				1
	Ear: red, bilateral				3
	Rust colored fur: abdomen				1
	side(s)				1

(continued)

Table 58. Summary of the F_1 Female Clinical Observations During the Post Mating Holding Period
(page 3 of 3)

Day[a]	Observation[b]	Butyl Benzyl Phthalate (ppm in the feed)			
		0	750	3750	11250
107	Alopecia: multiple areas				1
	Chromodacryorrhea: eye, right, gone				1
	Ear: red, bilateral				3
	Rust colored fur: abdomen, improving				1
	side(s)				1
108	Alopecia: multiple areas				1
	Chromodacryorrhea: eye, right				1
	Ear: red, bilateral				3
	Rust colored fur: abdomen				1
	side(s)				1
109	Alopecia: multiple areas				1
	Chromodacryorrhea: eye, right				1
	Ear: red, bilateral				3
	Rust colored fur: abdomen				1
	side(s)				1
110	Alopecia: multiple areas				1
	Chromodacryorrhea: eye, right, gone				1
	Ear: red, bilateral				3
	Rust colored fur: abdomen				1
	side(s)				1
111	Alopecia: multiple areas				1
	Ear: red, bilateral				3
	Rust colored fur: abdomen				1
	side(s)				1
112	Alopecia: multiple areas				1
	Ear: red, bilateral				3
	Rust colored fur: abdomen, improving				1
	side(s)				1
113	Alopecia: multiple areas				1
	Ear: red, bilateral				3
	Rust colored fur: abdomen				1
	side(s)				1

[a]Study day.
[b]Clinical observations are tabulated once per day per animal.

Table 59. Summary and Statistical Analysis of the F_1 Male Organ Weights, Relative Organ Weights, Sperm Evaluations and Selected Gross Necropsy Findings (page 1 of 5)

	Butyl Benzyl Phthalate (ppm in the feed)			
	0	750	3750	11250
No. of Males on Study	30	30	30	30
Sacrifice Body Weight (g)[a]				
	601.30 ‡‡‡ ± 9.12 §§§ N=30	606.55 ± 10.10 N=29[b]	626.94 ± 9.64 N=28[b]	547.34 *** ± 11.01 N=28[d]
Brain Weight (g)[a]				
	2.2046 ‡‡ ± 0.0155 §§ N=30	2.2016 ± 0.0167 N=29	2.2259 ± 0.0134 N=28	2.1429 * ± 0.0231 N=28
Pituitary Weight (g)[a]				
#	0.0168 ± 0.0003 N=30	0.0163 ± 0.0002 N=29	0.0166 ± 0.0003 N=27[e]	0.0165 ± 0.0004 N=28
Thyroid Weight (g)[a]				
	0.0305 ± 0.0012 § N=30	0.0311 ± 0.0009 N=29	0.0321 ± 0.0011 N=28	0.0281 ± 0.0008 N=28
Liver Weight (g)[a]				
	23.4280 ‡‡ ± 0.5172 N=30	23.5671 ± 0.5312 N=29	25.8734 ** ± 0.6483 N=28	24.5673 ± 0.5678 N=28
Pancreas Weight (g)[a]				
	0.7532 ‡ ± 0.0250 § N=30	0.8243 ± 0.0228 N=29	0.8562 * ± 0.0337 N=28	0.8616 * ± 0.0352 N=28
Spleen Weight (g)[a]				
	0.8792 ‡‡ ± 0.0258 § N=30	0.8768 ± 0.0269 N=29	0.9392 ± 0.0268 N=28	0.7987 ± 0.0239 N=28
Paired Kidney Weight (g)[a]				
	4.3343 ‡‡‡ ± 0.0820 § N=30	4.4658 ± 0.0719 N=29	4.8475 *** ± 0.0770 N=28	4.1499 ± 0.0842 N=28
Paired Adrenal Weight (g)[a]				
	0.0583 ± 0.0018 N=30	0.0594 ± 0.0017 N=29	0.0600 ± 0.0017 N=28	0.0584 ± 0.0013 N=28

(continued)

Table 59. Summary and Statistical Analysis of the F_1 Male Organ Weights, Relative Organ Weights, Sperm Evaluations and Selected Gross Necropsy Findings (page 2 of 5)

	Butyl Benzyl Phthalate (ppm in the feed)			
	0	750	3750	11250
Paired Testes Weight (g)[a]				
#	3.5980 ŧŧŧ ŸŸŸ ± 0.0505 N=29[f]	3.6494 ± 0.0474 N=29	3.6231 ± 0.1143 N=27[f]	2.8585 ÞÞÞ ± 0.1792 N=27[f]
Paired Epididymis Weight (g)[a]				
	1.3507 ‡‡ §§ ± 0.0278 N=30	1.3791 ± 0.0166 N=28[g]	1.3854 ± 0.0371 N=27[h]	1.2076 * ± 0.0535 N=19[h]
Prostate Weight (g)[a]				
	0.7556 ‡‡‡ §§§ ± 0.0371 N=30	0.6654 ± 0.0327 N=29	0.7039 ± 0.0298 N=28	0.5626 *** ± 0.0293 N=28
Seminal Vesicles with Coagulating Gland Weight (g)[a]				
#	2.1455 ŧŧŧ ŸŸŸ ± 0.0525 N=30	2.1580 ± 0.0497 N=29	2.1289 ± 0.0521 N=28	1.7515 ÞÞÞ ± 0.0937 N=24[i]
Relative Brain Weight (% sacrifice weight)[a]				
	0.3691 ‡‡‡ §§§ ± 0.0062 N=30	0.3653 ± 0.0056 N=29	0.3572 ± 0.0057 N=28	0.3944 ** ± 0.0060 N=28
Relative Pituitary Weight (% sacrifice weight)[a]				
	0.0028 ‡‡‡ §§§ ± 0.0001 N=30	0.0027 ± 0.0000 N=29	0.0026 ± 0.0000 N=27[e]	0.0030 * ± 0.0001 N=28
Relative Thyroid Weight (% sacrifice weight)[a]				
	0.0051 ± 0.0002 N=30	0.0051 ± 0.0002 N=29	0.0051 ± 0.0002 N=28	0.0052 ± 0.0002 N=28
Relative Liver Weight (% sacrifice weight)[a]				
	3.8905 ‡‡‡ §§§ ± 0.0506 N=30	3.8876 ± 0.0649 N=29	4.1182 * ± 0.0652 N=28	4.4942 *** ± 0.0670 N=28
Relative Pancreas Weight (% sacrifice weight)[a]				
	0.1256 ‡‡‡ §§§ ± 0.0039 N=30	0.1368 ± 0.0043 N=29	0.1371 ± 0.0056 N=28	0.1570 *** ± 0.0054 N=28

(continued)

Table 59. Summary and Statistical Analysis of the F_1 Male Organ Weights, Relative Organ Weights, Sperm Evaluations and Selected Gross Necropsy Findings (page 3 of 5)

	Butyl Benzyl Phthalate (ppm in the feed)			
	0	750	3750	11250
Relative Spleen Weight (% sacrifice weight)[a]				
	0.1468 ± 0.0046 N=30	0.1451 ± 0.0045 N=29	0.1502 ± 0.0042 N=28	0.1461 ± 0.0034 N=28
Relative Paired Kidney Weight (% sacrifice weight)[a]				
	0.7228 ‡‡ ± 0.0129 § N=30	0.7385 ± 0.0106 N=29	0.7749 ** ± 0.0107 N=28	0.7603 ± 0.0110 N=28
Relative Paired Adrenal Weight (% sacrifice weight)[a]				
	0.0097 ‡ ± 0.0003 §§ N=30	0.0098 ± 0.0002 N=29	0.0096 ± 0.0003 N=28	0.0108 * ± 0.0003 N=28
Relative Paired Testes Weight (% sacrifice weight)[a]				
#	0.5995 ± 0.0101 ŸŸ N=29[f]	0.6059 ± 0.0121 N=29	0.5834 ± 0.0217 N=27[f]	0.5212 ± 0.0305 N=27[f]
Relative Paired Epididymis Weight (% sacrifice weight)[a]				
	0.2259 ± 0.0056 N=30	0.2295 ± 0.0040 N=28[g]	0.2228 ± 0.0073 N=27[h]	0.2171 ± 0.0087 N=19[h]
Relative Prostate Weight (% sacrifice weight)[a]				
	0.1265 ‡ ± 0.0065 § N=30	0.1105 ± 0.0056 N=29	0.1127 ± 0.0048 N=28	0.1029 ** ± 0.0052 N=28
Relative Seminal Vesicles with Coagulating Gland Weight (% sacrifice weight)[a]				
#	0.3579 ± 0.0087 Ÿ N=30	0.3574 ± 0.0084 N=29	0.3414 ± 0.0092 N=28	0.3160 ± 0.0161 N=24[i]
Percent Motile Sperm[a]				
#	68.6 † ± 4.0 ŸŸ N=30	74.0 ± 1.7 N=29	71.7 ± 3.7 N=28	52.1 Þ ± 6.4 N=27[h]
Percent Progressively Motile Sperm[a]				
#	57.3 †† ± 3.8 ŸŸ N=30	61.2 ± 2.1 N=29	60.1 ± 3.7 N=28	42.1 Þ ± 5.7 N=27[h]

(continued)

Table 59. Summary and Statistical Analysis of the F_1 Male Organ Weights, Relative Organ Weights, Sperm Evaluations and Selected Gross Necropsy Findings (page 4 of 5)

	Butyl Benzyl Phthalate (ppm in the feed)			
	0	750	3750	11250
Epididymal Sperm Concentration (mil/g)[a]				
#	825.59 † ± 38.16 ŸŸ N=30	860.78 ± 25.91 N=29	845.35 ± 40.82 N=28	649.51 Þ ± 70.25 N=27[h]
Spermatid Head Concentration (mil/g)[a]				
	47.22 ± 6.12 § N=29[f]	53.62 ± 6.66 N=29	47.01 ± 5.82 N=28	30.30 ± 6.54 N=28
Daily Sperm Production per Testis[a]				
	18.18 ± 2.32 § N=29[f]	21.39 ± 2.77 N=29	19.13 ± 2.28 N=28	11.96 ± 2.99 N=28
Efficiency of Daily Sperm Production[a]				
	10.24 ± 1.33 § N=29[f]	11.63 ± 1.45 N=29	10.20 ± 1.26 N=28	6.57 ± 1.42 N=28
Percent Abnormal Sperm[a]				
	5.99 ± 3.18 N=29[j]	2.77 ± 0.31 N=29	5.61 ± 3.08 N=27	3.27 ± 0.63 N=20[h,j]
Number of Males with One or More Urinary Tract Malformations[k,l]				
	5	8	6	5
Percent Males with One or More Urinary Tract Malformations[k,l,m]				
	16.67 ± 6.92 N=30	26.67 ± 8.21 N=30	20.00 ± 7.42 N=30	16.67 ± 6.92 N=30
Number of Males with One or More Reproductive Tract Malformations[k,n]				
	1	0	3	16
Percent Males with One or More Reproductive Tract Malformations[k,m,n]				
	3.33 ŁŁ ± 3.33 ΨΨΨ N=30	0.00 ± 0.00 N=30	10.00 ± 5.57 N=30	53.33 ΦΦΦ ± 9.26 N=30

(continued)

Table 59. Summary and Statistical Analysis of the F_1 Male Organ Weights, Relative Organ Weights, Sperm Evaluations and Selected Gross Necropsy Findings (page 5 of 5)

[a]Reported as the mean ± S.E.M.
[b]Decrease in N is due to male 1163 being found dead on study day 78.
[c]Decrease in N is due to male 1157 being euthanized moribund on study day 21 and male 1033 being found dead on study day 83.
[d]Decrease in N is due to males 1085 and 1115 being euthanized moribund on study day 75.
[e]Decrease in N is due the head being missing at time of weighing the pituitary.
[f]Decrease in N is due to one or both testis being missing.
[g]Decrease in N is due to one weight being a statistical outlier and therefore it was excluded.
[h]Decrease in N is due to one or more males having all or part of one or both epididymis missing.
[i]Decrease in N is due to one or more males having all or part of the seminal vesicles missing.
[j]Decrease in N is due to there being no sperm on any of the morphology slides for one or more males.
[k]See Table 60 for a list of the gross necropsy findings.
[l]Includes hydronephrosis.
[m]Reported as the percentage ± the standard error of the percentage.
[n]Includes missing epididymis (whole or part), epididymis reduced in size, missing testis, testis reduced in size, missing seminal vesicle, seminal vesicle reduced in size, seminal vesicle abnormally shaped, missing coagulating gland and hypospadias.
[#]Levene's test for homogeneity of variances was significant ($p<0.05$), therefore robust regression methods were used to test all treatment effects.
[‡]$p<0.05$; ANOVA Test.
[‡‡]$p<0.01$; ANOVA Test.
[‡‡‡]$p<0.001$; ANOVA Test.
[§]$p<0.05$; Test for Linear Trend.
[§§]$p<0.01$; Test for Linear Trend.
[§§§]$p<0.001$; Test for Linear Trend.
[*]$p<0.05$; Dunnett's Test.
[**]$p<0.01$; Dunnett's Test.
[***]$p<0.001$; Dunnett's Test.
[†]$p<0.05$; Wald Chi-square Test for overall treatment effect in robust linear regression model.
[††]$p<0.01$; Wald Chi-square Test for overall treatment effect in robust linear regression model.
[†††]$p<0.001$; Wald Chi-square Test for overall treatment effect in robust linear regression model.
[Þ]$p<0.05$; Individual t-test for pairwise comparisons to control in robust linear regression model.
[ÞÞÞ]$p<0.001$; Individual t-test for pairwise comparisons to control in robust linear regression model.
[Ÿ]$p<0.05$; Linear trend test in robust regression model.
[ŸŸ]$p<0.01$; Linear trend test in robust regression model.
[ŸŸŸ]$p<0.001$; Linear trend test in robust regression model.
[£]$p<0.01$; Chi-Square Test.
[ΦΦΦ]$p<0.001$; Fishers' Exact Test.
[ψψψ]$p<0.001$; Cochran-Armitage Test.

Table 60. Summary of F_1 Male Gross Necropsy Findings and Microscopic Findings (page 1 of 4)

Gross Findings

Scheduled Sacrifice

Finding	Butyl Benzyl Phthalate (ppm in the feed)			
	0	750	3750	11250
Alopecia: limb(s)		1	1	1
multiple areas		1		
Chromodacryorrhea: eye, left		1		
eye, right			1	
Coagulating Gland: missing, bilateral				1
Ear: red and thickened, bilateral			1	
thickened, right				1
Epididymis: caput and corpus missing, left			1	
caput missing, bilateral				4
caput missing, left				2
caput missing, right				1
cauda missing, bilateral				1
cauda reduced in size, bilateral				2
missing, bilateral				1
reduced in size, bilateral			2	3
reduced in size, left				1
reduced in size, right	1			3
Hypospadias				2
Kidney: hydronephrosis, bilateral		1		
hydronephrosis, right	5	6	6	3
multiple cysts on external and internal surfaces, bilateral	1			
multiple white granules, right	3			
Liver: right caudal lobe misshapen		1		
Nipples: 4				1
6				1
Pancreas: multiple white granular deposits				1
reddish discoloration		1	1	
Preputial Gland: abscess, left				1
Rust colored fur: limb(s)		1		
multiple areas	1			
side(s)			1	
Seminal Vesicles: missing, bilateral				3
missing, left				1
reddened, left				1
reduced in size and misshapened				1
reduced in size and round instead of horn shaped				1
reduced in size, bilateral				3
reduced in size, right				1
Testis: missing, left			1	
missing, right	1			1
reduced in size, bilateral			2	8
reduced in size, left				1
reduced in size, right				3

(continued)

Table 60. Summary of F_1 Male Gross Necropsy Findings and Microscopic Findings (page 2 of 4)

Gross Findings

Unscheduled Sacrifice

Finding	Butyl Benzyl Phthalate (ppm in the feed)			
	0	750	3750	11250
Adrenal Gland: enlarged, bilateral				2
Epididymis: missing, bilateral				1
reduced in size, right				1
Hemorrhagic cystitis				2
Kidney: discolored, bilateral				1
hydronephrosis, bilateral				2
hydronephrosis, right		1		
Lung: discolored, diffuse, all lobes				1
Nipples: 3				1
6				1
Prostate: hemorrhagic				2
Prostatitis				2
Seminal Vesicles: hemorrhagic, bilateral				1
Testis: reduced in size, bilateral				1
reduced in size, right				1
Urinary Bladder: distended and hemorrhagic				1
enlarged and contained clotted blood				1

Microscopic Findings

Finding	Butyl Benzyl Phthalate (ppm in the feed)			
	0	750	3750	11250
ADRENAL GLAND				
Number Examined	0	0	0	1
No Findings				
EPIDIDYMIS				
Number Examined	30	7	30	24
Aspermia	1		2	8
Dilatation, Duct				1
Inflammation, Chronic	1		1	4
Malformation				1
Mineralization, Epithelium				1

(continued)

Table 60. Summary of F_1 Male Gross Necropsy Findings and Microscopic Findings (page 3 of 4)

Microscopic Findings

Finding	Butyl Benzyl Phthalate (ppm in the feed)			
	0	750	3750	11250
KIDNEYS				
Number Examined	6	4	6	5
Calculus, Microscopic, Pelvis	2			
Dilatation, Collecting Tubule, Papilla				1
Hydronephrosis	5	4	6	4
Inflammation, Cortex, Acute				1
Inflammation, Perirenal Tissue, Acute				1
Necrosis, Papilla				1
Nephropathy		1		
Polycystic Kidney	1			
LIVER				
Number Examined	20	21	22	12
Cyst, Bile Duct	1			
Fibrosis, Periductular	1			
Necrosis, Hepatocyte		2	1	
LUNG				
Number Examined	0	0	0	1
No Findings				
PANCREAS				
Number Examined	10	1	2	13
Atrophy, Acinar Cell	3			4
Hyperplasia, Islet Cell	6		1	
Inflammation, Chronic	3			2
PENIS				
Number Examined	0	0	0	2
No Findings				
PITUITARY GLAND				
Number Examined	10	1	2	12
No Findings				
PREPUTIAL GLAND				
Number Examined	0	0	0	1
Inflammation, Chronic				1
PROSTATE GLAND				
Number Examined	30	7	30	30
Inflammation, Acute				2
Inflammation, Chronic	17	3	18	13

(continued)

Table 60. Summary of F_1 Male Gross Necropsy Findings and Microscopic Findings (page 4 of 4)

Microscopic Findings

Finding	Butyl Benzyl Phthalate (ppm in the feed)			
	0	750	3750	11250
SEMINAL VESICLE				
Number Examined	30	7	30	26
Hypoplasia				1
Inflammation, Acute				1
Inflammation, Chronic				1
SKIN				
Number Examined	0	1	0	0
No Findings				
TESTIS (left)				
Number Examined	30	7	29	29
Atrophy, Seminiferous Tubule	3		2	15
Degeneration, Seminiferous Tubule			2	1
Dilatation, Duct, Rete Testis				7
THYROID				
Number Examined	10	1	2	12
Ultimobranchial Cyst	3			
URINARY BLADDER				
Number Examined	0	0	0	2
Inflammation, Acute				2

Table 61. Summary and Statistical Analysis of the F_1 Female Organ Weights, Relative Organ Weights, Ovarian Follicle Counts, Vaginal Cytology at Necropsy and Selected Gross Necropsy Findings
(page 1 of 5)

	Butyl Benzyl Phthalate (ppm in the feed)			
	0	750	3750	11250
No. of Females on Study	30	30	30	30
Sacrifice Body Weight (g)[a]				
	338.70 ‡‡‡ ± 5.00 §§§ N=30	342.17 ± 5.43 N=30	349.21 ± 3.97 N=30	319.11 ** ± 3.98 N=30
Brain Weight (g)[a]				
	1.9424 ‡ ± 0.0215 N=30	1.9325 ± 0.0180 N=30	1.9968 ± 0.0139 N=30	1.9120 ± 0.0197 N=30
Pituitary Weight (g)[a]				
	0.0198 ‡ ± 0.0005 § N=30	0.0194 ± 0.0005 N=30	0.0203 ± 0.0004 N=29[b]	0.0183 ± 0.0005 N=30
Thyroid Weight (g)[a]				
	0.0225 ± 0.0011 N=29[c]	0.0219 ± 0.0011 N=30	0.0241 ± 0.0009 N=30	0.0226 ± 0.0011 N=30
Liver Weight (g)[a]				
#	18.3484 ± 0.4701 N=30	18.5975 ± 0.4107 N=30	19.7664 ± 0.5531 N=30	18.3974 ± 0.9278 N=30
Pancreas Weight (g)[a]				
	0.7945 ± 0.0313 N=30	0.7530 ± 0.0220 N=30	0.8252 ± 0.0293 N=29[d]	0.7935 ± 0.0253 N=30
Spleen Weight (g)[a]				
	0.5702 ± 0.0145 N=30	0.5900 ± 0.0133 N=30	0.6125 ± 0.0129 N=30	0.5804 ± 0.0150 N=30
Paired Kidney Weight (g)[a]				
#	2.9431 ††† ± 0.0404 N=29[e]	3.0153 ± 0.0480 N=30	3.1909 ÞÞÞ ± 0.0518 N=30	2.9038 ± 0.0674 N=30
Paired Adrenal Weight (g)[a]				
	0.0787 ± 0.0029 N=29[f]	0.0837 ± 0.0025 N=30	0.0795 ± 0.0033 N=30	0.0758 ± 0.0039 N=30

(continued)

Table 61. Summary and Statistical Analysis of the F_1 Female Organ Weights, Relative Organ Weights, Ovarian Follicle Counts, Vaginal Cytology at Necropsy and Selected Gross Necropsy Findings
(page 2 of 5)

	Butyl Benzyl Phthalate (ppm in the feed)			
	0	750	3750	11250
Paired Ovary Weight (g)[a]				
	0.1455 ± 0.0038 N=30	0.1501 ± 0.0046 N=29[g]	0.1507 ± 0.0045 N=30	0.1486 ± 0.0050 N=30
Uterus Weight (g)[a]				
	0.5703 ‡ ± 0.0213 §§ N=30	0.5705 ± 0.0230 N=30	0.6223 ± 0.0293 N=30	0.6846 * ± 0.0454 N=30
Relative Brain Weight (% sacrifice weight)[a]				
	0.5762 ‡ ± 0.0090 §§ N=30	0.5675 ± 0.0076 N=30	0.5737 ± 0.0072 N=30	0.6014 ± 0.0087 N=30
Relative Pituitary Weight (% sacrifice weight)[a]				
	0.0059 ± 0.0002 N=30	0.0057 ± 0.0002 N=30	0.0058 ± 0.0001 N=29[b]	0.0057 ± 0.0002 N=30
Relative Thyroid Weight (% sacrifice weight)[a]				
	0.0067 ± 0.0003 N=29[c]	0.0064 ± 0.0003 N=30	0.0069 ± 0.0002 N=30	0.0071 ± 0.0003 N=30
Relative Liver Weight (% sacrifice weight)[a]				
#	5.4343 ± 0.1392 N=30	5.4521 ± 0.1146 N=30	5.6711 ± 0.1533 N=30	5.7361 ± 0.2643 N=30
Relative Pancreas Weight (% sacrifice weight)[a]				
	0.2361 ± 0.0096 N=30	0.2212 ± 0.0068 N=30	0.2374 ± 0.0086 N=29[d]	0.2490 ± 0.0077 N=30
Relative Spleen Weight (% sacrifice weight)[a]				
	0.1683 ± 0.0035 § N=30	0.1730 ± 0.0038 N=30	0.1761 ± 0.0043 N=30	0.1826 ± 0.0052 N=30
Relative Paired Kidney Weight (% sacrifice weight)[a]				
	0.8731 ± 0.0144 N=29[e]	0.8833 ± 0.0116 N=30	0.9152 ± 0.0138 N=30	0.9087 ± 0.0159 N=30

(continued)

Table 61. Summary and Statistical Analysis of the F_1 Female Organ Weights, Relative Organ Weights, Ovarian Follicle Counts, Vaginal Cytology at Necropsy and Selected Gross Necropsy Findings
(page 3 of 5)

	Butyl Benzyl Phthalate (ppm in the feed)			
	0	750	3750	11250
Relative Paired Adrenal Weight (% sacrifice weight)[a]				
	0.0233 ± 0.0008 N=29[f]	0.0247 ± 0.0008 N=30	0.0229 ± 0.0010 N=30	0.0238 ± 0.0013 N=30
Relative Paired Ovary Weight (% sacrifice weight)[a]				
#	0.0430 ± 0.0009 N=30	0.0440 ± 0.0012 N=29[g]	0.0432 ± 0.0012 N=30	0.0468 ± 0.0017 N=30
Relative Uterus Weight (% sacrifice weight)[a]				
#	0.1689 † ± 0.0065 ŸŸ N=30	0.1674 ± 0.0068 N=30	0.1791 ± 0.0088 N=30	0.2170 ÞÞ ± 0.0159 N=30
Paired Ovarian Follicle Count[a,h]				
	368.4 ± 26.3 N=10			414.9 ± 56.5 N=10
VAGINAL CYTOLOGY EVALUATION AT NECROPSY:[i]				
No. Females Evaluated	30	30	30	30
No. in Proestrus	4	6	7	5
% in Proestrus	13.33	21.43	25.93	17.24
No. in Estrus	5	5	5	8
% in Estrus	16.67	17.86	18.52	27.59
No. in Metestrus	16	14	15	15
% in Metestrus	53.33	50.00	55.56	51.72
No. in Diestrus	5	3	0	1
% in Diestrus	16.67 Ψ	10.71	0.00	3.45
No. Stage Not Determined	0	2	3	1
No. No Cells Present	0	0	0	0

(continued)

Table 61. Summary and Statistical Analysis of the F_1 Female Organ Weights, Relative Organ Weights, Ovarian Follicle Counts, Vaginal Cytology at Necropsy and Selected Gross Necropsy Findings
(page 4 of 5)

	Butyl Benzyl Phthalate (ppm in the feed)			
	0	750	3750	11250
Number of Females with One or More Urinary Tract Malformations[j,k]	3	3	1	2
Percent Females with One or More Urinary Tract Malformations[j,k,l]	10.00 ± 5.57 N=30	10.00 ± 5.57 N=30	3.33 ± 3.33 N=30	6.67 ± 4.63 N=30
Number of Females with One or More Reproductive Tract Malformations[j,m]	0	0	0	0
Percent Females with One or More Reproductive Tract Malformations[j,l,m]	0.00 ± 0.00 N=30	0.00 ± 0.00 N=30	0.00 ± 0.00 N=30	0.00 ± 0.00 N=30

[a]Reported as the mean ± S.E.M.
[b]Decrease in N is due to the head being missing at time of weighing the pituitary.
[c]Decrease in N is due to the thyroid weight for one female being a statistical outlier and therefore it was excluded.
[d]Decrease in N is due to the pancreas weight for one female being a statistical outlier and therefore it was excluded.
[e]Decrease in N is due to the paired kidney weight for one female being a statistical outlier and therefore it was excluded.
[f]Decrease in N is due to the paired adrenal weight for one female inadvertently not being recorded.
[g]Decrease in N is due to the paired ovary weight for one female inadvertently not being recorded.
[h]Ovarian follicle counts were done for 10 control females and 10 females in the 11250 ppm Butyl Benzyl Phthalate dose group.
[i]For presentation and statistical analysis purposes those females in two stages were pooled in the following manner: proestrus/estrus and estrus/proestrus were considered proestrus; estrus/metestrus and metestrus/estrus were considered estrus; metestrus/diestrus and diestrus/metestrus were considered metestrus; and diestrus/proestrus and proestrus/diestrus were considered diestrus. The females for which the stage could not be determined or no cells were present were not included in the statistical analysis.
[j]See Table 62 for a list of the gross necropsy findings.
[k]Includes hydronephrosis.
[l]Reported as the percentage ± the standard error of the percentage.
[m]Does not include retained fetus in uterus, fluid filled uterus, fluid filled vagina, bloody ovary or cyst on ovary or end of uterine horn.
[#]Levene's test for homogeneity of variances was significant ($p<0.05$), therefore robust regression methods were used to test all treatment effects.
[‡]$p<0.05$; ANOVA Test.
[‡‡‡]$p<0.001$; ANOVA Test.
[§]$p<0.05$; Test for Linear Trend.
[§§]$p<0.01$; Test for Linear Trend.
[§§§]$p<0.001$; Test for Linear Trend.

(continued)

Table 61. Summary and Statistical Analysis of the F_1 Female Organ Weights, Relative Organ Weights, Ovarian Follicle Counts, Vaginal Cytology at Necropsy and Selected Gross Necropsy Findings
(page 5 of 5)

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*p<0.05; Dunnett's Test.
**p<0.01; Dunnett's Test.
†p<0.05; Wald Chi-square Test for overall treatment effect in robust regression model.
†††p<0.001; Wald Chi-square Test for overall treatment effect in robust regression model.
ŸŸp<0.01; Linear trend test in robust regression model.
ÞÞp<0.01; Individual t-test for pairwise comparisons to control in robust regression model.
ÞÞÞp<0.001; Individual t-test for pairwise comparisons to control in robust regression model.
Ψp<0.05; Cochran-Armitage Test.

Table 62. Summary of F_1 Female Gross Necropsy Findings and Microscopic Findings (page 1 of 2)

Gross Findings

Scheduled Sacrifice

Finding	Butyl Benzyl Phthalate (ppm in the feed) 0	750	3750	11250
Alopecia: limb(s)	1		4	3
multiple areas	3		2	1
Chromodacryorrhea: eye, left		1	1	
eye, right	1			
Ear: red and thickened, bilateral		1	1	5
red, bilateral		1		
Kidney: hydronephrosis, bilateral	1	2	1	
hydronephrosis, left		1		1
hydronephrosis, right	2			1
multiple clear cysts, bilateral	1			
multiple cysts, right				1
Liver: irregular surface, all lobes	1			
Neck: 6 x 3 x 0.5 cm white firm irregular subcutaneous mass				1
Ovary: bloody, left			1	
cyst, left				1
Rust colored fur: abdomen				1
multiple areas		1		1
shoulder(s)		1	1	
side(s)				1
Thyroid Gland: enlarged, left		1		
Uterine Horn: 1 cm fluid filled cyst at ovarian end, right				1
Uterus: fluid filled			1	3
Vagina: fluid filled			1	

Microscopic Findings

Finding	Butyl Benzyl Phthalate (ppm in the feed) 0	750	3750	11250
Cervix				
Number Examined	10	2	5	20
No Findings				
Kidneys				
Number Examined	1	0	0	2
Hydronephrosis				1
Polycystic Kidney	1			1
Liver				
Number Examined	20	20	20	9
Cyst, Bile Duct	1			
Cytologic Alteration, Hepatocyte				5
Fibrosis, Periductular	1			

(continued)

Table 62. Summary of F_1 Female Gross Necropsy Findings and Microscopic Findings (page 2 of 2)

Microscopic Findings

Finding	Butyl Benzyl Phthalate (ppm in the feed)			
	0	750	3750	11250
Ovary				
Number Examined	10	2	5	20
No Findings				
Oviduct				
Number Examined	10	2	5	20
No Findings				
Pancreas				
Number Examined	10	0	0	8
Atrophy, Acinar Cell	1			2
Inflammation, Chronic				1
Pituitary Gland				
Number Examined	10	0	0	8
No Findings				
Thyroid				
Number Examined	10	0	0	8
Cyst, Follicle				1
Ectopic Thymus	2			
Ultimobranchial Cyst	3			3
Uterus				
Number Examined	10	2	5	20
Hydrometra			1	1
Vagina				
Number Examined	10	2	5	20
Inflammation, Acute			1	

APPENDIX I

Analytical Chemistry Report

Dose Formulation and Analysis Report

Final: September 10, 2001
(Draft: July 31, 2001)

DOSE FORMULATION AND ANALYSIS REPORT

RTI Project No.: 65C-07269
RTI Master Protocol No.: RTI-761
RTI Study Code: Rt00-BBP

BUTYL BENZYL PHTHALATE IN PURINA CERTIFIED GROUND RODENT CHOW® (No. 5002)

September 10, 2001

(Draft Date: July 31, 2001)

Prepared by

Dolores R. Brine 9/10/01

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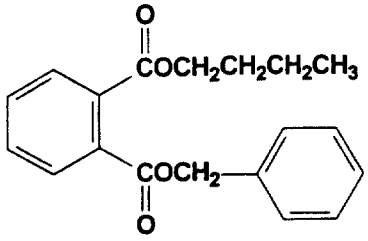
BUTYL BENZYL PHTHALATE

RTI Log Nos.: 9781-02-01 (Bulk chemical) 9781-02-02 (Analytical aliquot)	Amount Received: 105.5 kg (Bulk chemical) 50 g (Analytical aliquot)
CAS No.: 85-68-7	Received from: Solutia, Inc.
RTI Receipt Date: February 2, 2000	Batch Number: DL1120N
Appearance: Clear viscous liquid	Purity: 98.5% (Monsanto Company)
Storage Conditions: Room temperature	

STRUCTURE	MOL. WT.	FORMULA
	312.39	$C_{19}H_{20}O_4$

SUMMARY

A sample of butyl benzyl phthalate (RTI Log No. 9781-02-02) was submitted for dose formulation studies in support of RTI Project No. 65C-07269. The dose formulation studies included mixing studies of the test chemical and vehicle, dose storage stability studies, and simulated dosing stability studies.

Mixing/homogeneity studies conducted on dose formulations of butyl benzyl phthalate in Purina certified ground rodent chow® (No. 5002) at 700 and 12000 ppm showed a 0.5% variation in dose concentration among the three sampling locations for the low dose (700 ppm) and a 1.2% variation in dose concentration among the three sampling locations for the high dose (12000 ppm). No problems were experienced with the preparation of neat chemical and vehicle dose formulations.

The stability studies conducted on formulations of butyl benzyl phthalate in feed at 700 and 12000 ppm showed test chemical to be stable in dose formulations in feed for at least 54 days when the formulations are stored in sealed amber glass bottles in a freezer. The simulated dosing stability studies showed test chemical in dose formulations in feed at 700 and 12000 ppm to be stable for at least 12 days under conditions that simulate dosing of animals (in rat feed jars open to air and light at ambient temperature).

The performance of the method for dose analysis was evaluated using standards in the concentration range from 500 to 1200 ppm of test chemical in feed. (Extracts of formulations above the calibration range were diluted into the calibration range with blank feed extract prior

to analysis.) Linearity was confirmed for the vehicle standards data (correlation coefficient = 0.9984). Accuracy was confirmed with relative errors less than or equal to 4.1%; precision was demonstrated with relative standard deviations less than or equal to 2.3% for triplicate determinations.

Analyses were performed to determine the butyl benzyl phthalate content of dose formulations mixed on July 5, July 11, August 14, September 11, October 9, and December 4, 2000 and on February 26, 2001 for the Rt00-BBP study. Dose formulations of butyl benzyl phthalate in feed prepared at 750, 3750, and 11250 ppm and used in the animal study were found to contain 90.9 to 107% of the nominal concentrations of test chemical. The relative standard deviations for triplicate analyses were less than or equal to 5.6%. No test chemical was detected in the vehicle blanks (estimated detection limit of 35 ppm).

BUTYL BENZYL PHTHALATE

DOSE FORMULATION IN PURINA CERTIFIED GROUND RODENT CHOW® (No. 5002)

1.0 INTRODUCTION

The purpose of this work was to confirm the homogeneity of dose formulations of test chemical in Purina certified ground rodent chow® (No. 5002), to determine the stability of test chemical in the dose formulations, to develop procedures for dose formulation and analysis, and to determine the test chemical content in dose formulations to be used in animal studies.

2.0 CHEMICAL HANDLING

RTI Notebook No.: 9781 pp.: 2

The bulk chemical (105.5 kg) was received from Solutia, Inc. on February 2, 2000, in five 5-gal metal drums, and was stored at room temperature.

An aliquot of bulk chemical (50 g) was removed for use in dose formulation studies. This aliquot was stored in a glass bottle in a metal can at room temperature.

3.0 DOSE FORMULATION STUDIES - PURINA CERTIFIED GROUND RODENT CHOW® (No. 5002)

This section presents the results of mixing/homogeneity studies of butyl benzyl phthalate in Purina certified ground rodent chow® (No. 5002) at the dose concentrations proposed for use in animal studies. In addition, storage stability studies and dose simulation stability studies are also described. The concentrations of the chemical in Purina certified ground rodent chow® (No. 5002) were specified by the Research Triangle Institute Reproductive and Developmental Toxicology Laboratory.

3.1 Low Dose Mixing Study (700 ppm)

RTI Notebook No.: 9357 pp.: 83-87, 92-93, 99-103

3.1.1 Parameters

Nominal Concentration: 700 ppm

Vehicle: Purina certified ground rodent chow® (No. 5002)

Blender: Lowe 5 cubic foot blender

Batch Size: 30 kg

3.1.2 Premix Feed Preparation

The low dose formulation (700 ppm) was prepared by weighing butyl benzyl phthalate (21.03 g) onto a small amount of premix feed in a stainless steel bucket. The chemical was mixed into the feed by stirring. Additional premix feed was added, and the mixture was stirred until all the premix feed had been added. Care was taken to break up any lumps that appeared.

3.1.3 Bulk Mixing and Sampling

A 12-kg portion of blank feed was layered evenly in the blender. The premix feed was added in equal amounts to the left and right blender ports and evenly spread over the blank feed. Residual amounts of the premix feed were removed from the beaker by adding, stirring, and discharging (into the blender) three portions of approximately 1000 g of blank feed. A second 12-kg portion of blank feed was then added to the blender in an even layer. The blender ports were sealed and blending was begun. The blender was operated for 20 minutes.

Aliquots (200 g) of the dose formulation were collected from the left, right, and bottom blender ports for homogeneity testing. An aliquot (3 kg) of the bulk feed was collected for use in stability studies as the feed was being discharged from the blender into a polyethylene bag. The feed samples were stored in a freezer prior to analysis.

3.1.4 Analytical Method

3.1.4.1 Sample Preparation

Triplicate aliquots (approximately 10 g) of each homogeneity sample were weighed into scintillation vials and transferred to separate 250-mL wide-mouth amber glass bottles.

An aliquot (40 mL) of hexane was added to each bottle. The bottles were capped, sonicated without heat for 15 minutes, and shaken on a platform shaker at low setting for 60 minutes. After the feed had settled for at least 5 minutes, an aliquot (approximately 18-20 mL) of each extract was transferred to a separate scintillation vial and centrifuged for 5 minutes. An aliquot (1.00 mL) of each supernatant was transferred to a separate GC autosampler vial containing 0.1 mL of internal standard solution (2.00 mg of diethylphthalate/mL of hexane). The vials were capped, and the samples were mixed prior to gas chromatographic analysis.

3.1.4.2 Preparation of Calibration Standards

The vehicle calibration standards were the method performance evaluation standards described in Section 4.3.1.

3.1.4.3 Gas Chromatographic Analysis

Each dose formulation sample and vehicle standard was analyzed by gas chromatography (GC) in a single injection as described below. See Figure 1 for typical chromatograms of butyl benzyl phthalate extracted from feed.

Instrument: Hewlett-Packard 5890 series II gas chromatograph, 7673 autosampler

Chromatography Data System: Waters Millennium^{32} client/server CIMS, Vs. 3.05

Column: Capillary, J&W DB-1701 (15 m X 0.22 mm ID, 0.25 μ film)

Column Temperature: 160 °C for 5 min, 160 to 240 °C at 20 °C/min, 240 °C for 7 min

Carrier Gas: Helium (0.76 mL/min at 250 °C)

Auxillary Gas: Helium (29.3 mL/min)

Injector Type: Split (1:19), fast injection

Injector Temperature: 250 °C

Injection Solvent: Hexane

Injection Volume: 1 μL

Detector: FID

Detector Temperature: 280 °C

Retention Times: butyl benzyl phthalate, 10.9-12.6 minutes
diethylphthalate (internal standard), 3.2-5.3 minutes

3.1.4.4 Calculations

The ratio of the area of the butyl benzyl phthalate peak to the area of the internal standard peak was calculated for each injection. The linear regression equation (concentration *versus* peak area ratio) for the vehicle standards data was computed, and the correlation coefficient, r, was determined. See Figure 2 for a plot of the vehicle standards data. The concentration of test chemical in each sample was determined from the linear regression equation, the peak area ratio, and a factor to normalize to 10.0 g of feed.

The results are presented in terms of percent recovery. Recovery values are based on a theoretical concentration of 700 ppm of test material in feed.

3.1.5 Homogeneity Results (Low Dose - 700 ppm)

Sampling Location	Butyl Benzyl Phthalate Found (ppm)[a]	% Recovery[b]
Left	671	95.9
	668	95.4
	653	93.3
		$\bar{x}$ = 94.9 (1.5% RSD)
Right	659	94.1
	662	94.6
	662	94.6
		$\bar{x}$ = 94.4 (0.3% RSD)
Bottom	656	93.7
	666	95.1
	664	94.9
		$\bar{x}$ = 94.6 (0.8% RSD)

[a] Quantitation was based on the linear regression equation $y = 0.001482\,x - 0.004297$; $r = 0.9984$. The mean of all assays is 662 ppm (0.9% RSD, n=9).

[b] Recovery values are based on a nominal concentration of 700 ppm of butyl benzyl phthalate in feed.

3.1.6 Conclusions

The results of the homogeneity study indicate that the procedure for mixing butyl benzyl phthalate in Purina certified ground rodent chow® (No. 5002) at 700 ppm produced a uniform mixture suitable for use in toxicity studies. The percent variation in dose concentration among the three sampling locations was 0.5%. The relative standard deviations for triplicate sample analyses were ≤ 1.5%.

3.2 High Dose Mixing Study (12000 ppm)

RTI Notebook No.: 9357 pp.: 83-87, 92-93, 99-103

3.2.1 Parameters

Nominal Concentration: 12000 ppm

Vehicle: Purina certified ground rodent chow® (No. 5002)

Blender: Lowe 5 cubic foot blender

Batch Size: 30 kg

3.2.2 Premix Feed Preparation

The high dose formulation (12000 ppm) was prepared by weighing butyl benzyl phthalate (360.14 g) onto a small amount of premix feed in a stainless steel bucket and mixing the chemical into the feed by stirring. Additional premix feed was added with stirring until all the premix feed had been added. Care was taken to break up any lumps that appeared.

3.2.3 Bulk Mixing and Sampling

A 12-kg portion of blank feed was layered evenly in the blender. The premix feed was added in equal amounts to the left and right blender ports and evenly spread over the blank feed. Residual amounts of the premix feed were removed from the beaker by adding, stirring, and discharging (into the blender) three portions of approximately 1000 g of blank feed. A second 12-kg portion of blank feed was then added to the blender in an even layer. The blender ports were sealed and blending was begun. The blender was operated for 20 minutes.

Aliquots (200 g) of the dose formulation were collected from the left, right, and bottom blender ports for homogeneity testing. An aliquot (3 kg) of the bulk feed was collected for use in stability studies as the feed was being discharged from the blender into a polyethylene bag. The feed samples were stored in a freezer prior to analysis.

3.2.4 Analytical Method

3.2.4.1 Sample Preparation

Triplicate aliquots (approximately 10 g) of each homogeneity sample were weighed into scintillation vials and transferred to separate 250-mL wide-mouth amber glass bottles. Aliquots (4 X 10 g) of blank feed were weighed into scintillation vials and transferred to separate 250-mL wide-mouth amber glass bottles to serve for dilution of the high dose extracts.

An aliquot (40 mL) of hexane was added to each bottle. The bottles were capped, sonicated without heat for 15 minutes, and shaken on a platform shaker at low setting for 60 minutes. After the feed had settled for at least 5 minutes, an aliquot (approximately 18-20 mL) of each extract was transferred to a separate scintillation vial and centrifuged for 5 minutes. An aliquot (0.350 mL) of each supernatant was transferred to a separate 5-mL volumetric flask. The samples were diluted to volume with blank feed extract and mixed.

An aliquot (1.00 mL) of each dilution above was transferred to a separate GC autosampler vial containing 0.1 mL of internal standard solution (2.00 mg of diethylphthalate/mL of hexane). The vials were capped, and the samples were mixed prior to gas chromatographic analysis.

3.2.4.2 Preparation of Calibration Standards

The vehicle calibration standards were the method performance evaluation standards described in Section 4.3.1.

3.2.4.3 Gas Chromatographic Analysis

Each dose formulation sample and vehicle standard was analyzed by gas chromatography in a single injection as described in Section 3.1.4.3. See Figure 1 for typical chromatograms of butyl benzyl phthalate extracted from feed.

3.2.4.4 Calculations

The ratio of the area of the butyl benzyl phthalate peak to the area of the internal standard peak was calculated for each injection. The linear regression equation (concentration *versus* peak area ratio) for the vehicle standards data was computed, and the correlation

coefficient, r, was determined. See Figure 2 for a plot of the vehicle standards data. The concentration of test chemical in each sample was determined from the linear regression equation, the peak area ratio, a factor to normalize to 10.0 g of feed, and a factor to account for extract dilution.

The results are presented in terms of percent recovery. Recovery values are based on a theoretical concentration of 12000 ppm of test material in feed.

3.2.5 Homogeneity Results (High Dose - 12000 ppm)

Sampling Location	Butyl Benzyl Phthalate Found (ppm)[a]	% Recovery[b]
Left	11700	97.5
	11900	99.2
	12100	101
		$\bar{x}$ = 99.2 (1.8% RSD)
Right	11700	97.5
	11800	98.3
	11900	99.2
		$\bar{x}$ = 98.3 (0.9% RSD)
Bottom	11900	99.2
	11800	98.3
	12100	101
		$\bar{x}$ = 99.5 (1.4% RSD)

[a] Quantitation was based on the linear regression equation $y = 0.001482\,x - 0.004297$; $r = 0.9984$. The mean of all assays is 11900 ppm (1.3% RSD, n=9).

[b] Recovery values are based on a nominal concentration of 12000 ppm butyl benzyl phthalate in feed.

3.2.6 Conclusions

The results of the homogeneity study indicate that the procedure for mixing butyl benzyl phthalate in Purina certified ground rodent chow® (No. 5002) at 12000 ppm produced a uniform mixture suitable for use in toxicity studies. The percent variation in dose concentration among the three sampling locations was 1.2%. The relative standard deviations for triplicate sample analyses were ≤ 1.8%.

3.3 Low Dose Stability Studies (700 ppm)

3.3.1 Dose Storage Stability Study (Low Dose - 700 ppm)

This section describes a stability study of butyl benzyl phthalate in Purina certified ground rodent chow® (No. 5002) at 700 ppm under conditions that will be used for storage of dose formulations.

RTI Notebook No.: 9357 pp.: 85, 88, 115-136

3.3.1.1 Parameters

Vehicle: Purina certified ground rodent chow® (No. 5002)

Nominal Concentration: 700 ppm

Storage Conditions: Frozen (-15 to -19 °C); 20, 40, and 54 days; sealed in amber glass bottles

3.3.1.2 Dose Preparation and Storage

The preparation of the dose formulation is described in Section 3.1. Three aliquots of the dose formulation (large enough to almost fill the bottles) were transferred to separate 250-mL wide-mouth amber glass bottles. The bottles were capped, sealed with tape, and stored in a freezer until the date of analysis.

3.3.1.3 Sample Preparation

At the end of each designated storage period, a dose formulation aliquot was removed from freezer storage and allowed to warm to room temperature. Triplicate aliquots (10 g) of feed were weighed into scintillation vials and transferred to separate 250-mL wide-mouth amber glass bottles.

An aliquot (40 mL) of hexane was added to each bottle. The bottles were capped, sonicated for 15 minutes without heat, and shaken on a platform shaker at low setting for 60 minutes. After the feed had settled for at least 5 minutes, an aliquot (approximately 18-20 mL) of each extract was transferred to a separate scintillation vial and centrifuged for 5 minutes. An aliquot (1.00 mL) of each supernatant was transferred to a separate GC autosampler vial containing 0.1 mL of internal standard solution (2.00 mg of diethylphthalate/mL of hexane). The vials were capped, and the samples were mixed prior to gas chromatographic analysis.

3.3.1.4 Preparation of Calibration Standards

The vehicle calibration standards were the method performance evaluation standards described in Section 4.3.1.

3.3.1.5 Gas Chromatographic Analysis

The chromatographic conditions are described in Section 3.1.4.3.

3.3.1.6 Calculations

The ratio of the area of the butyl benzyl phthalate peak to the area of the internal standard peak was calculated for each injection. The linear regression equation (concentration *versus* peak area ratio) was computed for the vehicle calibration standards data and the correlation coefficient, r, was determined. See Figure 2 for a plot of the calibration standards data. The concentration of test chemical in each sample was determined from the linear regression equation, the peak area ratio, and a factor to normalize to 10.0 g of feed.

The results are reported in terms of percent recovery. The values obtained on each day of analysis are compared to those obtained on the reference day in the following way.

$$\% \ Recovery = \frac{concentration \ on \ day \ d}{mean \ concentration \ on \ reference \ day} X \ 100.$$

The reference day samples are the nine homogeneity samples described in Section 3.1.

3.3.1.7 Results of the Dose Storage Stability Study (Low Dose - 700 ppm)

Time (days)	Storage Temperature	Butyl Benzyl Phthalate Found (ppm)[a]	% Recovery[b]
0	NA[c]	662[d]	NA
20	-15 to -19 °C	617	93.2
		612	92.4
		624	94.3
			$\bar{x}$ = 93.3 (1.0% RSD)
40	-15 to -19 °C	684	103
		661	99.8
		731	110
			$\bar{x}$ = 104 (5.0% RSD)
54	-15 to -19 °C	645	97.4
		643	97.1
		644	97.3
			$\bar{x}$ = 97.3 (0.2% RSD)

[a] Quantitation was based on the linear regression equation $y = 0.001482 x - 0.004297$; $r = 0.9984$.

[b] $\% \ Recovery = \frac{concentration \ on \ day \ d}{mean \ concentration \ on \ reference \ day} X \ 100$; the mean concentration on the reference day is 662 ppm (0.9% RSD, n=9).

[c] Not applicable.

[d] The reference day samples are the nine homogeneity samples described in Section 3.1.

3.3.1.8 Conclusions

The results of the storage stability study indicate that dose formulations of butyl benzyl phthalate in Purina certified ground rodent chow® (No. 5002) at 700 ppm are stable for at least 54 days when stored in sealed amber glass bottles at freezer temperature (-15 to -19 °C).

3.3.2 Dose Simulation Stability Study (Low Dose – 700 ppm)

This section describes a stability study of butyl benzyl phthalate in Purina certified ground rodent chow® (No. 5002) at 700 ppm under conditions that simulate dosing of animals.

RTI Notebook No.: 9357 pp.: 85, 88, 104-114

3.3.2.1 Parameters

Vehicle: Purina certified ground rodent chow® (No. 5002)

Nominal Concentration: 700 ppm

Storage Conditions: Room temperature (21 °C); 5 and 12 days in feeding jars exposed to air and light

3.3.2.2 Dose Preparation and Storage

The preparation of the dose formulation is described in Section 3.1. Three feeding jars were filled almost full with the dose formulation and placed in a fume hood exposed to air and light.

3.3.2.3 Analytical Method

At the end of each designated storage period, triplicate aliquots (10 g) of the feed in one of the feeding jars were weighed into scintillation vials and transferred to separate 250-mL wide-mouth amber glass bottles. The samples were worked up and analyzed as described in Sections 3.3.1.3 through 3.3.1.6.

3.3.2.4 Results of the Dose Simulation Stability Study (Low Dose - 700 ppm)

Time (Days)	Butyl Benzyl Phthalate Found (ppm)[a]	% Recovery[b]	Mean % Recovery
0	662[c]	NA[d]	NA
5	642	97.0	97.8 (0.8% RSD)
	652	98.5	
	648	97.9	
12	639	96.5	96.1 (0.7% RSD)
	631	95.3	
	639	96.5	

[a] Quantitation was based on the linear regression equation $y = 0.001482\,x - 0.004297$; $r = 0.9984$.

[b] $\%\ Recovery = \frac{concentration\ on\ day\ d}{mean\ concentration\ on\ reference\ day} X\ 100$; the mean concentration on the reference day is 662 ppm (0.9% RSD, n=9).

[c] The reference day samples are the nine homogeneity samples described in Section 3.1.

[d] Not applicable.

3.3.2.5 Conclusions

Results of the dose simulation stability study indicate that dose formulations of butyl benzyl phthalate in Purina certified ground rodent chow® (No. 5002) at 700 ppm are stable for at least 12 days under conditions that simulate dosing.

3.4 High Dose Stability Studies (12000 ppm)

3.4.1 Dose Storage Stability Study (High Dose - 12000 ppm)

This section describes a stability study of butyl benzyl phthalate in Purina certified ground rodent chow® (No. 5002) at 12000 ppm under conditions that will be used for storage of dose formulations.

RTI Notebook No.: 9357 pp.: 85, 88, 115-136

3.4.1.1 Parameters

Vehicle: Purina certified ground rodent chow® (No. 5002)

Nominal Concentration: 12000 ppm

Storage Conditions: Frozen (-15 to -19 °C); 20, 40, and 54 days; sealed in amber glass bottles

3.4.1.2 Dose Preparation and Storage

The preparation of the dose formulation is described in Section 3.2. Four aliquots of the dose formulation (large enough to almost fill the bottles) were transferred to separate 250-mL wide-mouth amber glass bottles. The bottles were capped, sealed with tape, and stored in a freezer until the date of analysis.

3.4.1.3 Analytical Method

At the end of each designated storage period, a dose formulation aliquot was removed from freezer storage and allowed to warm to room temperature. Triplicate aliquots (10 g) of feed were weighed into scintillation vials and transferred to separate 250-mL wide-mouth amber glass bottles. An aliquot (10 g) of blank feed was weighed into a scintillation vial and transferred to a 250-mL wide-mouth amber glass bottle to serve for dilution of the high dose extracts.

An aliquot (40 mL) of hexane was added to each bottle. The bottles were capped, sonicated for 15 minutes without heat, and shaken on a platform shaker at low setting for 60 minutes. After the feed had settled for at least 5 minutes, an aliquot (approximately 18-20 mL) of each extract was transferred to a separate scintillation vial and centrifuged for 5 minutes. An aliquot (0.350 mL) of each supernatant was transferred to a separate 5-mL volumetric flask. The samples were diluted to volume with blank feed extract and mixed.

An aliquot (1.00 mL) of each dilution above was transferred to a GC autosampler vial containing 0.1 mL of internal standard solution (2.00 mg of diethylphthalate/mL of hexane). The vials were capped, and the samples were mixed prior to gas chromatographic analysis as described in Section 3.1.4.3. The vehicle calibration standards were the method performance evaluation standards described in Section 4.3.1.

The ratio of the area of the butyl benzyl phthalate peak to the area of the internal standard peak was calculated for each injection. The linear regression equation (concentration *versus* peak area ratio) was computed for the vehicle calibration standards data, and the correlation coefficient, r, was determined. See Figure 2 for a plot of the calibration standards data. The concentration of test chemical in each sample was determined from the linear

regression equation, the peak area ratio, a factor to normalize to 10.0 g of feed, and a factor to account for extract dilution.

The results are reported in terms of percent recovery. The values obtained on each day of analysis are compared to those obtained on the reference day in the following way.

$$\%\ Recovery = \frac{concentration\ on\ day\ d}{mean\ concentration\ on\ reference\ day} X\ 100.$$

The reference day samples are the nine homogeneity samples described in Section 3.2.

3.4.1.4 Results of the Dose Storage Stability Study (High Dose - 12000 ppm)

Time (days)	Storage Temperature	Butyl Benzyl Phthalate Found (ppm)[a]	% Recovery[b]
0	NA[c]	11900[d]	NA
20	-15 to -19 °C	11000	92.4
		11100	93.3
		11000	92.4
			$\bar{x}$ = 92.7 (0.6% RSD)
40	-15 to -19 °C	11300	95.0
		11400	95.8
		11400	95.8
			$\bar{x}$ = 95.5 (0.5% RSD)
54	-15 to -19 °C	11200	94.1
		11500	96.6
		11500	96.6
			$\bar{x}$ = 95.8 (1.5% RSD)

[a] Quantitation was based on the linear regression equation $y = 0.001482\ x - 0.004297;\ r = 0.9984.$

[b] $\%\ Recovery = \frac{concentration\ on\ day\ d}{mean\ concentration\ on\ reference\ day} X\ 100;$ the mean concentration on the reference day is 11900 ppm (1.3% RSD, n=9).

[c] Not applicable.

[d] The reference day samples are the nine homogeneity samples described in Section 3.2.

3.4.1.5 Conclusions

The results of the storage stability study indicate that dose formulations of butyl benzyl phthalate in Purina certified ground rodent chow® (No. 5002) at 12000 ppm are stable for at least 54 days when stored in sealed amber glass bottles at freezer temperature (-15 to -19 °C).

3.4.2 Dose Simulation Stability Study (High Dose - 12000 ppm)

This section describes a stability study of butyl benzyl phthalate in Purina certified ground rodent chow® (No. 5002) at 12000 ppm under conditions that simulate dosing of animals.

RTI Notebook No.: 9357 pp.: 85, 88, 104-114

3.4.2.1 Parameters

Vehicle: Purina certified ground rodent chow® (No. 5002)

Nominal Concentration: 12000 ppm

Storage Conditions: Room temperature (21 °C); 5 and 12 days in feeding jars exposed to air and light

3.4.2.2 Dose Preparation and Storage

The preparation of the dose formulation is described in Section 3.2. Three feeding jars were filled almost full with the dose formulation and placed in a fume hood exposed to air and light.

3.4.2.3 Analytical Method

At the end of each designated storage period, triplicate aliquots (10 g) of the feed in one of the feeding jars were weighed into scintillation vials and transferred to separate 250-mL wide-mouth amber glass bottles. An aliquot (10 g) of blank feed was weighed into a scintillation vial and transferred to a 250-mL wide-mouth amber glass bottle to serve for dilution of the high dose extracts. The samples were worked up and analyzed as described in Section 3.4.1.3.

3.4.2.4 Results of the Dose Simulation Stability Study (High Dose - 12000 ppm)

Time (Days)	Butyl Benzyl Phthalate Found (ppm)[a]	% Recovery[b]	Mean % Recovery
0	11900[c]	NA[d]	NA
5	10900	91.6	93.8 (2.3% RSD)
	11200	94.1	
	11400	95.8	
12	11100	93.3	92.7 (0.6% RSD)
	11000	92.4	
	11000	92.4	

[a] Quantitation was based on the linear regression equation $y = 0.001482\,x - 0.004297;\ r = 0.9984.$

[b] $\%\ Recovery = \frac{concentration\ on\ day\ d}{mean\ concentration\ on\ reference\ day} X\ 100;$ the mean concentration on the reference day is 11900 ppm (1.3% RSD, n=9).

[c] The reference day samples are the nine homogeneity samples described in Section 3.2.

[d] Not applicable.

3.4.2.5 Conclusions

Results of the dose simulation stability study indicate that dose formulations of butyl benzyl phthalate in Purina certified ground rodent chow® (No. 5002) at 12000 ppm are stable for at least 12 days under conditions that simulate dosing.

4.0 DOSE FORMULATION PROCEDURES

This section describes procedures for the mixing and storage of formulations of butyl benzyl phthalate in Purina certified ground rodent chow® (No. 5002). Also included are procedures for analyzing dose formulations of butyl benzyl phthalate in the 700 to 12000 ppm concentration range and for evaluating the performance of the dose analysis method.

4.1 Mixing Procedure

The following procedures are recommended for the preparation of 90 kg of a 750 ppm and an 11250 ppm dose formulation of butyl benzyl phthalate in Purina certified ground rodent chow® (No. 5002).

4.1.1 Premix Feed Preparation

To prepare a 750 ppm dose formulation, weigh an aliquot (67.5 g) of the test chemical onto a small amount of premix feed in a 5-gal stainless steel bucket. Mix the chemical into the feed with a stainless steel spoon. Add premix feed in portions until all the premix feed (5 kg) has been used. Take care to break up any lumps that appear.

To prepare a 11250 ppm dose formulation, weigh two aliquots (506.25 g each) of the test chemical onto portions of premix feed in separate 5-gal stainless steel buckets. Mix the chemical into the feed with a stainless steel spoon. Add premix feed to each bucket in portions with mixing until all the premix feed (2 X 5 kg) has been used. Take care to break up any lumps that appear.

4.1.2 Bulk Mixing and Sampling

To prepare a 750 ppm dose formulation, layer a 40-kg portion of blank feed evenly in the blender. Add the premix feed in equal amounts to the left and right blender ports and evenly spread it over the blank feed. Remove residual amounts of the premix feed from the bucket by adding, stirring, and discharging (into the blender) three portions of approximately 1667 g of blank feed. Add a second 40-kg portion of blank feed to the blender in an even layer. Seal the blender ports and begin blending. Operate the blender for 20 minutes.

To prepare an 11250 ppm dose formulation, layer a 37-kg portion of blank feed evenly in the blender. Add the premix feed in equal amounts to the left and right blender ports and evenly spread it over the blank feed. Remove residual amounts of the premix feed from the buckets by adding, stirring, and discharging (into the blender) three portions of approximately 1000 g of blank feed for each bucket. Add a second 37-kg portion of blank feed to the blender in an even layer. Seal the blender ports and begin blending. Operate the blender for 20 minutes.

If different amounts of formulation or different test chemical concentrations are needed, adjust the amount of the test material and Purina certified ground rodent chow® (No. 5002) accordingly.

Collect analytical and archive samples (200 g) from the middle of the feed as it is being discharged into a polyethylene bag. Store analytical and archive samples in a freezer until they are analyzed.

4.2 Storage Conditions

Formulations containing butyl benzyl phthalate in Purina certified ground rodent chow® (No. 5002) at concentrations from 700 to 12000 ppm can be stored for a period of at least 54 days at freezer temperature (-15 to -19 °C) in sealed amber glass bottles. Prior to use, the formulations should be brought to room temperature.

4.3 Dose Analysis

4.3.1 Method Performance Evaluation

RTI Notebook No.: 9357 pp.: 63-64, 68-83, 94-98, 100

This section describes a procedure for evaluating the performance of the analytical method used for dose analysis and the method performance evaluation results. The evaluation was conducted using vehicle standards prepared at 500 to 1200 ppm of test chemical in Purina certified ground rodent chow® (No. 5002). (Extracts of formulations above the calibrated range are diluted into this calibration range with blank feed prior to analysis.) The vehicle standards were used to determine the precision, accuracy, linearity, and recovery for the dose analysis method. The vehicle standards were prepared at six concentrations; the lowest and highest concentration standards were prepared in triplicate, and the four mid-concentration standards were prepared singly. A single blank vehicle sample was prepared. The accuracy and precision for analysis of dose formulations at 12000 ppm were also determined.

4.3.1.1 Preparation of Standard Solutions

An internal standard solution (2.00 mg/mL) was prepared by accurately weighing 100 mg of diethylphthalate (Aldrich Chemical Co.) into a glass scintillation vial, dissolving the material in hexane, quantitatively transferring the solution to a 50-mL volumetric flask, diluting to volume with hexane, and mixing.

A 24.0 mg/mL compound stock solution (Solution A) was prepared by weighing 600 mg of butyl benzyl phthalate into a scintillation vial, dissolving the compound in hexane, transferring the solution quantitatively to a 25-mL volumetric flask, diluting to volume with hexane, and mixing.

A 20.0 mg/mL compound stock solution (Solution B) was prepared by weighing 500 mg of butyl benzyl phthalate into a scintillation vial, dissolving the compound in hexane, transferring the solution quantitatively to a 25-mL volumetric flask, diluting to volume with hexane, and mixing.

The following dilutions were prepared by transferring an aliquot of one of the stock solutions (Solutions A or B) to a separate volumetric flask, diluting to volume with hexane, and mixing.

Dilution	Stock Used	Volume of Stock Added (mL)	Total Volume (mL)	Concentration of the Dilution (mg/mL)
A1	Solution A	5.00	10.0	12.0
B1	Solution B	5.00	10.0	10.0
A2	Solution A	3.50	10.0	8.40
B2	Solution B	3.50	10.0	7.00
A3	Solution A	2.50	10.0	6.00
B3	Solution B	2.50	10.0	5.00

4.3.1.2 Preparation of Vehicle Standards

Eleven aliquots (10 g) of blank Purina certified ground rodent chow® (No. 5002) feed were weighed into a scintillation vial and transferred to separate 250-mL wide-mouth amber glass screw-top bottles. An aliquot (1.00 mL) of one of the test chemical dilutions above (or hexane for the vehicle blank) was added to each feed aliquot as shown in the table below, and the samples were mixed. This procedure produced vehicle standards at the concentrations listed.

Vehicle Standard	Dilution Added	Amount of Dilution or Solution Added (mL)	Concentration in feed (ppm)
MA1a	A1	1.00	1200
MA1b	A1	1.00	1200
MA1c	A1	1.00	1200
MB1	B1	1.00	1000
MA2	A2	1.00	840
MB2	B2	1.00	700
MA3	A3	1.00	600
MB3a	B3	1.00	500
MB3b	B3	1.00	500
MB3c	B3	1.00	500
MBlank	Hexane	1.00	0

An aliquot (39 mL) of hexane was added to each bottle. The bottles were capped, sonicated without heat for 15 minutes, and shaken on a platform shaker at low setting for 60 minutes. The feed was allowed to settle for at least 5 minutes. Approximately 18-20 mL of each extract was transferred to a separate scintillation vial and centrifuged for 5 minutes. An aliquot of each supernatant (1.00 mL) was transferred to a separate GC autosampler vial containing 0.1 mL of internal standard solution (2.00 mg of diethylphthalate/mL of hexane). The vials were capped, and the samples were mixed prior to gas chromatographic analysis as described in Section 4.3.1.5.

4.3.1.3 Preparation of Solvent Standards

An aliquot (1.00 mL) of each test chemical dilution was transferred to a separate 125-mL amber glass wide-mouth bottle, and 39 mL of hexane was added. The solutions were mixed, and an aliquot (1.00 mL) of each solution was transferred to a separate GC autosampler vial containing 0.1 mL of internal standard solution (2.00 mg of diethylphthalate/mL of hexane). The samples were mixed and analyzed by gas chromatography as described in Section 4.3.1.5.

4.3.1.4 Preparation of Samples for Determination of Accuracy and Precision at High Dose Concentrations (12000 ppm)

In order to determine the accuracy and precision for analysis of the test chemical in dose formulations prepared at 12000 ppm, triplicate vehicle standards at 12000 ppm were prepared and analyzed.

Three aliquots (10 g) of blank feed were weighed into a scintillation vial and transferred to separate 250-mL wide-mouth amber glass bottles. An aliquot (5.00 mL) of Solution A was added to each feed sample, and the samples were mixed. This procedure produced vehicle standards containing 12000 ppm of butyl benzyl phthalate in feed. Aliquots of blank feed (2 X 10 g) were weighed into scintillation vials and transferred to separate 250-mL wide-mouth amber glass bottles to serve for dilution of the high dose extracts.

An aliquot (35 mL) of hexane was added to each bottle containing dose formulation at 12000 ppm; 40 mL of hexane was added to each bottle containing blank feed. The bottles were capped, sonicated for 15 minutes without heat, and shaken on a platform shaker at low setting for 60 minutes. The feed was allowed to settle for at least 5 minutes. Approximately 18-20 mL of each extract was transferred to a separate scintillation vial and centrifuged for 5 minutes. An aliquot (0.700 mL) of each supernatant from high dose formulations was transferred to a separate 10-mL volumetric flask. The samples were diluted to volume with blank feed extract and mixed.

An aliquot (1.00 mL) of each dilution above was transferred to a GC autosampler vial containing 0.1 mL of internal standard solution (2.00 mg of diethylphthalate/mL of hexane). The vials were capped, and the samples were mixed prior to gas chromatographic analysis.

4.3.1.5 Gas Chromatographic Analysis

Each vehicle standard and solvent standard was analyzed by gas chromatography in a single injection as described below. See Figure 1 for typical chromatograms of butyl benzyl phthalate extracted from feed.

Instrument: Hewlett-Packard 5890 series II gas chromatograph, 7673 autosampler

Chromatography Data System: Waters Millennium^{32} client/server CIMS, Vs. 3.05

Column: Capillary, J&W DB-1701 (15 m X 0.22 mm ID, 0.25 μ film)

Column Temperature: 160 °C for 5 min, 160 to 240 °C at 20 °C/min, 240 °C for 7 min

Carrier Gas: Helium (0.76 mL/min at 250 °C)

Auxillary Gas: Helium (29.3 mL/min)

Injector Type: Split (1:19), fast injection

Injector Temperature: 250 °C

Injection Solvent: Hexane

Injection Volume: 1 μL

Detector: FID

Detector Temperature: 280 °C

Retention Times: butyl benzyl phthalate, 10.9-12.6 minutes
diethylphthalate (internal standard), 3.2-5.3 minutes

4.3.1.6 Calculations

For each injection, the ratio of the area of the butyl benzyl phthalate peak to the area of the internal standard peak was calculated. The average response (peak area ratio), $\bar{y}$, and the standard deviation, s, were computed for each standard prepared in triplicate. To estimate precision, the percent relative standard deviations (% RSD), were calculated as

$$\%\,RSD = \frac{s}{\bar{y}}\,X\,100.$$

for the standards analyzed in triplicate. The percent recovery was determined by comparing the area ratio for each vehicle standard to that of the corresponding solvent standard.

The linear regression equation (concentration *versus* peak area ratio) for the vehicle standards were computed and the correlation coefficient, r, was determined. The vehicle standards data were plotted for a visual evaluation of linearity. The accuracy of the method was examined by using the regression equation and peak area ratios to compute the concentration of butyl benzyl phthalate found in each vehicle standard. The computed values were divided by the theoretical concentrations, and the results expressed as percentages. Subtracting 100 from these percentages yielded the relative errors.

4.3.1.7 Results

The results of the method performance evaluation are presented in the following table. See Figure 2 for a plot of the vehicle standards data.

Nominal Conc. (ppm)	Instrument Vehicle Standards	Response Solvent Standards	Recovery (%)	Found Conc. (ppm)	Found/ Nominal (%)	Relative Error (%)
1200	1.76	1.79	98.3	1190	99.2	-0.8
1200	1.77		98.9	1200	100	0.0
1200	1.82		102	1230	103	2.5
	$\bar{y}$ = 1.78					
	(1.8% RSD)					
1000	1.45	1.48	98.0	981	98.1	-1.9
840	1.25	1.21	103	846	101	0.7
700	0.990	1.01	98.0	671	95.9	-4.1
600	0.885	0.889	99.6	600	100	0.0
500	0.766	0.736	104	520	104	4.0
500	0.732		99.5	497	99.4	-0.6
500	0.746		101	506	101	1.2
	$\bar{y}$ = 0.748					
	(2.3% RSD)					

Linear regression equation: $y = 0.001482\,x - 0.004297$ where y is peak area ratio and x is concentration.
Correlation coefficient (r): *0.9984*

The % RSD for triplicate analysis at 12000 ppm was 1.7%; the relative error of the mean for analysis at 12000 ppm was 1.7%.

4.3.1.8 Conclusions

The performance of the method was evaluated using standards in the concentration range of 500 to 1200 ppm of butyl benzyl phthalate in feed. (Samples above this range are diluted into this calibration range with blank feed prior to analysis.) Linearity was demonstrated (correlation coefficient of 0.9984) for the vehicle standards data. Accuracy was confirmed with relative errors less than or equal to 4.1%; precision was demonstrated with relative standard deviations less than or equal to 2.3% for triplicate determinations. The accuracy and precision of analysis at 12000 ppm were demonstrated with a relative error of the mean equal to 1.7% and a relative standard deviation for triplicate sample analysis of 1.7%. The limit of detection, estimated as three times the standard deviation of the lowest concentration vehicle standard prepared in triplicate, was estimated to be 35 ppm.

4.3.2 Analytical Method for Dose Analysis

This procedure is recommended for the analysis of dose formulations of butyl benzyl phthalate in Purina certified ground rodent chow® (No. 5002) in the concentration range from 700 to 12000 ppm.

4.3.2.1 Preparation of Standard Solutions

Prepare an internal standard solution (2.00 mg/mL) by weighing 100 mg of diethylphthalate (Aldrich Chemical Co.) into a glass scintillation vial, dissolving the material in hexane, quantitatively transferring the solution to a 50-mL volumetric flask, diluting to volume with hexane, and mixing well.

Prepare a 24.0 mg/mL compound stock solution (Solution A) by weighing 600 mg of butyl benzyl phthalate into a scintillation vial, dissolving the compound in hexane, transferring the solution quantitatively to a 25-mL volumetric flask, diluting to volume with hexane, and mixing well.

Prepare a 20.0 mg/mL compound stock solution (Solution B) by weighing 500 mg of butyl benzyl phthalate into a scintillation vial, dissolving the compound in hexane, transferring the solution quantitatively to a 25-mL volumetric flask, diluting to volume with hexane, and mixing well.

Prepare the following dilutions of the stock solutions (Solution A and B) by transferring an aliquot of one of the stock solutions to a separate volumetric flask, diluting to volume with hexane, and mixing.

Dilution	Stock Used	Volume of Stock Added (mL)	Total Volume (mL)	Concentration of the Dilution (mg/mL)
A1	Solution A	5.00	10.0	12.0
B1	Solution B	5.00	10.0	10.0
A2	Solution A	3.50	10.0	8.40
B2	Solution B	3.50	10.0	7.00
A3	Solution A	2.50	10.0	6.00
B3	Solution B	2.50	10.0	5.00

4.3.2.2 Preparation of Vehicle Standards

Weigh seven 10-g aliquots of blank feed into a scintillation vial and transfer each aliquot to a separate 250-mL wide-mouth amber glass bottle. Add an aliquot (1.00 mL) of hexane or one of the test chemical dilutions above to each sample as shown in the table below, and mix the samples to produce spiked vehicle standards at the concentrations listed. (Alternatively, the method performance evaluation standards described in Section 4.3.1 can be used.)

Vehicle Standard	Dilution Added	Amount of Dilution or Solution Added (mL)	Concentration in feed (ppm)
MA1	A1	1.00	1200
MB1	B1	1.00	1000
MA2	A2	1.00	840
MB2	B2	1.00	700
MA3	A3	1.00	600
MB3	B3	1.00	500
MBlank	Hexane	1.00	0

Add an aliquot (39 mL) of hexane to each bottle. Cap the bottles, sonicate them without heat for 15 minutes, and shake them on a platform shaker at low setting for 60 minutes. Allow

the feed to settle for at least 5 minutes. Transfer approximately 18-20 mL of each extract to a separate scintillation vial and centrifuge for 5 minutes. Transfer an aliquot (1.0 mL) of each supernatant to a separate GC autosampler vial containing 0.1 mL of internal standard solution (2.00 mg of diethylphthalate/mL of hexane). Cap the vials and mix the samples prior to gas chromatographic analysis as described in Section 4.3.1.5.

4.3.2.3 Sample Preparation

Allow all dose formulations to warm to room temperature before proceeding. Weigh triplicate aliquots (10 g) of each formulation into a scintillation vial and transfer each aliquot to a separate 250-mL wide-mouth amber glass bottle. Weigh two aliquots (10 g) of blank feed into a scintillation vial, and transfer the aliquots to separate 250-mL amber glass bottles to serve for dilution of the extracts from high dose formulations.

Add an aliquot (40 mL) of hexane to each bottle, cap the bottles, sonicate the samples without heat for 15 minutes, and shake them on a platform shaker at low setting for 60 minutes. Allow the feed to settle for at least 5 minutes. Transfer approximately 18-20 mL of each extract to a separate scintillation vial, and centrifuge the extracts for 5 minutes.

Transfer an aliquot (1.00 mL) of each supernatant from dose formulations prepared at 0 and 750 ppm to a separate GC autosampler vial containing 0.1 mL of internal standard solution (2.00 mg of diethylphthalate/mL of hexane). Cap the vials and mix the samples.

Transfer an aliquot (1.00 mL) of each supernatant from dose formulations prepared at 3750 ppm to a separate 5-mL volumetric flask, dilute to volume with extract from blank feed, and mix. Transfer an aliquot (1.00 mL) of each dilution to a separate GC autosampler vial containing 0.1 mL of internal standard solution (2.00 mg of diethylphthalate/mL of hexane). Cap the vials and mix the samples.

Transfer an aliquot (0.350 mL) of each supernatant from dose formulations prepared at 11250 ppm to a separate 5-mL volumetric flask, dilute to volume with extract from blank feed, and mix. Transfer an aliquot (1.00 mL) of each dilution to a separate GC autosampler vial containing 0.1 mL of internal standard solution (2.00 mg of diethylphthalate/mL of hexane). Cap the vials and mix the samples.

4.3.2.4 Gas Chromatographic Analysis

Analyze each vehicle standard and dose analysis sample by gas chromatography in a single injection as described in Section 4.3.1.5. See Figure 3 for typical chromatograms of butyl benzyl phthalate dose formulations in feed.

4.3.2.5 Calculations

For each injection, calculate the area ratio of the butyl benzyl phthalate peak to the area of the internal standard peak as

$$peak\ area\ ratio = \frac{area\ of\ butyl\ benzyl\ phthalate\ peak}{area\ of\ internal\ standard\ peak}.$$

Compute the linear regression equation (concentration *versus* peak area ratio) for the vehicle calibration standards data.

Calculate the correlation coefficient, r, for the vehicle standards data.

Plot the data for a visual evaluation of linearity.

Calculate the concentration of butyl benzyl phthalate in the dose formulation samples (ppm) from the peak area ratio for each sample, the linear regression equation, a factor to normalize to 10.0 g of feed, and a dilution factor when appropriate.

4.3.3 Results of Dose Analyses

The analytical method described in Section 4.3.2 was used to determine the butyl benzyl phthalate content of dose formulations prepared on July 5, July 11, August 14, September 11, October 9, and December 4, 2000 and on February 26, 2001 for the Rt00-BBP study. The results of these analyses can be found in the Appendix. See Figure 2 for a plot of the vehicle standards data for instrument calibration and Figure 3 for typical chromatograms.

4.3.4 Conclusions

Dose formulations of butyl benzyl phthalate in feed prepared for the Rt00-BBP study at 750, 3750, and 11250 ppm and used in the animal study were found to contain 90.9 to 107% of the nominal concentrations of test chemical. The relative standard deviations for triplicate analyses were less than or equal to 5.6%. No test chemical was detected in the vehicle blanks (estimated detection limit of 35 ppm).

5.0 CONTRIBUTORS

Personnel contributing to the analysis of butyl benzyl phthalate are listed below.

Dose Formulation Studies and Dose Analyses

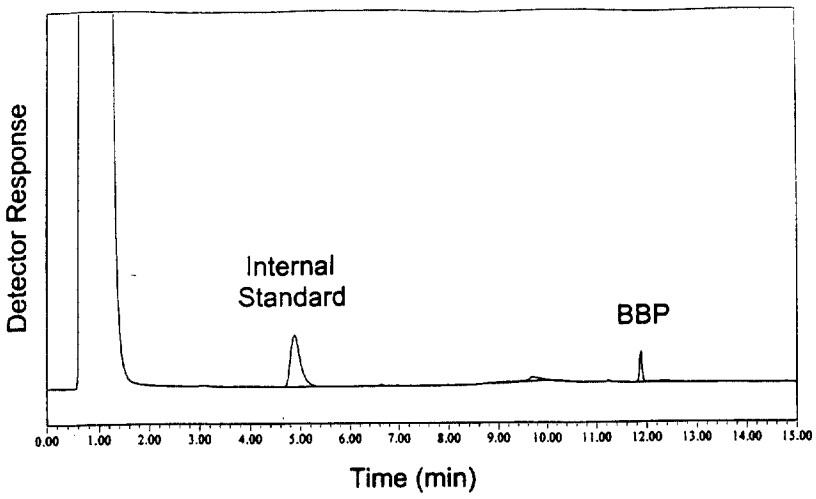
Dolores R. Brine
Graham M. West
Abir K. Khaled

Report Preparation

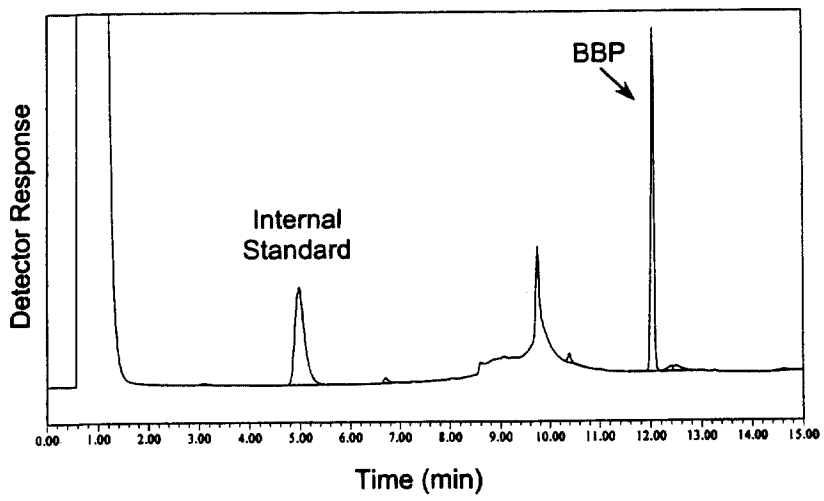
Calliandra B. Harris

Task Leader

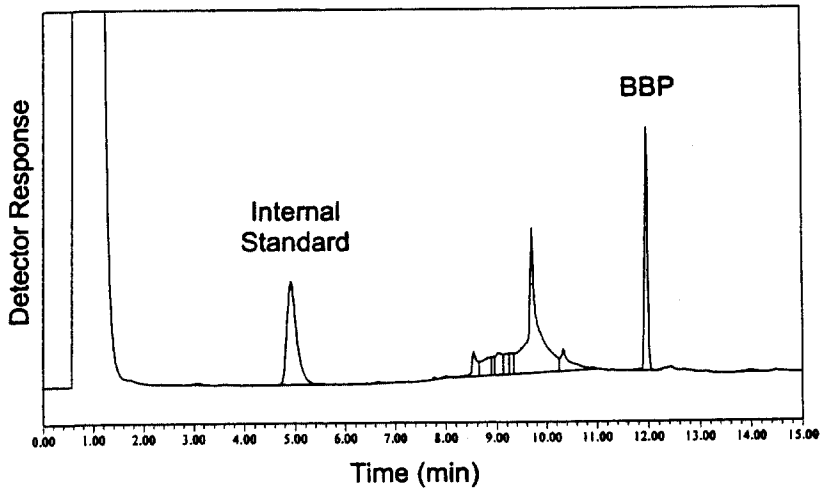
Dolores R. Brine



Homogeneity Sample
Bottom 2 (700 ppm)



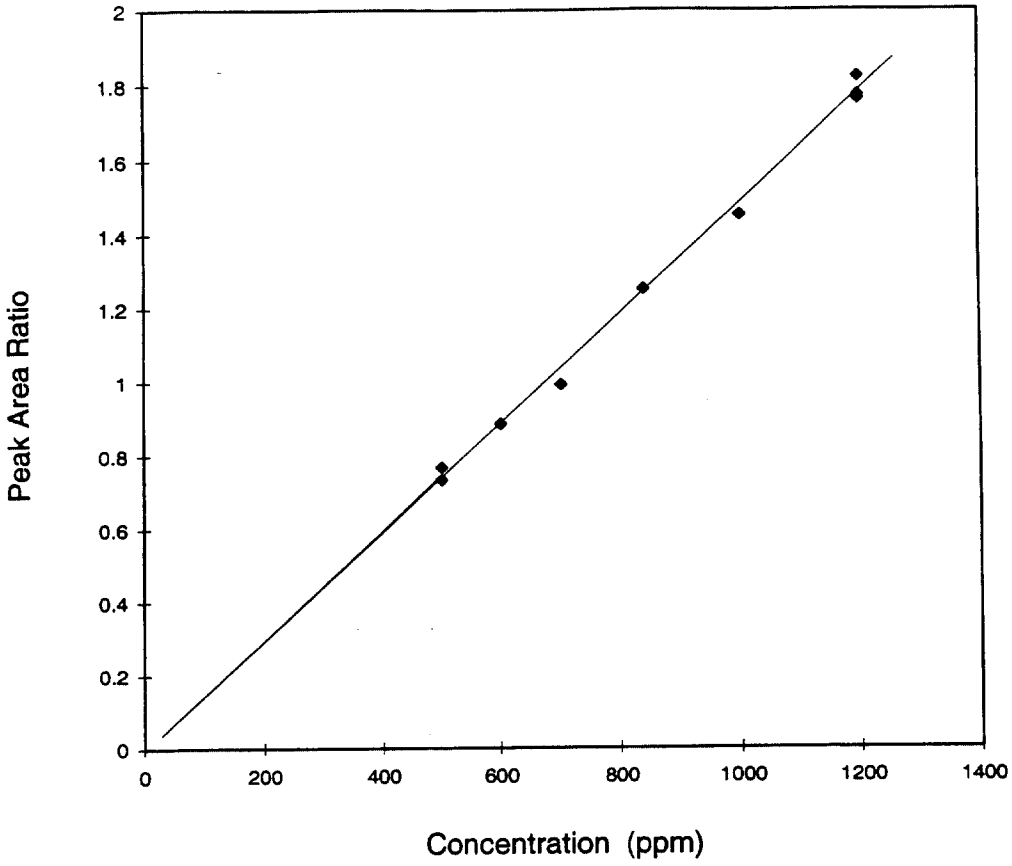
Dose Simulation
Stability
Study Sample 1, Day 5
(12000 ppm)



Vehicle Standard
(500 ppm)

Figure 1. GAS CHROMATOGRAMS OF BUTYL BENZYL PHTHALATE EXTRACTED FROM FEED

Instrument: Hewlett-Packard 5890 series II GC and Millennium^{32} Client/Server CIMS
Column: Capillary, J&W DB-1701
Detector: FID
Column Temperature: 160 °C for 5 min, 160 to 240 °C at 20 °C/min, 240 °C for 7 min



Regression Equation: y = 0.001482 x - 0.004297
Correlation Coefficient: 0.9984

Figure 2. PLOT OF VEHICLE STANDARDS DATA FOR METHOD PERFORMANCE EVALUATION FOR BUTYL BENZYL PHTHALATE IN FEED

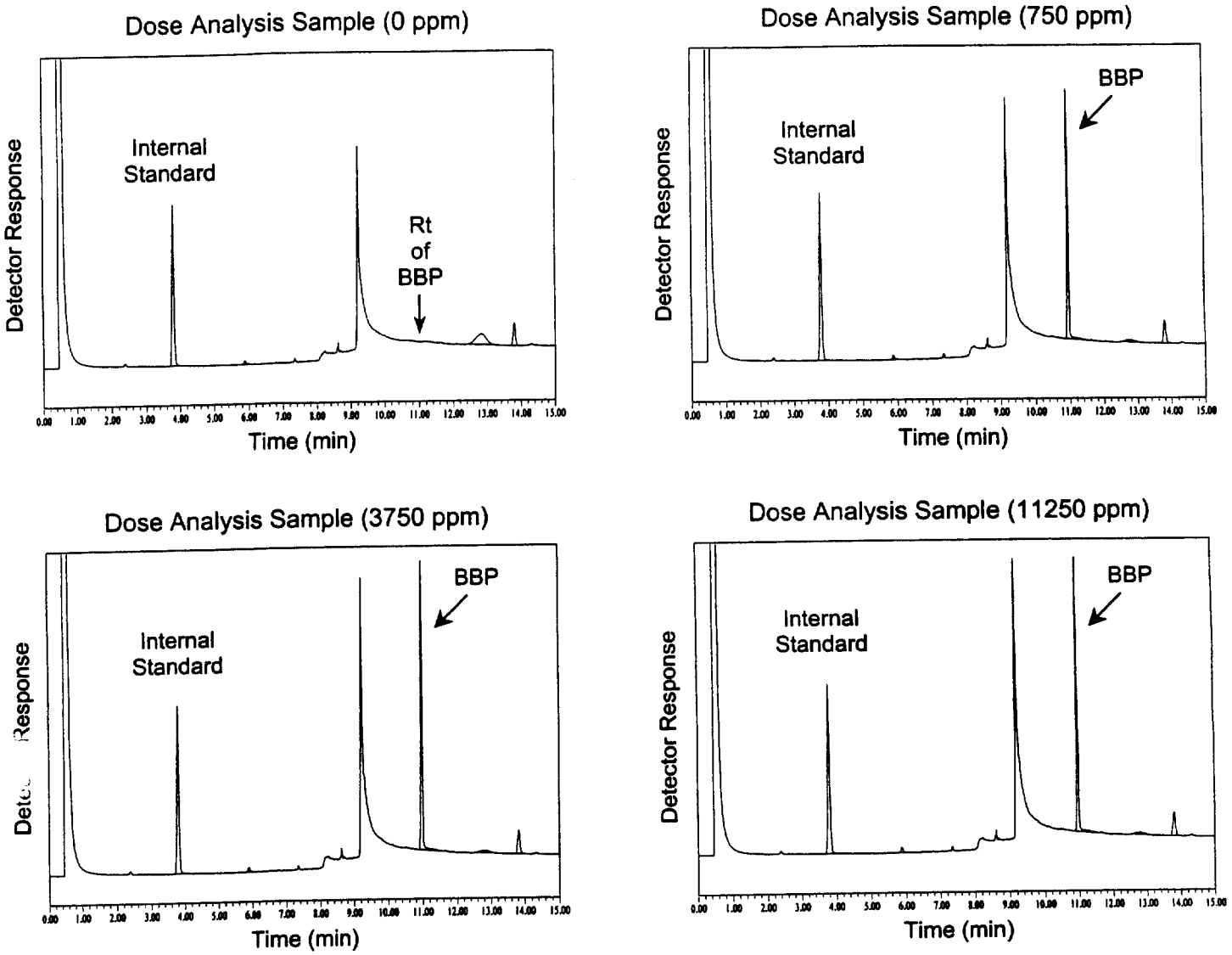


Figure 3. GAS CHROMATOGRAMS OF BUTYL BENZYL PHTHALATE DOSE FORMULATIONS IN FEED

APPENDIX

DOSE ANALYSIS FOR BUTYL BENZYL PHTHALATE IN PURINA CERTIFIED GROUND RODENT CHOW® (No. 5002)

DOSE ANALYSIS RESULTS
BUTYL BENZYL PHTHALATE IN PURINA CERTIFIED RODENT CHOW® (No. 5002)

Mix Date: July 5, 2000
Analysis Dates: July 6-12, 2000
(Draft Date: July 17, 2000)
Date of This Report: August 10, 2001

RTI Project No.: 65C-07269
RTI Master Protocol No.: RTI-761
RTI Study Code: Rt00-BBP
RTI Notebook No.: 9357 pp. 137-154, 164

RTI Log No.	Sample Label	Color Code	Nominal Concentration (ppm)	Approximate Amount Received
9781-10-09	Butyl Benzyl Phthalate in Purina 5002 Project #: 7269-200[a] Rt00-BBP Amount : 200 g Rx: 02322 Date: 07-05-00 Storage: FREEZE	Green	0	200 g
9781-10-10	Butyl Benzyl Phthalate in Purina 5002 Project #: 7269-200[a] Rt00-BBP Amount : 200 g Rx: 15072 Date: 07-05-00 Storage: FREEZE	Yellow	750	200 g
9781-10-11	Butyl Benzyl Phthalate in Purina 5002 Project #: 7269-200[a] Rt00-BBP Amount : 200 g Rx: 27002 Date: 07-05-00 Storage: FREEZE	Red	3,750	200 g
9781-10-12	Butyl Benzyl Phthalate in Purina 5002 Project #: 7269-200[a] Rt00-BBP Amount : 200 g Rx: 66181 Date: 07-05-00 Storage: FREEZE	Blue	11,250	200 g

[a] *Sic.*

Experimental

Concentration of Vehicle Standards: 500, 600, 700, 840, 1000, and 1200 ppm
Calibration Equation: $y = 0.001482\,x - 0.004297$
Correlation Coefficient: 0.9984

Results

The results obtained in this work are shown in the following table. The results were transmitted by electronic mail to Rochelle W. Tyl by Graham M. West on July 12, 2000, at 8:49 AM.

RTI Log No.	Color Code	Nominal Concentration (ppm)	Found Conc. (ppm) (% RSD, n=3)	% of Nominal Concentration Found
9781-10-09	Green	0	ND[a]	NA[b]
9781-10-10	Yellow	750	682 (2.1)	90.9
9781-10-11	Red	3,750	3,570 (5.3)	95.2
9781-10-12	Blue	11,250	9,730 (1.8)	86.5[c]

[a] Not detected. The estimated limit of detection is 35 ppm.

[b] Not applicable.

[c] This value represents the average for six replicate determinations. This dose was deemed unsuitable for use in the animal study.

Analyst: Graham M. West

Signature: [signature] for GMW

Task Leader: Dolores R. Brine

Signature: Dolores R. Brine

DOSE ANALYSIS RESULTS
BUTYL BENZYL PHTHALATE IN PURINA CERTIFIED RODENT CHOW® (No. 5002)

Mix Date: July 11, 2000
Analysis Date: July 13, 2000
(Draft Date: July 17, 2000)
Date of this Report: August 10, 2001

RTI Project No.: 65C-07269
RTI Master Protocol No.: RTI-761
RTI Study Code: Rt00-BBP
RTI Notebook No.: 9357 pp. 155-162, 165

RTI Log No.	Sample Label	Color Code	Nominal Concentration (ppm)	Approximate Amount Received
9781-11-03	Butyl Benzyl Phthalate in Purina 5002 Project #: 7269-200[a] Rt00-BBP Amount : 200 g Rx: 66181 Date: 07-11-00 Storage: FREEZE	Blue	11,250	200 g

[a] *Sic.*

Experimental

Concentration of Vehicle Standards: 500, 600, 700, 840, 1000, and 1200 ppm
Calibration Equation: $y = 0.001482 x - 0.004297$
Correlation Coefficient: 0.9984

Results

The results obtained in this work are shown in the following table. The results were transmitted by electronic mail to Rochelle W. Tyl by Graham M. West on July 13, 2000, at 4:18 PM.

RTI Log No.	Color Code	Nominal Concentration (ppm)	Found Conc. (ppm) (% RSD, n=3)	% of Nominal Concentration Found
9781-11-03	Blue	11,250	12,000 (0.5)	107

Analyst: Graham M. West

Signature: [signature] for GMW

Task Leader: Dolores R. Brine

Signature: Dolores R. Brine

DOSE ANALYSIS RESULTS
BUTYL BENZYL PHTHALATE IN PURINA CERTIFIED RODENT CHOW® (No. 5002)

Mix Date: August 14, 2000
Analysis Dates: August 15-16, 2000
(Draft Date: September 6, 2000)
Date of This Report: August 10, 2001

RTI Project No.: 65C-07269
RTI Master Protocol No.: RTI-761
RTI Study Code: Rt00-BBP
RTI Notebook No.: 9357 pp. 166-181

RTI Log No.	Sample Label	Color Code	Nominal Concentration (ppm)	Approximate Amount Received
9781-12-09	Butyl Benzyl Phthalate in Purina 5002 Project #: 7269-200[a] Rt00-BBP Amount : 200 g Rx: 02322 Date: 08-14-00 Storage: FREEZE	Green	0	200 g
9781-12-10	Butyl Benzyl Phthalate in Purina 5002 Project #: 7269-200[a] Rt00-BBP Amount : 200 g Rx: 15072 Date: 08-14-00 Storage: FREEZE	Yellow	750	200 g
9781-12-11	Butyl Benzyl Phthalate in Purina 5002 Project #: 7269-200[a] Rt00-BBP Amount : 200 g Rx: 27002 Date: 08-14-00 Storage: FREEZE	Red	3,750	200 g
9781-12-12	Butyl Benzyl Phthalate in Purina 5002 Project #: 7269-200[a] Rt00-BBP Amount : 200 g Rx: 66181 Date: 08-14-00 Storage: FREEZE	Blue	11,250	200 g

[a] *Sic.*

Experimental

Concentration of Vehicle Standards: 500, 600, 700, 840, 1000, and 1200 ppm
Calibration Equation: $y = 0.001482 x - 0.004297$
Correlation Coefficient: 0.9984

Results

The results obtained in this work are shown in the following table. The results were transmitted by electronic mail to Rochelle W. Tyl by Graham M. West on August 16, 2000, at 11:09 AM.

RTI Log No.	Color Code	Nominal Concentration (ppm)	Found Conc. (ppm) (% RSD, n=3)	% of Nominal Concentration Found
9781-12-09	Green	0	ND[a]	NA[b]
9781-12-10	Yellow	750	755 (1.9)	101
9781-12-11	Red	3,750	4,020 (1.6)	107
9781-12-12	Blue	11,250	11,000 (0.9)	97.8

[a] Not detected. The estimated limit of detection is 35 ppm.
[b] Not applicable.

Analyst: Graham M. West

Signature: [signature] for GMW

Task Leader: Dolores R. Brine

Signature: Dolores R. Brine

DOSE ANALYSIS RESULTS
BUTYL BENZYL PHTHALATE IN PURINA CERTIFIED RODENT CHOW® (No. 5002)

Mix Date: September 11, 2000
Analysis Dates: September 12-13, 2000
(Draft Date: September 18, 2000)
Date of This Report: August 10, 2001

RTI Project No.: 65C-07269
RTI Master Protocol No.: RTI-761
RTI Study Code: Rt00-BBP
RTI Notebook No.: 9357 pp. 182-194

RTI Log No.	Sample Label	Color Code	Nominal Concentration (ppm)	Approximate Amount Received
9781-13-09	Butyl Benzyl Phthalate in Purina 5002 Project #: 7269-200[a] Rt00-BBP Amount : 200 g Rx: 02322 Date: 09-11-00 Storage: FREEZE	Green	0	200 g
9781-13-10	Butyl Benzyl Phthalate in Purina 5002 Project #: 7269-200[a] Rt00-BBP Amount : 200 g Rx: 15072 Date: 09-11-00 Storage: FREEZE	Yellow	750	200 g
9781-13-11	Butyl Benzyl Phthalate in Purina 5002 Project #: 7269-200[a] Rt00-BBP Amount : 200 g Rx: 27002 Date: 09-11-00 Storage: FREEZE	Red	3,750	200 g
9781-13-12	Butyl Benzyl Phthalate in Purina 5002 Project #: 7269-200[a] Rt00-BBP Amount : 200 g Rx: 66181 Date: 09-11-00 Storage: FREEZE	Blue	11,250	200 g

[a] *Sic.*

Experimental

Concentration of Vehicle Standards: 500, 600, 700, 840, 1000, and 1200 ppm
Calibration Equation: $y = 0.001482 x - 0.004297$
Correlation Coefficient: 0.9984

Results

The results obtained in this work are shown in the following table. The results were transmitted by electronic mail to Rochelle W. Tyl by Graham M. West on September 13, 2000, at 9:23 AM.

RTI Log No.	Color Code	Nominal Concentration (ppm)	Found Conc. (ppm) (% RSD, n=3)	% of Nominal Concentration Found
9781-13-09	Green	0	ND[a]	NA[b]
9781-13-10	Yellow	750	775 (5.6)	103
9781-13-11	Red	3,750	3,890 (0.9)	104
9781-13-12	Blue	11,250	11,600 (0.5)	103

[a] Not detected. The estimated limit of detection is 35 ppm.
[b] Not applicable.

Analyst: Graham M. West

Signature: [signature] for GMW

Task Leader: Dolores R. Brine

Signature: Dolores R Brine

DOSE ANALYSIS RESULTS
BUTYL BENZYL PHTHALATE IN PURINA CERTIFIED RODENT CHOW® (No. 5002)

Mix Date: October 9, 2000
Analysis Dates: October 10-11, 2000
(Draft Date: October 20, 2000)
Date of This Report: August 10, 2001

RTI Project No.: 65C-07269
RTI Master Protocol No.: RTI-761
RTI Study Code: Rt00-BBP
RTI Notebook No.: 9357 pp. 195-200
9965 pp. 1-7

RTI Log No.	Sample Label	Color Code	Nominal Concentration (ppm)	Approximate Amount Received
9781-14-09	Butyl Benzyl Phthalate in Purina 5002 Project #: 7269-200[a] Rt00-BBP Amount : 200 g Rx: 02322 Date: 10-09-00 Storage: FREEZE	Green	0	200 g
9781-14-10	Butyl Benzyl Phthalate in Purina 5002 Project #: 7269-200[a] Rt00-BBP Amount : 200 g Rx: 15072 Date: 10-09-00 Storage: FREEZE	Yellow	750	200 g
9781-14-11	Butyl Benzyl Phthalate in Purina 5002 Project #: 7269-200[a] Rt00-BBP Amount : 200 g Rx: 27002 Date: 10-09-00 Storage: FREEZE	Red	3,750	200 g
9781-14-12	Butyl Benzyl Phthalate in Purina 5002 Project #: 7269-200[a] Rt00-BBP Amount : 200 g Rx: 66181 Date: 10-09-00 Storage: FREEZE	Blue	11,250	200 g

[a] *Sic.*

Experimental

Concentration of Vehicle Standards: 500, 600, 700, 840, 1000, and 1200 ppm
Calibration Equation: $y = 0.001482 x - 0.004297$
Correlation Coefficient: 0.9984

Results

The results obtained in this work are shown in the following table. The results were transmitted by electronic mail to Rochelle W. Tyl by Graham M. West on October 11, 2000, at 10:39 AM.

RTI Log No.	Color Code	Nominal Concentration (ppm)	Found Conc. (ppm) (% RSD, n=3)	% of Nominal Concentration Found
9781-14-09	Green	0	ND[a]	NA[b]
9781-14-10	Yellow	750	740 (1.0)	98.7
9781-14-11	Red	3,750	3,610 (1.2)	96.3
9781-14-12	Blue	11,250	10,500 (1.1)	93.3

[a] Not detected. The estimated limit of detection is 35 ppm.
[b] Not applicable.

Analyst: Graham M. West

Signature: [signature] for GMW

Task Leader: Dolores R. Brine

Signature: Dolores R. Brine

DOSE ANALYSIS RESULTS
BUTYL BENZYL PHTHALATE IN PURINA CERTIFIED RODENT CHOW® (No. 5002)

Mix Date: December 4, 2000
Analysis Dates: December 5-6, 2000
(Draft Date: December 6, 2000)
Date of This Report: August 10, 2001

RTI Project No.: 65C-07269
RTI Master Protocol No.: RTI-761
RTI Study Code: Rt00-BBP
RTI Notebook No.: 9965 pp. 8-20

RTI Log No.	Sample Label	Color Code	Nominal Concentration (ppm)	Approximate Amount Received
9781-16-09	Butyl Benzyl Phthalate in Purina 5002 Project #: 7269-200[a] (Green) Rt00-BBP Amount : 200 g Rx: 02322 Date: 12-04-00 Storage: Freeze	Green	0	200 g
9781-16-10	Butyl Benzyl Phthalate in Purina 5002 Project #: 7269-200[a] (Yellow) Rt00-BBP Amount : 200 g Rx: 15072 Date: 12-04-00 Storage: Freeze	Yellow	750	200 g
9781-16-11	Butyl Benzyl Phthalate in Purina 5002 Project #: 7269-200[a] (Red) Rt00-BBP Amount : 200 g Rx: 27002 Date: 12-04-00 Storage: Freeze	Red	3,750	200 g
9781-16-12	Butyl Benzyl Phthalate in Purina 5002 Project #: 7269-200[a] (Blue) Rt00-BBP Amount : 200 g Rx: 66181 Date: 12-04-00 Storage: Freeze	Blue	11,250	200 g

[a] *Sic.*

Experimental

Concentration of Vehicle Standards: 500, 600, 700, 840, 1000, and 1200 ppm
Calibration Equation: $y = 0.001482\,x - 0.004297$
Correlation Coefficient: 0.9984

Results

The results obtained in this work are shown in the following table. The results were transmitted by electronic mail to Rochelle W. Tyl by Graham M. West on December 6, 2000, at 9:19 AM.

RTI Log No.	Color Code	Nominal Concentration (ppm)	Found Conc. (ppm) (% RSD, n=3)	% of Nominal Concentration Found
9781-16-09	Green	0	ND[a]	NA[b]
9781-16-10	Yellow	750	709 (0.6)	94.5
9781-16-11	Red	3,750	3,800 (0.5)	101
9781-16-12	Blue	11,250	10,700 (1.1)	95.1

[a] Not detected. The estimated limit of detection is 35 ppm.
[b] Not applicable.

Analyst: Graham M. West

Signature: [signature] for GMW

Task Leader: Dolores R. Brine

Signature: Dolores R. Brine

DOSE ANALYSIS RESULTS
BUTYL BENZYL PHTHALATE IN PURINA CERTIFIED RODENT CHOW® (No. 5002)

Mix Date: February 26, 2001
Analysis Dates: February 27- March 1, 2001
(Draft Date: April 6, 2001)
Date of This Report: August 10, 2001

RTI Project No.: 65C-07269
RTI Master Protocol No.: RTI-761
RTI Study Code: Rt00-BBP
RTI Notebook No.: 9965 pp. 21-44

RTI Log No.	Sample Label	Color Code	Nominal Concentration (ppm)	Approximate Amount Received
9781-19-09	Butyl Benzyl Phthalate in Purina 5002 meal (Green) Project #: 7269-200[a] Study Code: Rt00-BBP Amount # : 200 g Rx: 02322 Date: 02-26-00 Storage: FREEZE	Green	0	200 g
9781-19-10	Butyl Benzyl Phthalate in Purina 5002 meal (Yellow) Project #: 7269-200[a] Study Code: Rt00-BBP Amount # : 200 g Rx: 15072 Date: 02-26-00 Storage: FREEZE	Yellow	750	200 g
9781-19-11	Butyl Benzyl Phthalate in Purina 5002 meal (Red) Project #: 7269-200[a] Study Code: Rt00-BBP Amount # : 200 g Rx: 27002 Date: 02-26-00 Storage: FREEZE	Red	3,750	200 g
9781-19-12	Butyl Benzyl Phthalate in Purina 5002 meal (Blue) Project #: 7269-200[a] Study Code: Rt00-BBP Amount # : 200 g Rx: 66181 Date: 02-26-00 Storage: FREEZE	Blue	11,250	200 g

[a] *Sic.*

Experimental

Concentration of Vehicle Standards: 500, 600, 700, 840, 1000, and 1200 ppm
Calibration Equation: $y = 0.001482\,x - 0.004297$
Correlation Coefficient: 0.9984

Results

The results obtained in this work are shown in the following table. The results were transmitted by electronic mail to Rochelle W. Tyl by Abir K. Khaled on March 1, 2001, at 10:28 AM.

RTI Log No.	Color Code	Nominal Concentration (ppm)	Found Conc. (ppm) (% RSD, n=3)	% of Nominal Concentration Found
9781-19-09	Green	0	ND[a]	NA[b]
9781-19-10	Yellow	750	703 (1.0)	93.7
9781-19-11	Red	3,750	3,810 (1.5)	102
9781-19-12	Blue	11,250	10,600 (3.3)	94.2

[a] Not detected. The estimated limit of detection is 35 ppm.
[b] Not applicable.

Analyst: Abir K. Khaled

Signature: Khaled

Task Leader: Dolores R. Brine

Signature: Dolores R. Brine