Two Generation Toxicity Study in Sprague-Dawley Derived Rats with n-Propyl Bromide (nPB)

Summary and Conclusions

Two generations of Sprague-Dawley derived rats (F_0 and F_1) were exposed to n-propyl bromide (nPB) for 6 hours/day, 7 days/week via whole body inhalation for at least 70 days prior to mating, throughout mating, and through most of gestation and lactation. Exposure was temporarily stopped during the parturition period, from gestation day (GD) 20 to postnatal day (PND) 5. F_1 pups were culled on PND 4 and were exposed via lactation until PND 21 and then directly via inhalation until study termination. F_2 pups were exposed via lactation until PND 21 and sacrificed. Targeted ambient air nPB concentrations were 0, 100, 250, 500, and 750 ppm; nominal mean exposure concentrations were measured and shown to be close to the target concentrations: F_0 generation – 0, 99, 252, 505, and 750 ppm, respectively; F_1 generation – 0, 100, 252, and 502 ppm, respectively. No live litters were produced in the F_0 750 ppm generation; therefore, there was no F_1 treatment group exposed to this dose.

Three deaths unrelated to treatment occurred during the experiment: one F_0 500-ppm female and two F_1 males (1 control and 1 at 500 ppm). At 750 ppm, F_0 males displayed a decrease in mean weekly body weights and cumulative body weight gain throughout the study; F_0 females exhibited a reduction in mean body weight during pre-mating weeks 5-10 and decreased cumulative weight gain throughout the pre-mating period. At 500-ppm, F_0 females showed a non-significant reduction in mean body weight, weight gain, food consumption, and food efficiency during pre-mating, gestation, and lactation. Small, nonsignificant reductions in mean weekly body weight gains were also observed in the F_1 males and females in this dose group. Weight loss in the F_0 and F_1 500-ppm females during late gestation was associated with a reduction in the mean litter size.

In the F_0 generation, there was a statistically significant dose-response decrease in male and female fertility indices at 500 and 750 ppm and in the mating index at 750 ppm. Infertility was 100% in the 750 ppm group and 52% in the 500 ppm group. At 500 ppm, the mean number of implantation sites and size of live litters was also statistically significantly reduced. In the F_1 generation, no statistically significant decrease in the fertility or mating index was observed at any dose, although, there was a significant reduction in the number of implantation sites and size of live litters at 500 ppm.

Statistically significant changes in female reproductive parameters included a decrease in absolute and relative ovary weights at 750 ppm in the F_0 generation and an increase in the length of the estrus cycle at $\geq$ 500 ppm in F_0 females. Estrus cycle length was increased at 500 ppm in F_1 females; however, the increase was not reported to be statistically significant relative to controls. Estrus cycling was not observed in 2 F_0 females in the 500 ppm group, 3 F_0 females in the 750 ppm group, 3 F_1 females in the 250 ppm group, and 4 F_1 females in the 500 ppm group. Analysis of estrus cycle length by the study authors excluded ammenorheic females. ICF reanalyzed these data and included data from ammenorheic females in the analysis. Both estrus cycle length and mean number of

estrus cycles within a 3-week period prior to mating (following 7 weeks exposure) were evaluated. The mean number of estrus cycles that occurred within the 3-week period of observation was statistically significantly decreased at ≥ 250 ppm in F_0 females and at 500 ppm in F_1 females. ICF conducted a second analysis in which data from ammenorheic females were excluded; statistically significant decreases occurred at the same doses although the mean number of estrus cycles was slightly increased. ICF considered these findings to be toxicologically significant.

The number of primordial follicles and corpora lutea were compared between high-dose groups (750 ppm for F_0 females and 500 ppm for F_1 females) and controls; a statistically significant increase in primordial follicles and a decrease in corporal lutea were observed. However, these end points were not examined in lower dose groups and thus, a dose-response could not be determined.

Statistically significant changes in male reproductive and spermatogenic endpoints included: (1) decreased sperm motility in the F_0 generation at exposures ≥ 500 ppm, and in the F_1 generation at exposures; (2) decreased number of normal sperm at exposures ≥ 250 ppm in F_0 generation and at 100 and 500 ppm in the F_1 generation; (3) reduction in the absolute weight of the left and right cauda epididymes at exposures ≥ 500 ppm and in the relative weight of the right cauda epididymis at 750 ppm in the F_0 generation, and in the absolute weight of both epididymes at 500 ppm in the F_1 generation; (4) decreased absolute, but not relative, prostate weights in F_0 (≥ 250 ppm) and F_1 (500 ppm) males, and (5) decreased seminal vesicle weights in F_0 males at 750 ppm and F_1 males at 250 ppm males. In general, most of the organ weight changes were in absolute values, and were not statistically significant when weights were expressed relative to body weight, and did not show a clearcut dose-response. No associated macroscopic or microscopic tissue changes were observed except in the prostate gland which showed acute inflammation in F_1, but not F_0, males exposed to 500 ppm. The study authors concluded that the biological significance of these findings was unclear. Although the number of normal sperm in the F_0 generation was statistically decreased in the three highest dose group relative to concurrent controls, these numbers were within the normal background range and thus this finding was not considered to be toxicologically significant.

Statistically significant changes in organ weights that were observed in treated groups relative to controls included (1) decreased absolute brain weight in F_0 (≥ 250 ppm) and F_1 (≥ 100 ppm) males and in F_0 females at 750 ppm and F_1 females at 500 ppm ; (2) decreased absolute pituitary weights in F_0 (750 ppm) and F_1 (500 ppm) males, and (3) increased absolute and relative thymus weights in F_1 males. Most of these changes did not show a clear dose-response and none were associated with pathologic or histopathologic changes. Therefore, the authors concluded that the biological significance of these findings was unclear. Relative kidney weights were increased in F_0 males and females at 750 ppm and absolute kidney weight was increased in F_1 males at 500 ppm. Microscopic changes in the kidney were also observed and included significant increases in the incidence of minimal-to-mild pelvic mineralization, and/or secondary transitional epithelial hyperplasia following exposure to ≥ 500 ppm in F_0 females and increased mononuclear cell infiltrate at 500 ppm in F_1 males; however, most of these

effects did not show a clear dose-response and were not considered to be biologically significant by the study authors. Hepatic effects were also observed in both F_0 and F_1 males and females. An increase in mean relative liver weight occurred in F_0 animals at exposures ≥ 500 ppm and in F_1 animals at 500 ppm. Histopathology was observed in the F_1 generation and consisted of an increased incidence of minimal-to-mild centrilobular hepatocellular degeneration at ≥ 250 ppm in males and at 500 ppm in females. These findings were considered to be toxicologically significant. An increase in hepatic glycogen was also observed at 500 ppm in F_1 males and ≥ 100 ppm in F_2 females; however, the biologic significance of this result is unclear.

Based on decreased sperm motility in F_1 males, decreased mean number of estrus cycles in F_0 females, and mild liver histopathology, the study NOAEL is 100 ppm, with a LOAEL of 250 ppm. A summary of reproductive and developmental NOAELs for toxicologically-relevant end points for F_0, F_1, and F_2 generations is presented in Table S.1. One selected reproductive endpoint below is post-natal weight for F_1 and F_2 generation pups at certain exposure levels. Pup weight was measured every seven days and compared with controls. A statistically significant decrease in weight was observed for both generations of the 500 ppm females' pups, on post-natal day (PND) 28 in the F_1 generation and PND 14 and 21 in the F_2 generation.

Table S.1 Selected Reproductive and Related Endpoints for F_0, F_1, and F_2 Generations

F_0 Generation

Reproductive Endpoints		
Female Endpoints (Measure)	**NOAEL (ppm)**	**LOAEL (ppm)**
Decrease in fertility Index (%)	250	500**
Increase in mean estrus cycle length (days)[b]	250	500*
Decrease in mean no. of estrus cycles within 3-week period prior to mating – all females (N)[c]	100	250**
Decrease in mean no. of estrus cycles within 3-week period prior to mating – excluding acyclic females (N)[c]	100	250**
Decrease in mean no. implantation sites (N/animal)	250	500**
Decrease in litter size (N/litter)	250	500**
Male Endpoints	**NOAEL (ppm)**	**LOAEL (ppm)**
Decrease in fertility index (%)	250	500**
Decrease in sperm number in left. epididymis (no. sperm x 10^6/gram tissue)	500	750**
Decrease in motile sperm (%)	250	500**

Selected Organ Weights (n=25)[a]		
Male	**NOAEL (ppm)**	**LOAEL (ppm)**
Final mean body wt (g) (mean % decrease)	500	750**
Decrease in relative left testis (g/100 g)	500	750*
Decrease in absolute right cauda epididymis (g)	250	500**
Decrease in relative right cauda epididymis (g/ 100 g)	500	750**
Decrease in absolute left cauda epididymis (g)	250	500**
Decrease in absolute prostate gland (g)	100	250**
Female[a]	**NOAEL (ppm)**	**LOAEL (ppm)**
Decrease in relative liver (g/100 g)	500	750**
Decrease in relative kidney (g/100 g)	500	750**
Decrease in absolute ovary (g)	500	750**
Decrease in relative ovary (g/100 g)	500	750**

Other Important Endpoints	**NOAEL (ppm)**	**LOAEL (ppm)**
Liver histopathology	100	250*

F_1 Generation

Reproductive Endpoints		
Female Endpoint (Measure)	**NOAEL (ppm)**	**LOAEL (ppm)**
Mean length of estrus cycles (days)	500	N/A
Decrease in mean no. of estrus cycles within 3 weeks -all females[c]	250	500*
Decrease in mean no. of estrus cycles within 3 weeks -excluding acyclic females[d]	250	500*
Decrease in Implantation sites	250	500 **
Decrease in number of pups born	250	500 **
Male Endpoint	**NOAEL (ppm)**	**LOAEL (ppm)**
Decrease in motile sperm (%)	100	250*

Other Important Endpoints	**NOAEL (ppm)**	**LOAEL (ppm)**
Liver histopathology	250	500*

Selected Organ Weights (n=25)[a]		
Male (Measure)	**NOAEL (ppm)**	**LOAEL (ppm)**
Decrease in relative liver (g/100 g)	250	500 **
Decrease in absolute right cauda epididymis (g)	250	500**
Decrease in absolute left cauda epididymis (g)	250	500**

Pup Weight for F1	**NOAEL (ppm)**	**LOAEL (ppm)**
Decreased pup weight at postnatal day 28	250	500**

F_2 Generation

Pup Weight for F2	**NOAEL (ppm)**	**LOAEL (ppm)**
Decreased pup weight at postnatal day 14 and 21	250	500**

N/A = not available
a There were no statistically significant changes in mean final body weights of in any of the dose groups in either generation, with the exception of 750-ppm F_0 males.
[b] Statistical analysis calculated by ICF
[c] Calculated by ICF Poisson regression model
[d] Calculated by ICF excluding females that did not have a full estrus cycle and analyzed using a quasi-likelihood Poisson-like regression model
*Significantly different from control, $p<0.05$.
**Significantly different from control, $p<0.01$.
*** Significantly different from control, $p<0.001$.

Materials and Methods

In a two-generation reproductive toxicity study (Stump, 2001), groups of 25 male and female rats (Crl:CD®(SD) IGS BR) were exposed to 99.8% pure n-propyl bromide (nPB) via whole-body inhalation for 6 hours/day, 7 days/week at target concentrations of 0, 100, 250, 500 or 750 ppm. Compound impurities were as follows: 0.015% 2-bromopropane; unknown propyl ether 0.002%; 0.017% propyl ether; 0.004% 1,2-dibromopropane; 0.062% unknown 1,2-dibromopropanes; 0.046% unknown 1,2,3-tribromopropanes. These values are within the Office of Prevention, Pesticides and Toxic Substances (OPPTS) health effects guideline standards of purity and stability (OPPTS, 1998).

The animals were acclimatized for 15 days after receipt; all animals were housed singly except at mating and weaning. Animal husbandry and care were in accordance with appropriate OPPTS health effects harmonized guidelines (OPPTS, 1998). Exposure chambers were constructed of wire mesh and the animals were not given either water or food during exposures periods. The concentrations were monitored during exposure and

the measured mean exposure concentrations were as follows: F_0 generation – 0, 99, 252, 505, and 750 ppm; F_1 generation – 0, 100, 252, and 502 ppm. There was no 750 ppm group for the F_1 generation because no live litters were produced at this exposure level in the F_0 generation. Homogeneity of the test article's distribution within 4 different areas in the exposure chamber was measured during method development prior to exposures. The test article was distributed evenly in the exposure chamber, with a mean percent deviation from the reference concentration ranging from – 4.6% to + 4.3%.

The F_0 generation was ~ 6 weeks old at the start of exposure and exposed for 70 days prior to mating (1:1). Exposure to male animals of both generations continued throughout mating to the day prior to study termination. Exposure to female animals in both generations continued throughout mating, from gestation day (GD) 1- 21, and from postnatal (PND) 4 through the day prior to study termination. Thus, exposure was temporarily stopped around the time of parturition (GD 21-PND4). In the F_1 generation, pups were directly exposed via whole body inhalation to nPB on PND 22, following weaning on PND 21. Selected F_1 generation animals (4 pups/sex/litter, culled on PND 4) were exposed for 70 days prior to mating; females were not exposed during the period GD 21-PND 4. F_2 pups were exposed via lactation until PND 21 and sacrificed at that time.

All animals were observed twice daily for clinical signs, appearance, behavior, and mortality. Body weights, food consumption values were recorded daily. Developmental landmarks (balanopreputial separation and vaginal patency) were also evaluated. Culled F_1 pups were necropsied on PND 21 or 28 and culled F_2 pups were necropsied on PND 21. Parental animals were necropsied after weaning and selected organs were weighed and/or examined macroscopically and microscopically. Measured reproductive endpoints included sperm motility, morphology and numbers for males in all dose groups and ovarian primordial follicle counts and corpora lutea counts in the control and high-dose F_0 and F_1 females. Estrus cycle stage and length were monitored during the 3-week period prior to mating (following 7 weeks exposure) in both F_0 and F_1 groups

Statistical Analyses

Most analyses by Stump were conducted using two-tailed tests for a minimum significance level of 5% comparing each treated group to the control group. Each mean was presented with the standard deviation and the number of animals used to calculate the mean. Data obtained from nongravid animals were excluded from statistical analyses following the mating period. Statistical analyses were not performed when weekly food or body weight data for one or more animals were not available because the animals remained in the lactation phase. Statistical tests were performed using appropriate computing devises or programs and are referenced in the report tables. The following analyses used different statistical tests:

Table 1. Statistical Tests Used in Analyses

Statistical Test	Parameter
Chi-square test with Yates correction factor	Parental Mating and Fertility Indices
One-way ANOVA with Dunnet's Test	Parental Weekly Body Weights and Weight Changes, Gestation and Lactation Body Weights, Parental Food Consumption, Food Efficiency, Gestation length, Pre-coital Interval, Implantation Sites, Unaccounted Sites, Offspring Weights and Weight Changes, Absolute and Relative Organ Weights, Live Litter Size, Sperm Production Rate, Epididymal and Testicular Sperm Numbers, Ovarian Primordial Follicle Counts, Number of Pups Born, Balanopreputial Separation, Vaginal Patency
Kruskal-Wallis Test with Mann-Whitney U-Test	Sperm Motility, Percent Morphologically Normal Sperm, Pup Sexes at birth (% Males per litter), Proportional Postnatal Survival
Fisher's Exact Test	Histopathological Findings

Study Results

F_0 Generation

One 500-ppm F_0 female was euthanized *in extremis* due to an abnormality of the eye that was not considered to be treatment-related. No clinical signs of toxicity were noted that were considered to be treatment-related. In the F_0 animals, mean body weights in the 750-ppm males were reduced by 5.4-12.7% from week 3 through study termination (at week 19), which correlated with sporadic decreases in weekly weight gains and food efficiency. The 750-ppm males displayed reduced cumulative weight gains throughout the study. Generally, the 750-ppm F_0 males and females displayed comparable food consumption as compared to controls, showing only sporadic reductions in food efficiency. The mean body weight of 750-ppm females was reduced by 5.0-5.8% during weeks 5-10, as compared to controls, with associated reductions in cumulative weight gains during the pre-mating period. The 750-ppm females were never pregnant; therefore no data on weight and weight gain during parturition and/or lactation were available. The 500-ppm males displayed a general decrease in weight (4.7-6.3% during study weeks 12-19) and weight gain, but the data were only sporadically statistically different from controls. The 500-, 250-, and 100-ppm females and 250- and 100-ppm males did not display a treatment-related reduction in weight, weight gain, food consumption, or food efficiency. During gestation, a reduction in weight gain was observed on GD 11-14, 14-20, and 0-20 for the 500- and 250-ppm females. However, the only statistically

significant finding was a decrease at 500 ppm on GD 14-20 and GD 0-20; these results correlated with a statistically significant reduction in overall food consumption during these periods. Reduced gestation weights were statistically significant in the 500-ppm females on GD 14 and 20, and were attributed to a reduction in litter size. During lactation, slight nonsignificant reductions in body weight were observed in the 500-ppm females (Lactation Day (LD) 1-4 and LD 7-14).

Table 2. F_0 Females Reproductive Endpoints[a]

Endpoint	0 ppm	100 ppm	250 ppm	500 ppm	750 ppm
Fertility index (%) N	92.0 25	100 25	88.0 25	52.0** 25	0.0** 25
Mating index (%) N	96.0 25	100 25	100 25	84.0 25	68.0* 25
Evidence of mating w/out delivery (no.)	1	0	3	10	17
Estrus cycle length (days)[b] N	4.2±0.49 25	4.5±1.05 25	4.7±0.90 25	5.5±2.17* 23	5.6±1.79* 22
Mean no. of estrus cycles within 3 weeks[c] N	3.96±0.54 25	3.84±0.62 25	3.52±0.65** 25	2.88±1.17*** 25	2.56±1.26*** 25
Mean no. of estrus cycles within 3 weeks excluding ammenorheic females[d] N	3.96±0.54 25	3.84±0.62 25	3.52±0.65** 25	3.00±1.02*** 24	2.78±1.04*** 23
Implantation sites N	15.3±2.53 23	14.3±3.09 25	13.8±4.23 22	9.0±4.54** 11	NA
Number of born N	15.0±2.42 23	13.6±3.09 25	12.5±4.27 22	8.5±4.41** 11	NA
Unaccounted Sites N	0.3±0.57 23	0.7±0.95 25	1.3±1.36** 22	0.5±0.69 11	NA

[a] Data were provided on pp. 123-124, 207
[b] Statistical analysis calculated by ICF
[c] Calculated by ICF and analyzed using a quasi-likelihood Poisson-like regression model
[d] Calculated by ICF excluding females that did not have a full estrus cycle and analyzed using a quasi-likelihood Poisson-like regression model
*Significantly different from control, $p<0.05$.
**Significantly different from control, $p<0.01$.
*** Significantly different from control, $p<0.001$.

Table 3. Primordial Follicles and Corpora Lutea[a] for the F_0 Animals

N=25	0 ppm	500 ppm	750 ppm
Primordial follicles	107.8±55.80	NA	134.2±58.29
Corpora lutea	178.9±51.61	156.8±56.03	126.9±67.49**

[a] Data were derived from pp. 245 of the study report
**Significantly different from control, $p<0.01$.

A decrease in reproductive performance was statistically significant in the 500- and 750-ppm animals with fertility indices (both male and female) of 52.0 and 0.0%, respectively. No live litters were produced at 750 ppm. Historical control fertility indices for males

and females were 89.3% and 90.4%, respectively. Mating indices (both male and female) for the 500- and 750-ppm animals were 84.0% and 68.0%, respectively; however, only the 750-ppm value reached statistical significance as compared to controls (96.0%) and was lower than historical control data (96.0 and 97.0% for males and females, respectively). An increase in the number of females that displayed evidence of mating without delivery was observed and 2 females from the 500-ppm group were nongravid (i.e., showed no evidence of implantation). The mean number of days between pairing and coitus were extended in the 500- and 750-ppm groups, to 4.3 and 4.8 days, respectively, as compared to concurrent control (mean of 3.4 days) and historical control mean of 2.0-3.5 days) data. The mating interval could not be determined for 1 female in the control group, 4 females in the 500 ppm group, and 8 females in the 750-ppm group, because there was no evidence of mating. Implantation sites were reduced in the 500-ppm females and the mean number of unaccounted-for sites was comparable to controls. An increase in the number of unaccounted- for sites in the 250-ppm females was statistically significant with a slight decrease in the number of implantation sites; however, a clear dose-response was not apparent.

An increase in estrus cycle length was statistically significant in the 500- and 750-ppm females; the data are summarized in Table 2. However, the estrus cycle data reported by the study authors excluded females who showed no evidence of cycling (2 and 3 for the 500- and 750-ppm groups, respectively). Therefore, ICF evaluated the individual data by (1) counting the number of estrus cycles within the three-week period prior to mating; (2) including females who did not complete an estrus cycle; and (3) conducting statistical analysis of dose-response using a Poisson regression model. ICF also analyzed the F_1 generation estrus cycle data in a similar manner (See F_1 generation section for these results). A dose-dependent decrease in the number of estrus cycles was observed in females exposed to ≥ 250-ppm. For comparison purposes, ICF conducted a second analysis of the estrus cycle data, excluding acyclic females. Although exclusion of these individuals skewed the data, the statistical significance of the findings stayed at the same concentration levels. A similar dose response was observed that was significantly higher than controls at ≥ 250 ppm.

Table 4. F_0 Males Reproductive Endpoints[a]

Endpoint	0 ppm	100 ppm	250 ppm	500 ppm	750 ppm
Fertility index (%) N	92.0 25	100 25	88.0 25	52.0** 25	0.0** 25
Mating index (%) N	96.0 25	100 25	100 25	84.0 25	68.0* 25
Evidence of mating w/out delivery (no.)	1	0	3	8	17
L. Epididymis (no. sperm in million/gram tissue) N	471.9±81.11 25	459.4±101.94 25	480.1±80.21 25	429.3±101.46 25	369.6±90.66** 25
Motile sperm (%) N	86.8±11.90 25	88.8±7.22 25	83.4±10.41 25	71.9±9.27** 23	53.2±19.59** 15
Normal sperm N	99.7±0.6 25	99.7±0.52 25	99.3±0.83* 25	98.2±2.59** 24	90.6±8.74** 24
Normal shaped head	0.2±0.46	0.2±0.36	0.4±0.54	1.4±2.15	4.8±4.28

Endpoint	0 ppm	100 ppm	250 ppm	500 ppm	750 ppm
separated from flagellum N	 25	 25	 25	 24	 24
Head absent from flagellum N	0.1±0.22 25	0.1±0.24 25	0.3±0.46 25	0.5±0.77 24	4.6±5.91 24

[a] Data were provided on pp. 123, 195, 197-198
*Significantly different from control, $p<0.05$.
**Significantly different from control, $p<0.01$.

Spermatogenic analysis showed a reduction in sperm motility and in the mean number of morphologically normal sperm from the 750-ppm males. There were two types of sperm abnormalities; one in which abnormal heads were separated from the flagellum and another in which normal flagella were without the head. The 500-ppm males displayed a reduction in sperm motility and number of morphologically normal sperm cells, similarly abnormal sperm cell types. The number of normal sperm was statistically decreased in the 250-ppm males; however, they were very close to control values and not considered to be toxicologically significant. Relative to controls, the right epididymes were small on two 750- and one 500-ppm males. A 750-ppm male had a small left epididymis. The testes were small in one 500- and 750-ppm males and one 500-ppm male had soft testes.

Table 5a. Selected F_0 Male Organ Weights (n=25)[a]

Organ	0 ppm	100 ppm	250 ppm	500 ppm	750 ppm
Final Body Wt (g)	548±56.0	526±52.3	530±63.3	515±61.3	483±44.6**
Brain (g)	2.19±0.091	2.15±0.114	2.08±0.087**	2.10±0.177*	2.05±0.091**
Relative brain (g/100 g)	0.403±0.0401	0.411±0.0384	0.398±0.0554	0.410±0.0446	0.428±0.0453
Liver (g)	20.09±3.206	18.63±3.008	19.42±2.934	20.85±4.147	21.25±2.953
Relative liver (g/100 g)	3.662±0.4292	3.538±0.4238	3.675±0.4573	4.034±0.4949*	4.401±0.4206**
Kidney (g)	3.77±0.418	3.58±0.358	3.59±0.274	3.62±0.334	3.62±0.329
Relative kidney (g/100 g)	0.690±0.0649	0.684±0.0654	0.684±0.0629	0.708±0.0862	0.7454±0.0706**
Spleen (g)	0.91±0.167	0.86±0.144	0.85±0.120	0.87±0.110	0.92±0.157
Relative spleen (g/100 g)	0.167±0.0291	0.164±0.0231	0.162±0.0212	0.170±0.0231	0.192±0.0353**
L. testis (g)	1.79±0.186	1.79±0.217	1.69±0.146	1.77±0.227	1.75±0.188
Relative L. testis (g/100 g)	0.329±0.0413	0.343±0.0474	0.323±0.0422	0.348±0.0666	0.364±0.0426*
R. Cauda Epididymis (g)	0.3327±0.03631	0.3311±0.4453	0.3053±0.04188	0.2912±0.05206**	0.2405±0.04804**
R. Cauda Epididymis (g/ 100 g)	0.61±0.0096	0.064±0.0121	0.059±0.0098	0.057±0.0120	0.050±0.0097**
L Cauda Epididymis (g)	0.3252±0.03673	0.3242±0.03149	0.3050±0.03556	0.2877±0.03170**	0.2401±0.03529**
L. Cauda	0.060±0.0100	0.062±0.0087	0.058±0.0096	0.056±0.0084	0.050±0.0068

Organ	0 ppm	100 ppm	250 ppm	500 ppm	750 ppm
Epididymis (g/ 100 g)					
Prostate (g)	1.33±0.247	1.27±0.241	1.14±0.169**	1.14±0.232*	1.14±0.178**
Prostate (g/ 100 g)	0.244±0.0525	0.245±0.0597	0.218±0.0442	0.224±0.0503	0.237±0.0405

[a] Data were provided on pp.208-211 and 214-216 of the study report
*Significantly different from control, $p<0.05$.
**Significantly different from control, $p<0.01$.

A decrease in the absolute brain weight was statistically significant in males at ≥250 ppm as compared to controls; the study authors attributed this finding to a decrease in body weight and did not consider it to be toxicologically significant. However, the extent of the decrease in brain weight prompted further histopathological examinations of the brain to determine if any lesions could explain the weight loss. No corresponding lesions were found. A decrease in the absolute adrenal and relative spleen weights of the 750-ppm males was statistically significant; these decreases were also attributed to general weight loss relative to controls as no macroscopic or microscopic lesions were noted at necropsy.

Decreases in the absolute prostate (≥250 ppm), right epididymis (≥500 ppm), pituitary (750 ppm), and seminal vesicles/coagulating gland (750 ppm) weights were statistically significant although relative weights of these organs/tissues were not. Further, none of these decreases were dose related. A decrease in the left and right cauda epididymes absolute weights was statistically significant as compared to the controls. These decreases were considered to be treatment-related based on that spermatogenic effects observed in the 500- and 750-ppm males. Other organ weight changes included an increase in the relative left testis weight (750 ppm) and in relative liver (≥500 ppm), kidney (750 ppm), and spleen (750 ppm) weights as compared to concurrent controls. The liver and kidneys displayed microscopic lesions; these findings are summarized in Table 5a and 5b and discussed further below.

Table 5b. Selected F_0 Female Organ Weights (n=25)[a]

Organ	0 ppm	100 ppm	250 ppm	500 ppm[b]	750 ppm
Final Body Wt (g)	331±20.7	330±22.3	327±24.8	332±38.3	319±25.5
Brain (g)	1.96±0.078	1.92±0.094	1.94±0.084	1.89±0.105*	1.86±0.072**
Relative brain (g/100 g)	0.595±0.0446	0.585±0.0458	0.595±0.0454	0.577±0.0674	0.586±0.0431
Liver (g)	12.55±1.292	13.08±1.443	12.91±1.199	12.86±1.595	13.70±1.735
Relative liver (g/100 g)	3.800±0.3414	3.958±0.3716	3.948±0.2402	3.888±0.3862	4.302±0.4402**
Kidney (g)	2.22±0.211	2.27±0.244	2.25±0.177	2.27±0.217	2.37±0.253
Relative kidney (g/100 g)	0.672±0.0548	0.686±0.0660	0.692±0.0734	0.688±0.0646	0.746±0.0861**
Ovaries (g)	0.1227±0.02592	0.1265±0.02404	0.1152±0.02360	0.1119±0.01514	0.09575±0.0.02798**
Relative	0.037±0.0078	0.038±0.0068	0.035±0.0072	0.034±0.0056	0.031±0.0079**

Organ	0 ppm	100 ppm	250 ppm	500 ppm[b]	750 ppm
ovaries (g/100 g)					

[a] Data were provided on pp.212-213 and 218-219 of the study report
[b] N=24
*Significantly different from control, p<0.05.
**Significantly different from control, p<0.01.

An increase in the relative liver and kidney weights was statistically significant in the 750-ppm females. The 750-ppm females also displayed a significant decrease in relative ovary weights that correlated with the infertility of these animals. The 750-ppm females displayed a decrease in absolute brain weight that was attributed to the decreased weight of these animals and was not considered by the study authors to be toxicologically significant. A dose-dependent decrease in absolute (22%) and relative ovary weights (16%) was observed in the 750-ppm females, relative to controls.

Table 6. Selected Histopathology Data for the F_0 Animals (n=25)[a]

	Male					Female				
	0 ppm	**100 ppm**	**250 ppm**	**500 ppm**	**750 ppm**	**0 ppm**	**100 ppm**	**250 ppm**	**500 ppm[b]**	**750 ppm**
Kidneys										
Pelvic Mineralization	1	0	1	2	6	2	3	5	12*	14*
Transitional Cell Hyperplasia	0	0	1	3	4	1	0	2	6*	5
Testes										
Seminiferous Tubules Degeneration	1	2	0	3	6	N/A				
Ovaries										
Decreased Corpora Lutea	N/A					3	0	3	6	11*
Luteinized Follicular Cyst	N/A					2	4	3	5	9*
Follicular Cyst	N/A					7	1*	3	3	6
Interstitial Hyperplasia	N/A					3	0	0	3	7
Liver										
Centrilobular Hepatocellular Vacuolation	0	0	7*	22*	24*	0	0	0	6*	16*
Increased Glycogen	14	14	20	21	24*	15	18	22	23*	23*

[a] Data were derived from pp. 227-244 of the study report
*Significantly different from control, p<0.05.

The males manifested a slight nonsignificant increase in minimal mineralization of the pelvic kidney and the females displayed a minimal-to-mild increase that was statistically significant at ≥500 ppm. The kidneys of treated males had a slight nonsignificant increase in the incidence of minimal transitional cell hyperplasia. The females displayed a more pronounced effect and the incidence for the 500-ppm females was statistically significant from controls. However, statistical significance for this end point was not achieved at 750 ppm, indicating a lack of dose-response. Statistically significant increases in the

incidence of mild-to-minimal centrilobular hepatocellular vacuolation was noted in males (≥250 ppm) and females (≥500 ppm). An increase in liver glycogen was statistically significant for males at 750 ppm and for females at ≥500 ppm.

Increases in incidence and severity of seminiferous tubules degeneration were noted in the treated males at ≥500 ppm; although these incidences were not statistically significantly different from controls, the study authors considered these changes to be treatment-related because of spermatogenic effects observed at these doses. A statistically significant decrease in corpora lutea and an increase in luteinized follicular cysts were observed in the ovaries of 750 ppm females. Microscopic determination of the number of primordial follicles and corpora lutea in the ovaries are presented in Table 6; a significant increase in primordial follicles and decrease in corpora lutea was observed at 750 ppm, relative to controls. However, lower doses were not analyzed for these end points; therefore, no dose-response conclusions can be made.

F_1 Generation

One control male (at week 22) and one 50-ppm male (at week 18) were euthanized *in extremis* and the deaths were not attributed to treatment. In the F_1 animals, the 500-ppm group displayed a significant reduction in live litter size, attributed to reductions in body weight. Postnatal survival was unaffected by treatment. Body weights were increased in the 500-ppm pups during PND 1 (both sexes) and 4 (females), and decreased in the 250- and 500-ppm males on PND 28. Body weight gains were decreased in 500-ppm males on PND 4-7, 7-14, and 21-28; decreases in body weight gains were also observed at PND 21-28 in ≥100-ppm males and 750-ppm females, although mean body weights did not differ from controls. The absolute brain weights of the ≥ 100-ppm F_1 males and 100-ppm females sacrificed at PND 21 displayed a statistically significant reduction; however, relative weights were unaffected. An increase in the average day at balanopreputial separation of the 500-ppm males was statistically significant as compared with concurrent controls (9% increase); however, there was no dose-response. Vaginal patency was increased in the 500-ppm females by 5% compared with concurrent controls; however, this delay was not statistically significant.

Mean body weight gains were reduced in the 500-ppm F_1 males (weeks 19-20 and 20-21) as compared to controls; percent decrease in body weights gains ranged from 9.3-18.5% during weeks 19-37; 500-ppm females displayed reduced body weight gains (weeks 19-28) with a 6.7-11.8% decrease in body weight being observed, as compared to controls, during weeks 19-28. The 100-ppm males displayed a reduction in body weight during weeks 26-37; however, the decrease was not dose-related and not attributed to treatment. F_1 500-ppm females displayed reduced weight gain (during GD 7-11, 11-14, 14-20, and 0-20) and body weight (at GD 14 and 20) that coincided with a decrease in litter size (42%), number of implantations (37%), and food efficiency. Further, mean body weights and food consumption were reduced in the F_1 500-ppm females through LD 4, 7, and 14. The 250-ppm females displayed a reduction in mean body weight gains during GD 14-20 with a slight reduction in food efficiency (GD 7-11, 11-14, and 14-20) and a significant overall reduction in food efficiency (GD 0-20). The 100-ppm females displayed a reduction in mean body weight gains during GD 4-7 with a slight reduction in food efficiency (GD 4-7 and 11-14). However, weight gain and food efficiency data of the 250- and 100-ppm females were comparable to controls.

Table 7. F_1 Females Reproductive Endpoints[a]

Endpoint	0 ppm	100 ppm	250 ppm	500 ppm
Fertility index (%) N	100.0 25	84.0 25	80.0 25	100.0 25
Mating index (%) N	88.0 25	68.0 25	64.0 25	72.0 25
Evidence of mating w/out delivery (no.)	3	4	4	8
Estrus cycle length (days)[b] N	4.5±1.25 24	4.5±0.91 24	4.9±1.43 22	5.1±1.68 21
Mean no. of estrus cycles within 3 weeks[c] N	3.64±1.15 25	3.68±1.11 25	2.88±1.36 25	2.68±1.35* 25
Mean no. of estrus cycles within 3 weeks excluding ammenorheic females[d] N	3.64±1.15 25	3.68±1.11 25	3.13±1.10 23	2.91±1.12* 23
Implantation sites N	15.5±2.11 22	15.8±3.29 17	13.5±4.12 16	9.8±4.93** 17
Number of born N	14.9±1.97 22	15.1±3.35 17	13.1±4.12 16	8.6±4.51** 17
Unaccounted Sites N	0.5±0.86 22	0.6±1.22 17	0.4±0.63 16	1.2±1.09 17

[a] Data were provided on pp. 272-275 and 356 of the study report
[b] Statistical analysis calculated by ICF
[c] Calculated by ICF Poisson regression model
[d] Calculated by ICF excluding females that did not have a full estrus cycle and analyzed using a quasi-likelihood Poisson-like regression model
*Significantly different from control, $p<0.05$.
**Significantly different from control, $p<0.01$.

Mating, fertility, and number of unaccounted-for implantation sites were unaffected by treatment. Estrus cycle length was extended by 13% in the 500-ppm females (historical controls range is 4.1-5.1 days); however, the data were not significantly different from concurrent controls. Three 250-ppm females and four 500-ppm females did not complete an estrus cycle; the study authors excluded the data from these individuals from their statistical analysis. ICF re-evaluated the estrus cycle data by determining the number of estrus cycles within the 3 weeks prior to mating, including data from all females, and performing a statistical analysis of dose-response using a Poisson regression model. A significant reduction in estrus cycle number was observed at 500 ppm. ICF performed further analysis of the estrus cycle data; results from acyclic females were excluded from this analysis. Although exclusion of the results from these individuals skewed the distribution of the data, the statistical significance remained at the same concentration levels. Gestation length was not affected by treatment; only one 250-ppm female experienced dystocia, which was not considered by the study authors to be treatment-related.

Table 8. F_1 Males Reproductive Endpoints[a]

Endpoint	0 ppm	100 ppm	250 ppm	500 ppm
Fertility index (%) N	87.5 24	68.0 25	64.0 25	70.8 24
Mating index (%) N	100.0 24	84.0 25	80.0 25	100.0 24
Evidence of mating w/out delivery (no.)	3	8	9	7
L. Epididymis (no. sperm in million/gram tissue) N	640.1±141.82 23	609.1±115.22 23	573.7±97.17 24	635.3±160.50 24
Motile sperm (%) N	88.9±4.52 24	86.4±4.96 25	84.8±6.02* 25	74.4±14.06** 24
Normal sperm (%) N	99.5±0.79 24	98.9±0.95** 25	99.1±1.13 25	95.3±6.51** 24
Normal shaped head separated from flagellum (%) N	0.2±0.47 24	0.5±0.68 25	0.4±0.73 25	1.6±4.12 24
Head absent from flagellum (%) N	0.2±0.57 24	0.6±0.75 25	0.4±0.61 25	2.0±2.99 24

[a] Data were provided on pp. 274, 345-348 of the study report
*Significantly different from control, $p<0.05$.
**Significantly different from control, $p<0.01$.

Spermatogenic analysis indicated a reduction in sperm motility and the number of morphologically normal sperm in 500-ppm males. There were two types of sperm abnormalities; one in which sperm had a normal head separated from the flagellum and another in which normal flagella were without the head. The mean testicular and epididymal sperm counts were comparable among all treatment and control groups. The 250-ppm males displayed a significant reduction in sperm motility. At 100-ppm, males also showed a significant decrease in number of morphologically normal sperm cells; however, this value was within the range of historical control values, and there was no clear dose-response. Therefore, this finding was not considered to be toxicologically significant.

Table 9a. Selected F_1 Male Organ Weights[a]

Organ	0 ppm	100 ppm	250 ppm	500 ppm
Final Body Wt (g) N	583±65.7 24	543±63.9 25	562±55.3 25	526±43.3 24
Brain (g) N	2.21±0.092 24	2.11±0.111** 25	2.12±0.109** 25	2.01±0.079** 24
Relative brain (g/100 g) N	0.382±0.0379 24	0.392±0.0401 25	0.379±0.0365	0.384±0.0313 24
Liver (g) N	20.18±3.353 24	18.54±2.304 25	19.94±2.994 25	21.25±4.126 24
Relative liver (g/100 g) N	3.455±0.3775 24	3.424±0.3348 25	3.538±0.3198	4.017±0.5570** 24
Kidney (g)	3.73±0.455	3.37±0.354**	3.52±0.349	3.40±0.450*

Organ	0 ppm	100 ppm	250 ppm	500 ppm
N	24	25	25	24
Relative kidney (g/100 g) N	0.640±0.0548 24	0.624±0.0672 25	0.627±0.0523 25	0.645±0.0587 24
R. Cauda Epididymis (g) N	0.3178±0.03778 24	0.3129±0.0.3862 25	0.3029±0.03885 25	0.2720±0.03787** 24
Relative r. Cauda Epididymis (g/ 100 g)	0.055±0.0075 24	0.058±0.0104 25	0.054±0.0083 25	0.052±0.0073 24
L Cauda Epididymis (g) N	0.3304±0.03279 24	0.3325±0.03912 25	0.3221±0.03981 25	0.2967±0.04462** 24
Relative l. Cauda Epididymis (g/ 100 g) N	0.057±0.0061 24	0.062±0.0111 25	0.058±0.0089 25	0.056±0.0075 24
Thymus (g) N	0.2319±0.05952 24	0.2463±0.06420 25	0.2702±0.05165 25	0.2762±0.05771* 24
Relative thymus (g/100 g) N	0.040±0.0114 24	0.045±0.0110 25	0.048±0.0091* 25	0.052±0.0102** 24
Prostate (g) N	1.16±0.258 24	1.00±0.285 25	1.06±0.243 25	1.05±0.205 24
Relative prostate (g/100 g) N	0.200±0.0405 24	0.187±0.0595 25	0.190±0.0502 25	0.201±0.0371 24

[a] Data were provided on pp. 357-360, 363-366 of the study report
*Significantly different from control, $p<0.05$.
**Significantly different from control, $p<0.01$.

The kidneys (100- and 500-ppm males) displayed a decrease in absolute weight that was not considered to be treatment-related or toxicologically significant, due lack of a dose-response and absence of macroscopic or microscopic kidney lesions. Mean absolute brain weights were reduced in a non-dose related fashion for the ≥100-ppm males and 500-ppm females; therefore, these change were not considered to be toxicologically significant. A decrease in absolute pituitary weight was statistically significant in the 500-ppm males; however, no lesions were found that suggested an association with treatment. No microscopic or macroscopic lesions were observed in the epididymides but the effect was similar to those observed in the F_0 males. Absolute and relative thymus weights were increased in the 500-ppm F_1 males; however, no macroscopic or microscopic lesions were noted in this gland.

Table 9b. Selected F_1 Female Organ Weights (N=25)[a]

Organ	0 ppm	100 ppm	250 ppm	500 ppm
Final Body Wt (g)	321±27.3	325±28.1	318±26.7	309±29.5
Brain (g)	1.97±0.076	1.96±0.073	1.92±0.067	1.89±0.102**
Relative brain (g/100 g)	0.615±0.0.520	0.606±0.0549	0.608±0.0470	0.617±0.0588
Liver (g)	12.27±1.571	12.57±1.559	12.44±1.641	12.53±1.894
Relative liver (g/ 100 g)	3.811±0.2847	3.873±0.3744	3.914±0.4246	4.059±0.4546
Ovaries (g)	0.1131±0.01554	0.1077±0.03170	0.1056±0.02791	0.1062±0.02302
Relative ovaries (g/100 g)	0.035±0.0027	0.022±0.0032	0.022±0.0042	0.021±0.0045

[a] Data were provided on pp. 361-362, 367-369 of the study report
**Significantly different from control, $p<0.01$.

The F_1 males and females exposed to 500 ppm displayed an increase in relative liver weight as compared to controls; however, the values for females were not statistically significant. These increases were considered treatment-related, because associated histopathology was observed, consisting of an increase in hepatic centrilobular vacuolation. An statistically significant increase in the number of primordial follicles (19%) relative to controls was observed in the 500 ppm group. However, this end point was not measured in lower dose groups, and thus a dose-response could not be determined.

Table 10. Primordial Follicles and Corpora Lutea[a] for F_1 Animals

N=25	0 ppm	500 ppm
Primordial follicles	105.6±44.96	141.8±50.26*
Corpora lutea	171.7±40.89	171.7±49.18

[a] Data were derived from p. 390 of the study report
**Significantly different from control, p<0.01.

The liver (≥250-ppm males and ≥100-ppm females) and kidneys (500-ppm males and ≥250-ppm females) displayed treatment-related lesions that correlated with lesions found in the F_0 generations.

Table 11. Selected Histopathology Data for the F_1 Animals[a]

Incidence	Male				Female			
	0 ppm	100 ppm	250 ppm	500 ppm	0 ppm	100 ppm	250 ppm	500 ppm
Kidneys								
Pelvic Mineralization	0	1	0	3	4	5	7	8
Mononuclear Infiltrate	11	9	1*	20*	5	5	5	7
Transitional Cell Hyperplasia	1	1	2	1	2	3	2	2
Prostate								
Acute Inflammation	3	-	-	13*				
Ovaries								
Decreased Corpora Lutea					3	3	7	4
Luteinized Follicular Cyst					2	3	2	3
Follicular Cyst					5	5	7	10
Interstitial Hyperplasia					12	10	13	18
Liver								
Total Centrilobular Hepatocellular Vacuolation	0	0	15*	23*	0	0	2	6*
Increased glycogen	19	18	17	24*	16	24*	23*	23*

[a] Data were provided on pp. 375- of the study report. N=24, 25, 25, 24 males treated with 0, 100, 250, 500 ppm of nPB and 25 all treated females
*Significantly different from control, p<0.05.
**Significantly different from control, p<0.01.

In the F_2 animals, a 41% decrease in live animals born per dam (PND 0) was observed in the 500-ppm pups. The 500-ppm pup weights were reduced at PND 14 (16% males and 14% females) and PND 21 (18% males and 15% females); weight gain was reduced in the 500-ppm pups at PND 4-7 (33% males and 31%), 7-14 (26% males and 24% females), and 14-21 (20% males and 16% females). The absolute brain, thymus, and spleen weights were reduced in the 500-ppm F_2 males and the absolute thymus and splean

weights were decreased in 500-ppm females. A decrease in the relative brain and spleen weights in 500 ppm males and in relative spleen weights in 500-ppm females was also statistically significant.

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Appendix 1: List of Acronyms

ANOVA	Analysis of Variance
Crl:CD®(SD) IGS BR	Type of Sprague Dawley Rat Species
FSH	Follicle Stimulating Hormones
GD	Gestation Day
LD	Lactation Day
LOAEL	Low Observable Adverse Effect Level
nPB	normal-Propyl Bromide
PND	Postnatal Day
PPM	Parts per Million
NOAEL	No Observable Adverse Effect Level
OPPTS	The Office of Prevention, Pesticides and Toxic Substances