5.0 RESULTS: FLUTAMIDE

5.1 EPA 14-Day Assay for Flutamide

The EPA 14-Day Flutamide exposure assay was conducted from February 18, 2003, to February 25, 2003 (pre exposure assay), and from February 25, 2003, to March 11, 2003 (exposure assay).

5.1.1 Survival

All males and females in all treatments survived the EPA 14-Day Flutamide assay.

5.1.2 Vitellogenin

Vitellogenin concentrations in Control treatment females used during the EPA 14-Day Flutamide assay ranged from 533,500 ng/mL to 6,960,500 ng/mL (Figure 5.1). Among females exposed to the two flutamide concentrations, vitellogenin concentrations ranged from 1,781,000 ng/mL to 8,388,000 ng/mL. No significant differences in the mean vitellogenin concentration per treatment (Table 5.1) were detected (Kruskal-Wallis, $H = 3.49$, $p = 0.175$, df = 2). The achieved power for this endpoint was 41%, and the sample size required to detect a significant difference from the Control treatment at 80% power was 35 (Table 5.1).

Table 5.1. Summary statistics and power estimates for female vitellogenin concentrations (ng/mL) for the EPA 14-Day Flutamide assay.

Level	N	Mean	Stdev	CV	Achieved Power [1]	Sample Size Required [2]
control	15	3,077,733	1,532,872	50%	41%	35
low	16	3,489,000	1,686,463	48%		
high	15	4,188,333	1,706,450	41%		

[1] Calculated from natural log transformed data; with sample size = 15.
[2] To detect a significant difference from control treatment based on maximum achieved absolute difference; calculated on natural log transformed data.

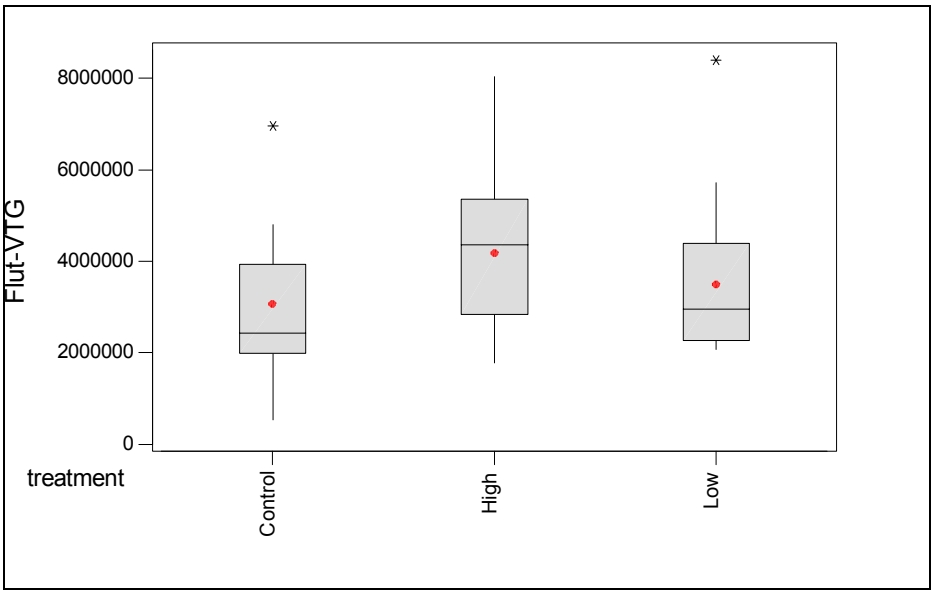

Figure 5.1. Boxplot of female vitellogenin concentration (ng/mL) by treatment for the EPA 14-Day Flutamide assay. The box represents the interquartile range, whiskers represent the data range, the horizontal line is the median value, the circle is the mean value, and the asterisk represents a probable outlier.

Vitellogenin concentrations in Control treatment males used during the EPA 14-Day Flutamide assay ranged from 0 ng/mL (not detected) to 12,700 ng/mL (Figure 5.2). Among most males exposed to the two flutamide concentrations, vitellogenin concentrations ranged from 0 ng/mL (not detected) to 15,212 ng/mL. One male exposed to the High flutamide concentration had a vitellogenin concentration of 353,600 ng/mL. No significant differences in the mean vitellogenin concentration per treatment (Table 5.2) were detected (Kruskal-Wallis, $H = 0.32$, $p = 0.850$, df = 2). The achieved power for this endpoint was 5%, and the sample size required to detect a significant difference from the Control treatment at 80% power was >1,000 (Table 5.2).

Table 5.2. Summary statistics and power estimates for male vitellogenin concentrations (ng/mL) for the EPA 14-Day Flutamide assay.

Level	N	Mean	Stdev	CV	Achieved Power [1]	Sample Size Required [2]
control	8	3,365	4,499	134%	5%	>1,000
low	8	4,497	5,469	122%		
high	8	44,563	124,870	280%		

[1] Calculated from natural log transformed data; with sample size = 8.
[2] To detect a significant difference from control treatment based on maximum achieved absolute difference; calculated on natural log transformed data.

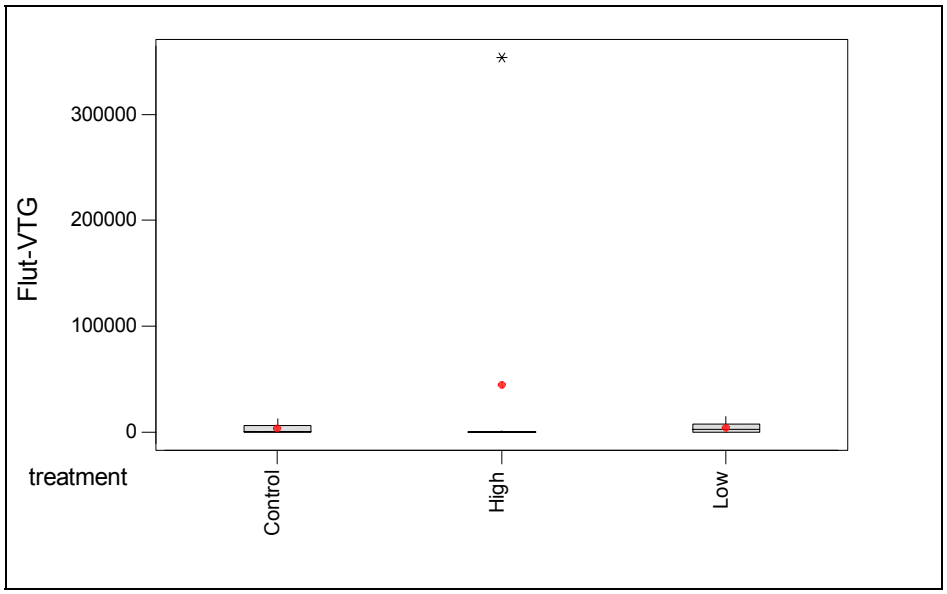

Figure 5.2. Boxplot of male vitellogenin concentration (ng/mL) by treatment for the EPA 14-Day Flutamide assay. The box represents the interquartile range, whiskers represent the data range, the horizontal line is the median value, the circle is the mean value and the asterisk represents a probable outlier.

5.1.3 Appearance / Secondary Sex Characteristics

All of the females used during the EPA 14-Day Flutamide assay exhibited typical female morphology (no fat pad, no tubercles, ovipositor present) with two exceptions. One female from the Control treatment lacked an ovipositor. One female from the High concentration that showed the dark vertical banding typically found in males.

Most males used during the EPA 14-Day Flutamide assay had typical male morphological features. One male from the High concentration lacked tubercles and a dorsal fat pad. Tubercles were present in all other males. Fat pads were present or pronounced in all other males from all treatments. Vertical banding was present or pronounced in all males.

5.1.4 Gonadosomatic Index

The range of GSI values calculated for females in the Control treatments varied from 1.0 to 19.7 (Figure 5.3), whereas the GSI values for the other treatments varied from 3.6 to 21.5 for females from the High concentration and from 8.1 to 16.3 for females from the Low concentration. The overall variability within each treatment was low to moderate (CVs = 17%–40%; Table 5.3). There were no significant differences in mean GSI values (Table 5.3) among treatments (Kruskal-Wallis, $H = 0.14$, $p = 0.933$, df = 2). The achieved power for this endpoint was 8%, and the sample size required to detect a significant difference from the Control treatment at 80% power was 442 (Table 5.3).

Table 5.3. Table E14FL GSI-1. Summary statistics and power estimates for female gonadosomatic index data for the EPA 14-Day Flutamide assay.

Level	N	Mean	Stdev	CV	Achieved Power [1]	Sample Size Required [2]
control	16	11.4	4.4	38%	8%	442
low	16	11.3	1.9	17%		
high	16	12.2	4.9	40%		

[1] Calculated from arcsine square-root transformed data; with sample size = 16.
[2] To detect a significant difference from control treatment based on maximum achieved absolute difference; calculated on arcsine square-root transformed data.

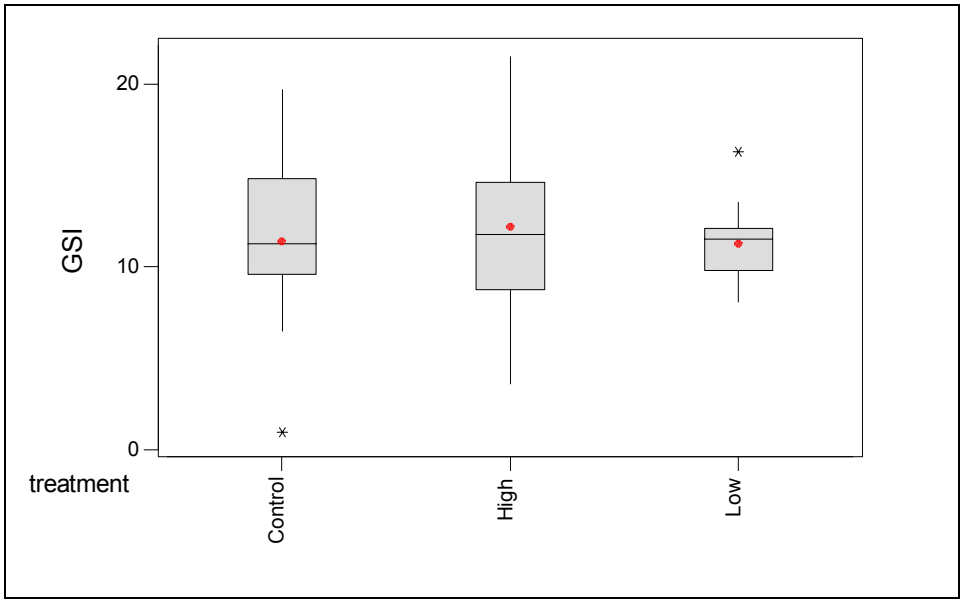

Figure 5.3. Boxplot of female GSI by treatment for the EPA 14-Day Flutamide assay. The box represents the interquartile range, whiskers represent the data range, the horizontal line is the median value, the circle is the mean value, and the asterisk represents a probable outlier.

The range of most GSI values calculated for males during the EPA 14-Day Flutamide assay, was small, ranging from 0.4 to 2.1 (Figure 5.4), which approximates the typical range for reproductively-active male fathead minnows. The highest and lowest male GSI values were 2.1 (one fish in the High concentration) and 0.4 (two fish in the Control treatment), respectively. Variability was moderate (CVs = 25%–48%). There were no significant differences in mean GSI values (Table 5.4) among treatments (Kruskal-Wallis, $H = 3.44$, $p = 0.180$, df = 2). The achieved power for this endpoint was 12%, and the sample size required to detect a significant difference from the Control treatment at 80% power was 84 (Table 5.4).

Table 5.4. Summary statistics and power estimates for male gonadosomatic index data for the EPA 14-Day Flutamide assay.

Level	N	Mean	Stdev	CV	Achieved Power [1]	Sample Size Required [2]
control	8	1.1	0.5	48%	12%	84
low	8	0.8	0.2	25%		
high	8	1.2	0.5	40%		

[1] Calculated from arcsine square-root transformed data; with sample size = 8.
[2] To detect a significant difference from control treatment based on maximum achieved absolute difference; calculated on arcsine square-root transformed data.

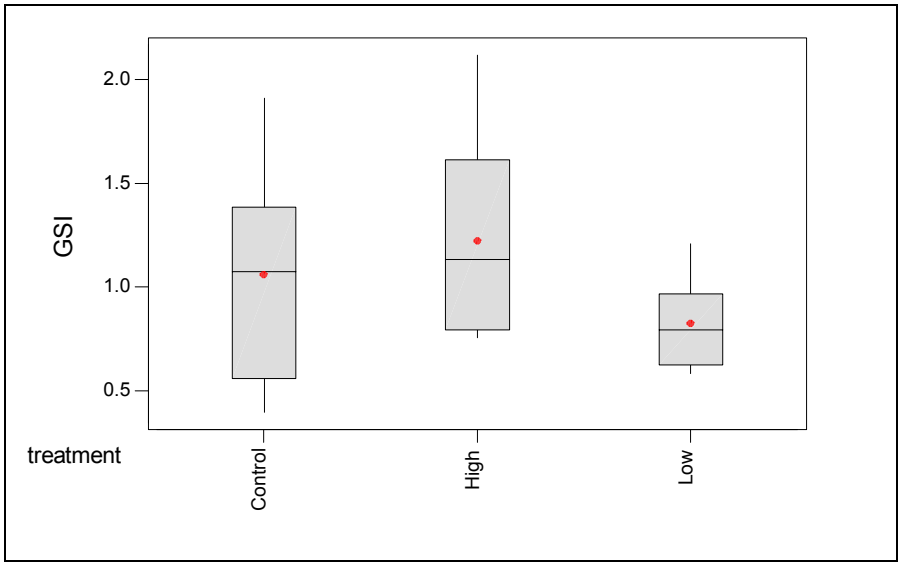

Figure 5.4. Boxplot of male GSI by treatment for the EPA 14-Day Flutamide assay. The box represents the interquartile range, whiskers represent the data range, the horizontal line is the median value, and the circle is the mean value.

5.1.5 Female Gonad Histology

Histological analyses were conducted on the ovaries of 47 females exposed to flutamide during the EPA 14-Day Assay.

General Ovary Staging—Statistical analysis of the mean ovarian staging from 12 microscope fields per female from the EPA 14-Day Flutamide assay revealed no significant differences among treatments (Kruskal-Wallis, $H = 4.73$, $p = 0.094$, df = 2).

Quantitative Ovarian Staging—One hundred cells in each of three sections per female were examined to quantitatively determine the developmental stage of the ovaries. Ova from females from the Control treatment and High concentration ranged from Stage 1a to Stage 5 (see Methods for a description of the stages), whereas ovaries in females from the Low-concentration treatment showed Stage 1a to Stage 4 development (Figure 5.5). Variability within treatments for each stage was very high as indicated by CVs that ranged as high as 303% (Table 5.5). Statistical analyses showed that there were no significant

difference among treatments in the proportion of cells in each of the five developmental stages (Table 5.5). Therefore, there was no effect on ovarian developmental stage associated with flutamide dose.

Table 5.5. Descriptive statistics of the proportion of ovarian cells in each developmental stage for females from the EPA 14-Day Flutamide assay and results of the Kruskal-Wallis Test (df = 2) comparing treatments.

	Control (n = 15)			Low (n = 16)			High (n = 16)			Kruskal-Wallis	
Stage	Mean	Stdev	CV	Mean	Stdev	CV	Value	Stdev	CV	*H*	*p*
1a	0.079	0.030	38%	0.094	0.037	39%	0.084	0.031	37%	0.95	0.621
1b	0.332	0.082	25%	0.305	0.040	13%	0.328	0.127	39%	2.39	0.302
2	0.174	0.039	23%	0.178	0.040	23%	0.158	0.071	45%	3.12	0.210
3	0.190	0.057	30%	0.188	0.044	23%	0.158	0.074	47%	2.19	0.335
4	0.193	0.090	47%	0.223	0.085	38%	0.215	0.116	54%	1.04	0.594
5	0.022	0.064	284%	0	0	–	0.033	0.100	303%	3.14	0.208

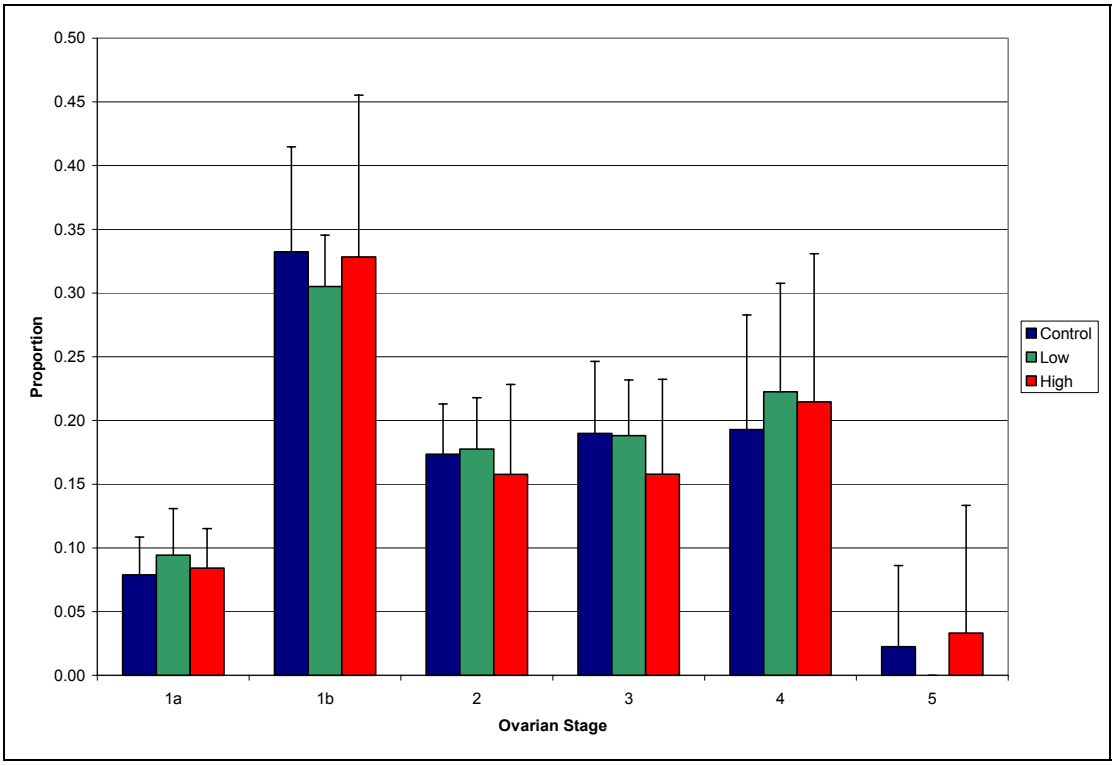

Figure 5.5. Frequency histogram showing the quantitative developmental staging of ovaries for each treatment of the EPA 14-Day Flutamide assay. For each treatment, the columns represent the grand mean proportion of cells in each stage and the bars represent the standard deviation.

Atretic Follicles—The mean proportion of atretic follicles per 300 follicles (counted per fish) ranged from 0.0004 follicles for females in the Control treatment to 0.015 follicles for females in the High concentration (Figure 5.6). There was a significant difference in the mean proportions of atretic follicles among treatments (Kruskal-Wallis, $H = 12.88$, $p = 0.002$, df = 2). The proportion of atretic follicles was

greater for females from the High concentration than from the Control treatment or the Low concentration.

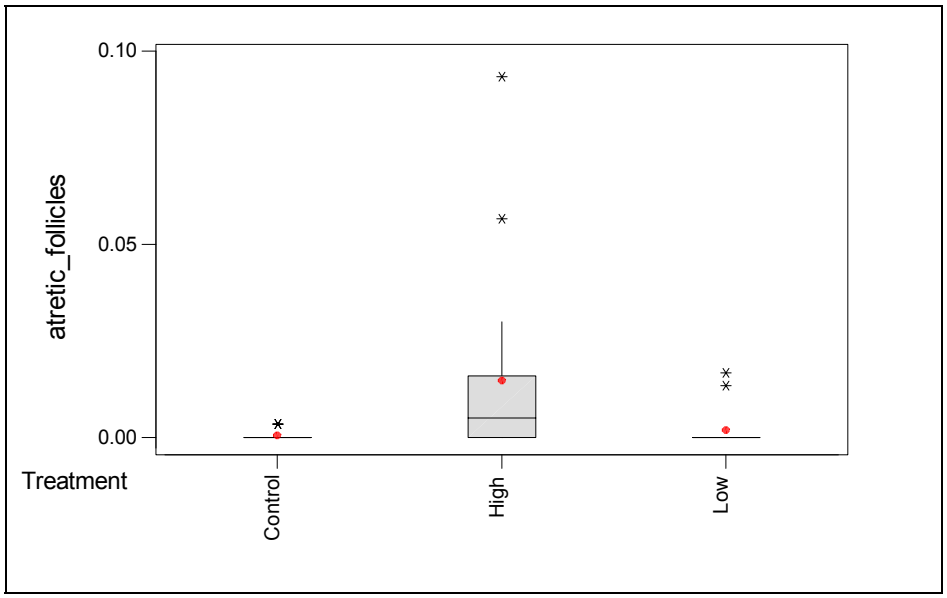

Figure 5.6. Boxplot of the proportion of atretic follicles per 300 follicles by treatment for the EPA 14-Day Flutamide assay. The box represents the interquartile range, whiskers represent the data range, the horizontal line is the median value, the circle is the mean value, and asterisks represent probable outliers.

Corpora Lutea—The mean proportion of corpora lutea per 300 follicles (counted per fish) ranged from 0.009 for females in the High concentration to 0.010 for females in the Low concentration (Figure 5.7). There were no significant differences in the mean proportion of corpora lutea among treatments (Kruskal-Wallis, $H = 0.56$, $p = 0.757$, df = 2). The value for the High concentration was significantly lower than those of the other two treatments.

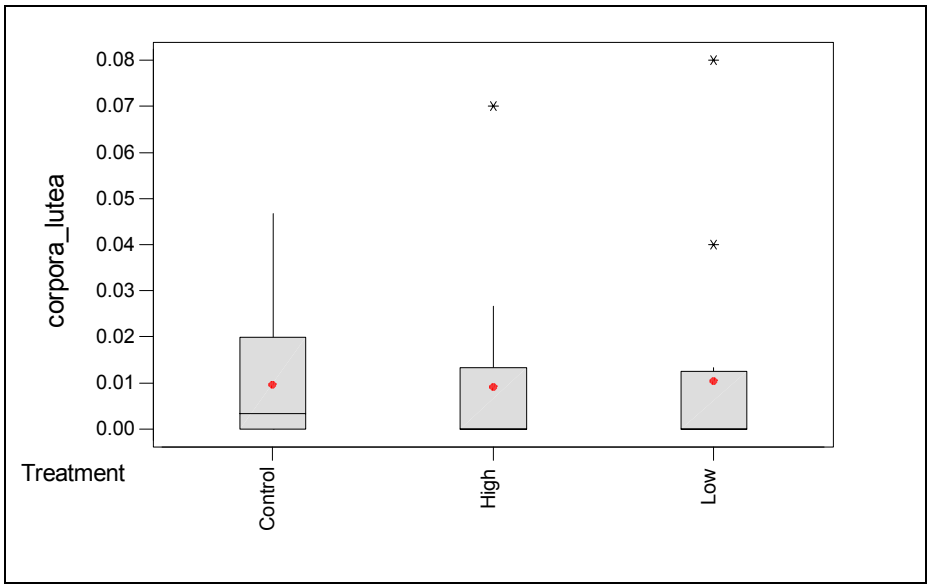

Figure 5.7. Boxplot of the proportion of corpora lutea per 300 follicles by treatment for the EPA 14-Day Flutamide assay. The box represents the interquartile range, whiskers represent the data range, the horizontal line is the median value, the circle is the mean value, and asterisks represent probable outliers.

Observations—One fish from the Control treatment had only eight ova present in the three ovarian section examined. This fish was not included in the data analyses. Another fish in the Control treatment was observed to have some cortical alveolus stage ova (Stage 2) that appeared abnormal because they had a very large yolk body next to the nucleus. Three fish exposed to the High flutamide concentration had very few ova or very few advanced stage ova in the ovaries.

5.1.6 Male Gonad Histology

General Testes Staging—Testes from 24 males exposed to flutamide during the EPA 14-Day Flutamide assay were examined to determine the general developmental condition. Males in all treatments had well-developed testes with all showing Stage 4 and Stage 5 development (see Methods for description of developmental stages). All of the 98 microscopic fields examined in the 8 Control treatment males showed Stage 4 (86 fields) or Stage 5 (12 fields) development. All of the 96 microscopic fields examined in the 8 Low-concentration treatment males showed Stage 4 (90 fields) or Stage 5 (6 fields) development. All of the 96 microscopic fields examined in the 8 High-concentration treatment males showed Stage 4 (81 fields) or Stage 5 (15 fields) development. Statistical analysis of the mean staging from 12 microscopic fields per fish revealed no significant differences among treatments (Kruskal-Wallis, $H = 0.70$, $p = 0.703$, df = 2).

Quantitative Testicular Staging—One hundred cells in each of three section per male were examined to quantitatively determine the developmental condition of the testes. The developmental stage all treatment testes ranged from Stage 2a to Stage 5 (Figure 5.8). Variability within treatments for each stage was very high as indicated by CVs that ranged as high as 170% (Table 5.6). Although statistical analyses showed that there were no significant differences among treatments in the proportion of cells in any developmental Stage, the probability value calculated for Stage 2a was slightly above the critical limit of 0.050 (Table 5.6). Therefore, there was no effect on testicular developmental stage associated with flutamide dose.

Table 5.6. Descriptive statistics of the proportion of testes cells in each developmental stage for males from the EPA 14-Day Flutamide assay and results of the Kruskal-Wallis Test (df = 2) comparing treatments.

	Control (n = 8)			Low (n = 8)			High (n = 8)			Kruskal-Wallis	
Stage	Mean	Stdev	CV	Mean	Stdev	CV	Value	Stdev	CV	*H*	*p*
1	0	0	–	0	0	–	0	0	–	–	–
2a	0.002	0.004	170%	0.006	0.005	83%	0.008	0.006	74%	5.82	0.054
2b	0.025	0.026	105%	0.015	0.013	85%	0.017	0.014	83%	0.43	0.805
3a	0.113	0.056	50%	0.135	0.058	43%	0.152	0.077	51%	1.86	0.394
3b	0.276	0.123	45%	0.287	0.076	26%	0.251	0.102	40%	0.59	0.746
4	0.174	0.079	45%	0.170	0.029	17%	0.124	0.068	55%	3.39	0.184
5	0.410	0.179	44%	0.386	0.085	22%	0.448	0.216	48%	0.23	0.893

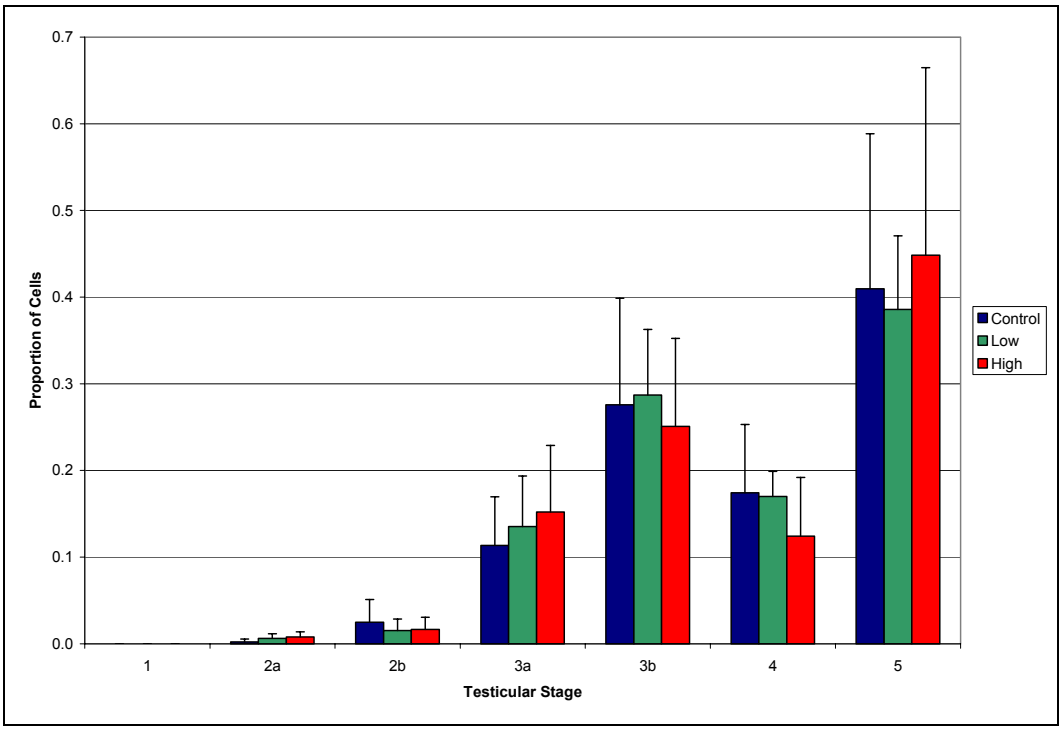

Figure 5.8. Frequency histogram showing the quantitative developmental staging of testes for each treatment of the EPA 14-Day Flutamide assay. For each treatment, the columns represent the grand mean proportion of cells in each stage and the bars represent the standard deviation.

Tubule Diameter—The diameter of the seminiferous tubules of males from the Control treatment ranged from 66.1 µm to 161.9 µm (Figure 5.9). Tubule diameters of males from the two test concentrations ranged from 92.2 µm to 164.2 µm. No significant differences in the mean tubule diameter per treatment (Table 5.7) were detected (Kruskal-Wallis, $H = 2.42$, $p = 0.297$, df = 2). The achieved power for this endpoint was 9%, and the sample size required to detect a significant difference from the Control treatment at 80% power was 152 (Table 5.7).

Table 5.7. Summary statistics and power estimates for male seminiferous tubule diameter data for the EPA 14-Day Flutamide assay.

Level	N	Mean	Stdev	CV	Achieved Power [1]	Sample Size Required [2]
control	8	118.6	29.0	24%	9%	152
low	8	108.4	10.8	10%		
high	8	125.1	22.2	18%		

[1] Calculated from natural log transformed data; with sample size = 8.
[2] To detect a significant difference from control treatment based on maximum achieved absolute difference; calculated on natural log transformed data.

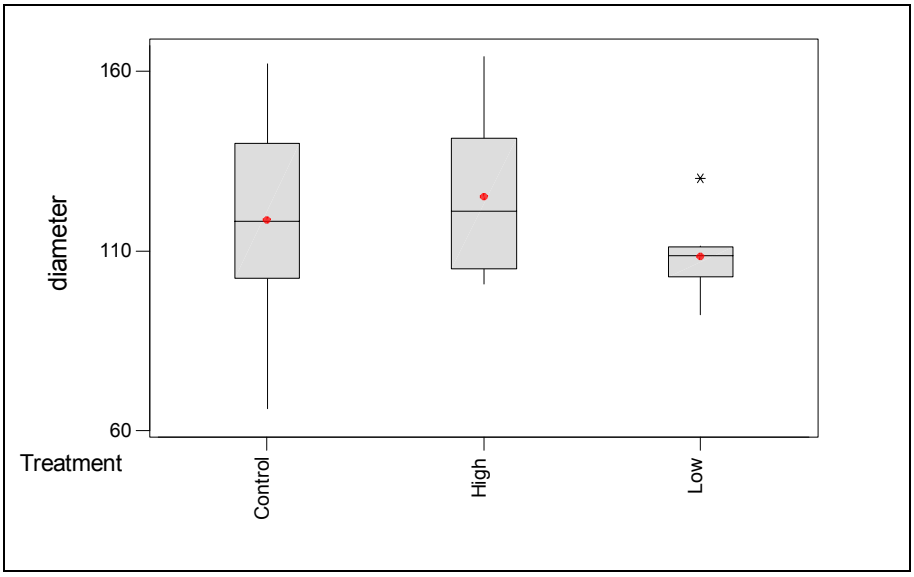

Figure 5.9. Boxplot of seminiferous tubule diameter (μm) by treatment for the EPA 14-Day Flutamide assay. The box represents the interquartile range, whiskers represent the data range, the horizontal line is the median value, the circle is the mean value, and the asterisk represents a probable outlier.

Observations—No Sertoli cell proliferation was observed. One male from the Control treatment was observed to have Leydig cell proliferation. The same male was also noted to have disorganized development of the seminiferous tubules with early developmental stages shed prematurely into tubule lumina. No testicular atrophy was recorded and no ovatestes were observed for any treatment.

5.1.7 Plasma Steroid Concentrations

Estradiol—Estradiol concentrations in Control-treatment females used during the EPA 14-Day Flutamide assay ranged from 0 pg/mL (not detected) to 4,453 pg/mL (Figure 5.10). Among females exposed to the two flutamide concentrations, estradiol concentrations ranged from 282 pg/mL to 5,837 pg/mL. No significant differences in the mean estradiol concentration per treatment (Table 5.8) were detected (Kruskal-Wallis, $H = 2.02$, $p = 0.365$, df = 2). The achieved power for this endpoint was 13%, and the

sample size required to detect a significant difference from the Control treatment at 80% power was 115 (Table 5.8).

Table 5.8. Summary statistics and power estimates for female estradiol concentrations (pg/mL) for the EPA 14-Day Flutamide assay.

Level	N	Mean	Stdev	CV	Achieved Power [1]	Sample Size Required [2]
control	12	2,241	1,338	60%	13%	115
low	15	2,351	1,294	55%		
high	15	1,693	1,221	72%		

[1] Calculated from natural log transformed data; with sample size = 12.
[2] To detect a significant difference from control treatment based on maximum achieved absolute difference; calculated on natural log transformed data.

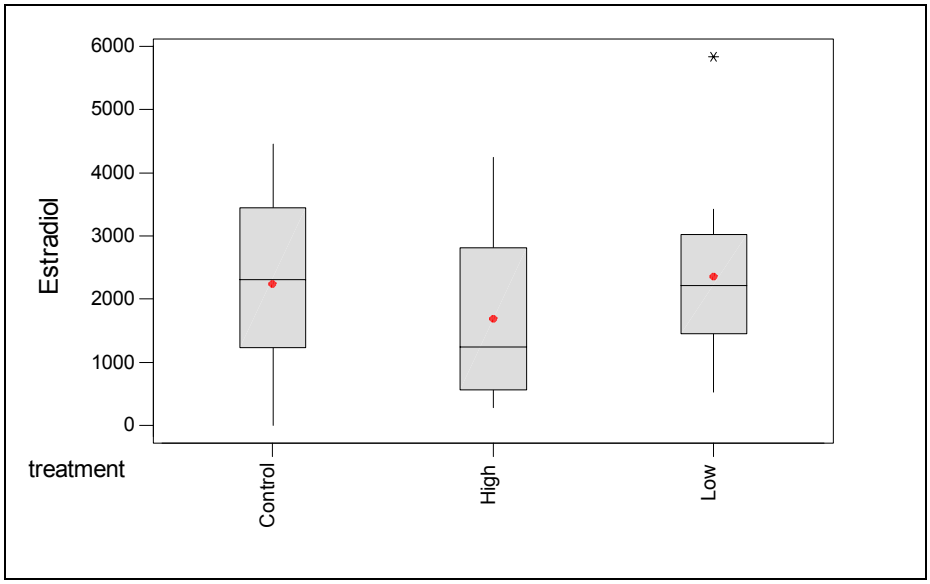

Figure 5.10. Boxplot of female estradiol concentration (pg/mL) by treatment for the EPA 14-Day Flutamide assay. The box represents the interquartile range, whiskers represent the data range, the horizontal line is the median value, the circle is the mean value, and the asterisk represents a probable outlier.

Estradiol concentrations in Control treatment males used during the EPA 14-Day Flutamide assay ranged from 210 pg/mL to 540 pg/mL (Figure 5.11). Among males exposed to the two flutamide concentrations, estradiol concentrations ranged from 0 pg/mL (not detected) to 992 pg/mL. A significant difference in the mean estradiol concentration per treatment (Table 5.9) was detected (Kruskal-Wallis, $H = 8.80$, $p = 0.012$, df = 2). The mean concentration of estradiol in males from the High concentration was less than that in males from the Low concentration. The achieved power for this endpoint was 22%, and the sample size required to detect a significant difference from the Control treatment at 80% power was 31 (Table 5.9).

Table 5.9. Summary statistics and power estimates for male estradiol concentrations (pg/mL) for the EPA 14-Day Flutamide assay.

Level	N	Mean	Stdev	CV	Achieved Power [1]	Sample Size Required [2]
control	7	302	110	36%	22%	31
low	8	400	245	61%		
high	7	200	97	48%		

[1] Calculated from natural log transformed data; with sample size = 7.
[2] To detect a significant difference from control treatment based on maximum achieved absolute difference; calculated on natural log transformed data.

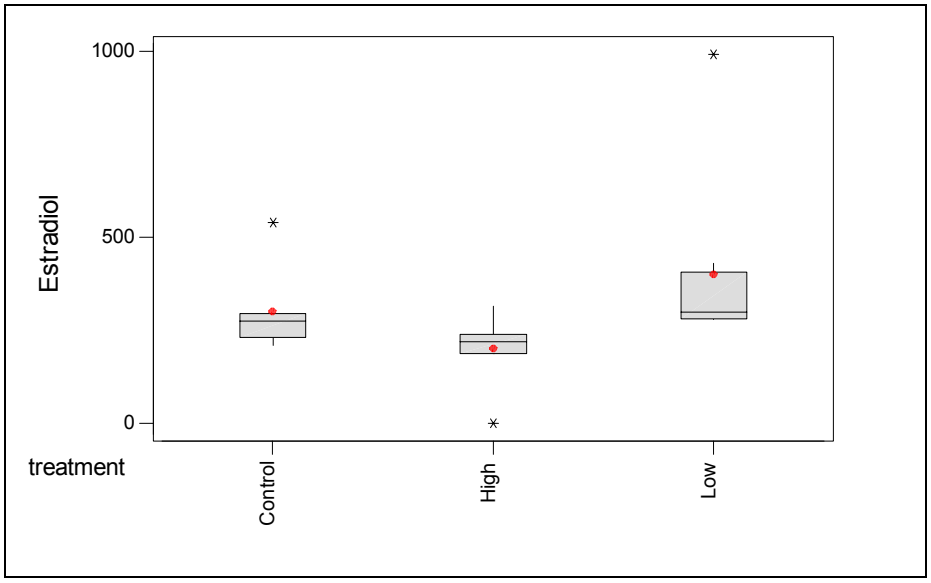

Figure 5.11. Boxplot of male estradiol concentration (pg/mL) by treatment for the EPA 14-Day Flutamide assay. The box represents the interquartile range, whiskers represent the data range, the horizontal line is the median value, the circle is the mean value, and the asterisks represent probable outliers.

Testosterone—Testosterone concentrations in Control-treatment females used during the EPA 14-Day Flutamide assay ranged from 0 pg/mL (not detected) to 2,989 pg/mL (Figure 5.12). Among females exposed to the two flutamide concentrations, testosterone concentrations ranged from 0 pg/mL (not detected) to 4,874 pg/mL. No significant differences in the mean testosterone concentration per treatment (Table 5.10) were detected (Kruskal-Wallis, $H = 2.59$, $p = 0.274$, df = 2). The achieved power for this endpoint was 7%, and the sample size required to detect a significant difference from the Control treatment at 80% power was 271 (Table 5.10).

Table 5.10. Summary statistics and power estimates for female testosterone concentrations (pg/mL) for the EPA 14-Day Flutamide assay.

Level	N	Mean	Stdev	CV	Achieved Power [1]	Sample Size Required [2]
control	11	1,227	851	69%	7%	271
low	9	1,298	791	61%		
high	10	2,613	1,884	72%		

[1] Calculated from natural log transformed data; with sample size = 9.
[2] To detect a significant difference from control treatment based on maximum achieved absolute difference; calculated on natural log transformed data.

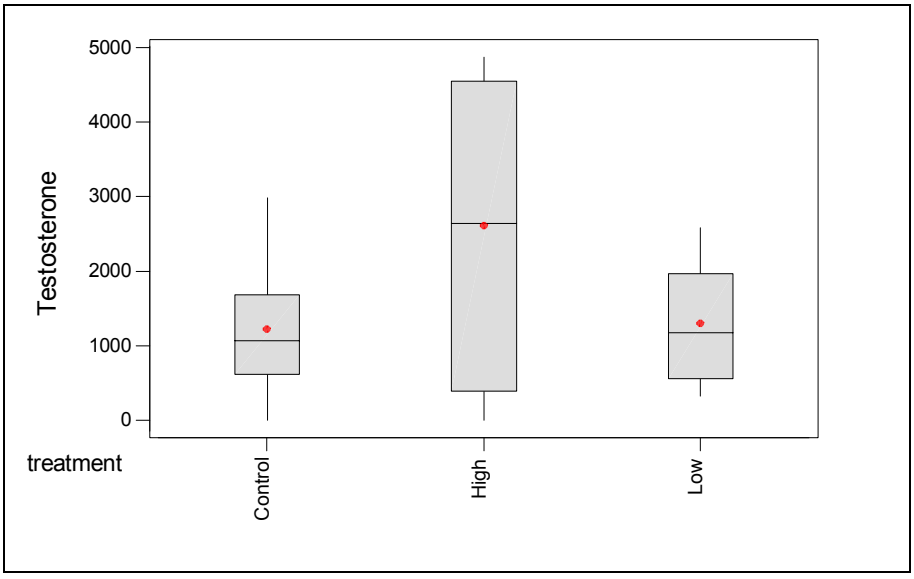

Figure 5.12. Boxplot of female testosterone concentration (pg/mL) by treatment for the EPA 14-Day Flutamide assay. The box represents the interquartile range, whiskers represent the data range, the horizontal line is the median value, and the circle is the mean value.

Testosterone concentrations in Control treatment males used during the EPA 14-Day Flutamide assay ranged from 949 pg/mL to 6,258 pg/mL (Figure 5.13). Among males exposed to the two flutamide concentrations, testosterone concentrations ranged from 1,161 pg/mL to 5,529 pg/mL. No significant differences in the mean testosterone concentration per treatment (Table 5.11) were detected (Kruskal-Wallis, $H = 0.25$, $p = 0.883$, df = 2). The achieved power for this endpoint was 6%, and the sample size required to detect a significant difference from the Control treatment at 80% power was 760 (Table 5.11).

Table 5.11. Summary statistics and power estimates for male testosterone concentrations (pg/mL) for the EPA 14-Day Flutamide assay.

Level	N	Mean	Stdev	CV	Achieved Power [1]	Sample Size Required [2]
control	7	2,853	2,159	76%	6%	760
low	8	2,552	1,173	46%		
high	7	2,715	1,357	50%		

[1] Calculated from natural log transformed data; with sample size = 7.
[2] To detect a significant difference from control treatment based on maximum achieved absolute difference; calculated on natural log transformed data.

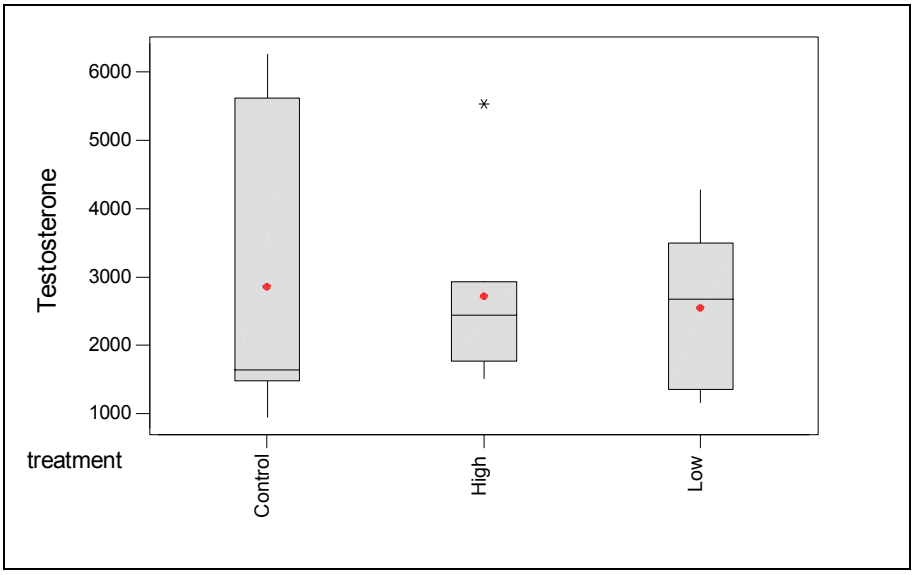

Figure 5.13. Boxplot of male testosterone concentration (pg/mL) by treatment for the EPA 14-Day Flutamide assay. The box represents the interquartile range, whiskers represent the data range, the horizontal line is the median value, the circle is the mean value, and the asterisk represents a probable outlier.

11-ketotestosterone—11-ketotesosterone was not detected in females from the Control treatment (five individuals), the Low concentration (six individuals), and the High concentration (six individuals) exposed during the EPA 14-Day Flutamide assay.

11-ketotesosterone concentrations in Control treatment males used during the EPA 14-Day Flutamide assay ranged from 5,287 pg/mL to 63,011 pg/mL (Figure 5.14). Among males exposed to the two flutamide concentrations, 11-ketotesosterone concentrations ranged from 3,942 pg/mL to 48,001 pg/mL. No significant differences in the mean 11-ketotesosterone concentration per treatment (Table 5.12) were detected (Kruskal-Wallis, $H = 0.37$, $p = 0.830$, df = 2). The achieved power for this endpoint was 6%, and the sample size required to detect a significant difference from the Control treatment at 80% power was 356 (Table 5.12).

Table 5.12. Summary statistics and power estimates for male 11-ketotesosterone concentrations (pg/mL) for the EPA 14-Day Flutamide assay.

Level	N	Mean	Stdev	CV	Achieved Power [1]	Sample Size Required [2]
control	7	24,269	24,145	99%	6%	356
low	8	23,583	12,787	54%		
high	7	21,815	13,712	63%		

[1] Calculated from natural log transformed data; with sample size = 7.
[2] To detect a significant difference from control treatment based on maximum achieved absolute difference; calculated on natural log transformed data.

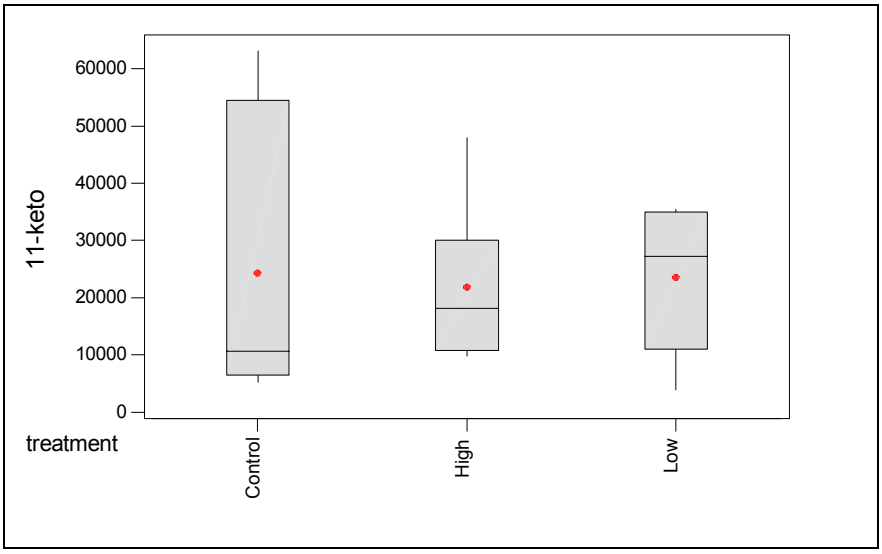

Figure 5.14. Boxplot of male 11-ketotesosterone concentration (pg/mL) by treatment for the EPA 14-Day Flutamide assay. The box represents the interquartile range, whiskers represent the data range, the horizontal line is the median value, and the circle is the mean value.

5.1.8 Fecundity

Total Fecundity—Variability among treatments in the total number of eggs produced per replicate during the EPA 14-Day Flutamide assay was very high (Figure 5.15). Total counts in the Control treatment ranged from 2,758 eggs to 4,346 eggs. Total counts for the High flutamide concentration treatment ranged from 1,004 eggs to 2,687 eggs. No eggs were laid after Day 10 in one High concentration replicate or after Day 12 in another replicate. The fecundity among replicates in the Low flutamide concentration ranged from 3,448 eggs to 4,876 eggs. A significant difference in the mean number of eggs (square-root transformed) produced per treatment (Table 5.13) was detected (Kruskal-Wallis, $H = 8.77$, $p = 0.012$, df = 2). The total fecundity of females in the High-concentration treatment was significantly less than it was for females in the Low concentration or the Control treatment. The achieved power for this assay was 79%, and the sample size required to detect a significant difference from the Control treatment at 80% power was 5 (Table 5.13).

Table 5.13. Summary statistics and power estimates for fecundity data for the EPA 14-Day Flutamide assay.

Level	N	Mean	Stdev	CV	Achieved Power [1]	Sample Size Required [2]
control	4	3539	684	19%	79%	5
low	4	4501	702	16%		
high	4	1690	820	48%		

[1] Calculated from square-root transformed data; with sample size = 4.
[2] To detect a significant difference from control treatment based on maximum achieved absolute difference; calculated on square-root transformed data.

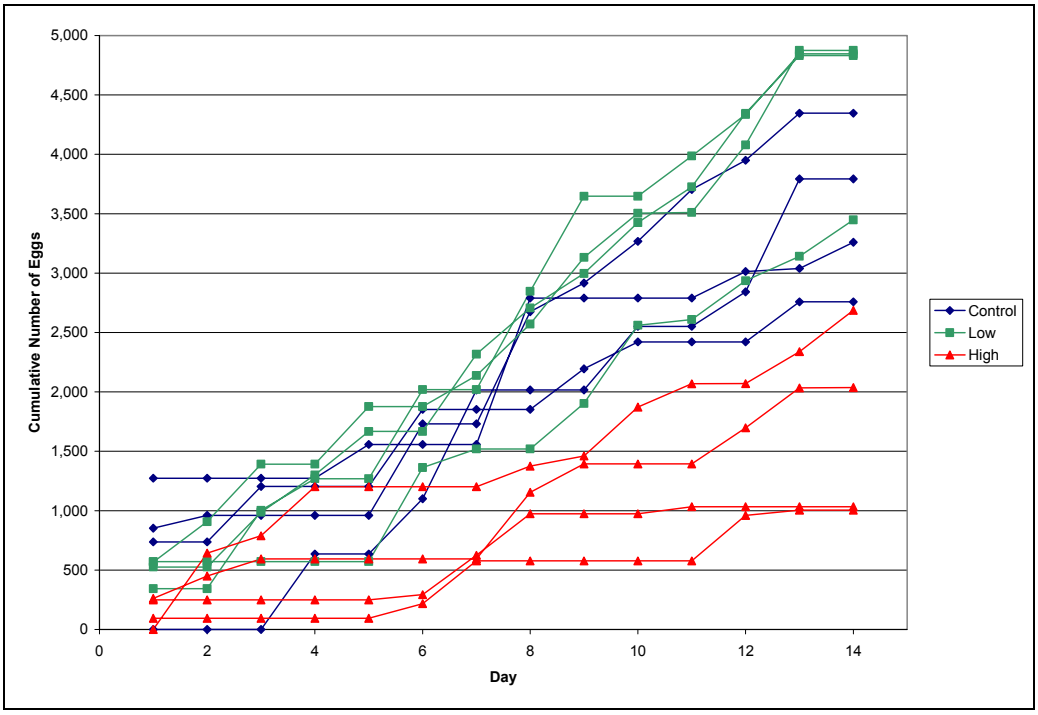

Figure 5.15. Total egg production by replicate per treatment for the EPA 14-Day Flutamide assay.

Fecundity per Female Reproductive Day—During the EPA 14-Day Flutamide assay, the maximum number of female reproductive days was achieved for all treatments as no fish died during the assay (Table 5.14). The number of eggs produced per female reproductive day in the Control treatment varied from 49.3 eggs to 77.6 eggs and from 61.6 eggs to 87.1 eggs in the Low concentration (Figure 5.16). For the High concentration, the number of eggs produced per female reproductive day ranged from 17.9 eggs to 48.0 eggs, with fish in two of the replicates producing fewer than 20 eggs per day (17.9, 18.5). Because no fish died during the assay, the statistical results reported here are the same as those reported for total fecundity. A significant difference in the mean number of eggs laid per day per treatment (Table 5.14) occurred. The number of eggs laid per day in the High-concentration treatment was significantly less than it was for females in the Low concentration or the Control treatment. The achieved power for this assay was 77%, and the sample size required to detect a significant difference from the Control treatment at 80% power was 5 (Table 5.14).

Table 5.14. Summary statistics and power estimates for fecundity per female reproductive day for the EPA 14-Day Flutamide assay.

Level	Mean Number of Reproductive Days [1]	N	Mean	Stdev	CV	Achieved Power [2]	Sample Size Required [3]
control	56	4	63.2	12.2	19%	77%	5
low	56	4	80.4	12.5	16%		
high	56	4	30.2	14.6	48%		

[1] Maximum number = 56.
[2] Calculated from natural log transformed data; with sample size = 4.
[3] To detect a significant difference from control treatment based on maximum achieved absolute difference; calculated on natural log transformed data.

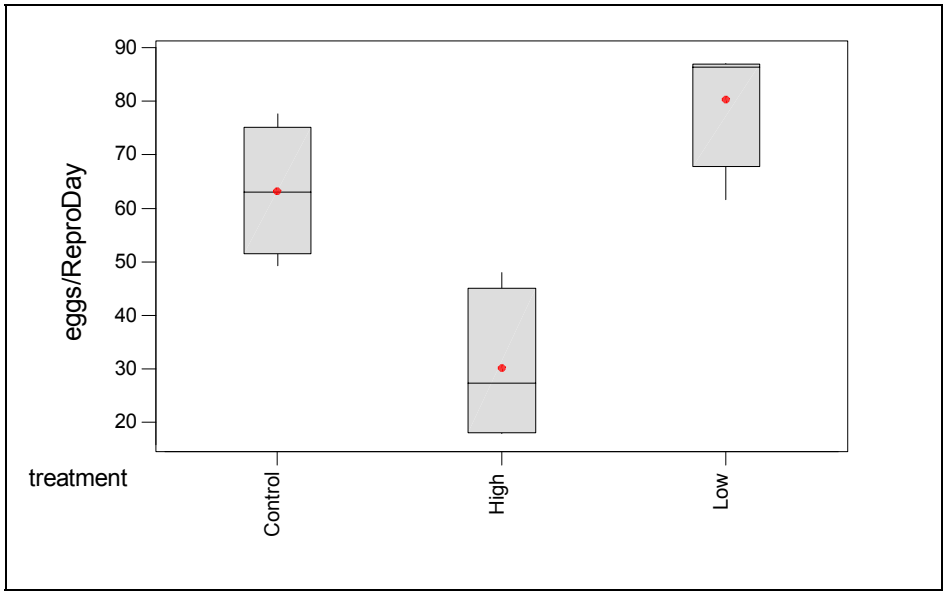

Figure 5.16. Boxplot of the number of eggs produced per female reproductive day by treatment for the EPA 14-Day Flutamide assay. The box represents the interquartile range, whiskers represent the data range, the horizontal line is the median value, and the circle is the mean value.

Eggs on Tiles/Dishes—The mean number of eggs laid on the tiles among the treatments during the EPA 14-Day Flutamide assay varied from 1,518 eggs for the High concentration to 4,148 eggs for the Low concentration (Appendix E, Table 3.3). The number of eggs on dishes ranged from 172 eggs for the High concentration to 353 eggs for the Low concentration. Because of the variability in the total number of eggs laid per treatment, the proportional difference in the number of eggs on dishes versus those on tiles [1–(# eggs on dishes ÷ # eggs on tiles)] was calculated. The proportional difference ranged from 0.78 (one High concentration replicate) to 0.96 (one Control-treatment replicate) (Appendix E, Figure 3.2). There were no significant differences in this mean proportional difference among treatments (Kruskal-Wallis, $H = 1.04$, $p = 0.595$, df = 2).

5.1.9 Fertilization Success

Total Fertilization—The total (tiles + dishes) fertilization-success rates for all treatment replicates during the EPA 14-Day Flutamide assay were high, ranging from 0.993 (one High-concentration replicate) to 1.00 (two High-concentration replicates, three Control-treatment replicates) (Figure 5.17). No significant differences in mean fertilization-success rates (Table 5.15) among treatments were detected (Kruskal-Wallis, $H = 1.53$, $p = 0.465$, df = 2). The achieved power for this assay was 17%, and the sample size required to detect a significant difference from the Control treatment at 80% power was 22 (Table 5.15).

Table 5.15. Summary statistics and power estimates for the proportion of eggs fertilized for the EPA 14-Day Flutamide assay.

Level	N	Mean	Stdev	CV	Achieved Power [1]	Sample Size Required [2]
control	4	1.000	0.001	0.1%	17%	22
low	4	1.000	0.000	0.0%		
high	4	0.997	0.003	0.3%		

[1] Calculated from arcsine square-root transformed data; with sample size = 4.
[2] To detect a significant difference from control treatment based on maximum achieved absolute difference; calculated on arcsine square-root transformed data.

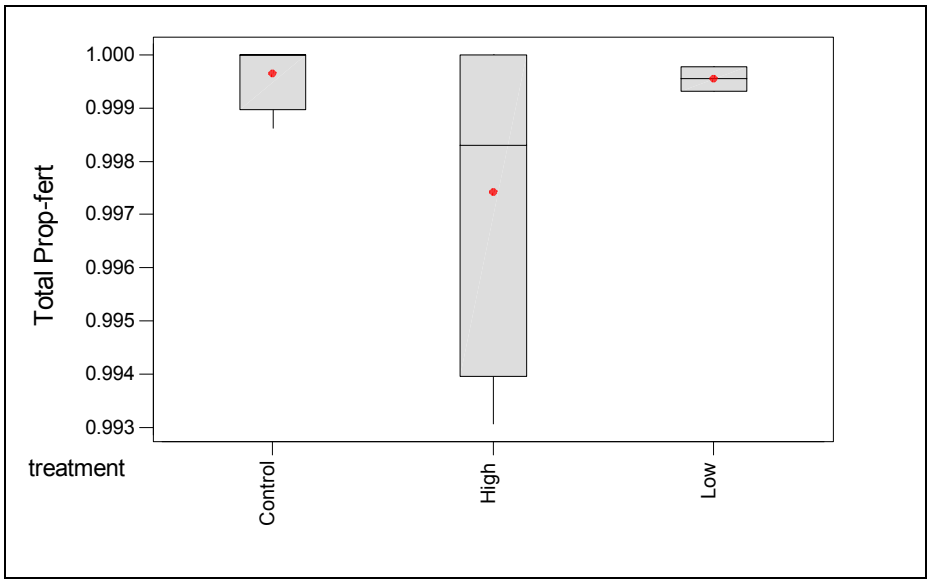

Figure 5.17. Boxplot of the proportion of eggs fertilized by treatment for the EPA 14-Day Flutamide assay. The box represents the interquartile range, whiskers represent the data range, the horizontal line is the median value, and the circle is the mean value.

Fertilization of Eggs on Tiles and Dishes— The fertilization-success rates for most treatment replicates for eggs laid on tiles during the EPA 14-Day Flutamide assay were high, ranging from 0.992 (one High concentration replicate) to 1.00 (two High concentration replicates, three Control-treatment replicates) (Appendix E, Figure 3.3). No significant differences in mean fertilization-success rates (Appendix E, Table 3.4) among treatments were detected (Kruskal-Wallis, $H = 1.53$, $p = 0.465$, df = 2). The fertilization-success rates for all treatment replicates for eggs laid on dishes during the assay were very

high, ranging from 0.997 (one Control-treatment replicate) to 1.00 (all remaining replicates from all treatments) (Appendix E, Figure 3.4). No significant differences in mean fertilization-success rates (Appendix E, Table 3.4) among treatments were detected (Kruskal-Wallis, $H = 2.00$, $p = 0.368$, df = 2).

5.1.10 Hatchability and Larval Development

Eggs were collected during the 14-day pre-exposure period for the evaluation of hatchability. The mean proportion of fertilized eggs that hatched in the Control treatment was 0.96 (sd = 0.05), 0.94 (sd = 0.03) in the Low concentration, and 0.98 (sd = 0.02) in the High concentration. There were no significant differences among treatments in the proportion of eggs that hatched (Kruskal-Wallis, $H = 2.20$, $p = 0.333$, df = 2).

Eggs were collected during the EPA 14-Day Flutamide assay for the evaluation of hatchability. The proportion of fertilized eggs that hatched ranged from 0.94 to 1.00 in the Control treatment and from 0.80 to 1.00 for the two test concentrations (Figure 5.18). There were no significant differences among treatments in the proportion of eggs that hatched (Kruskal-Wallis, $H = 2.99$, $p = 0.224$, df = 2). The achieved power for this endpoint was 20%, and the sample size required to detect a significant difference from the Control treatment at 80% power was 18 (Table 5.16).

Table 5.16. Summary statistics and power estimates for the proportion of fertile eggs that hatched for the EPA 14-Day Flutamide assay.

Level	N	Mean	Stdev	CV	Achieved Power [1]	Sample Size Required [2]
control	4	0.98	0.03	3%	20%	18
low	5	0.97	0.04	4%		
high	5	0.91	0.08	9%		

[1] Calculated from arcsine square-root transformed data; with sample size = 4.
[2] To detect a significant difference from control treatment based on maximum achieved absolute difference; calculated on arcsine square-root transformed data.

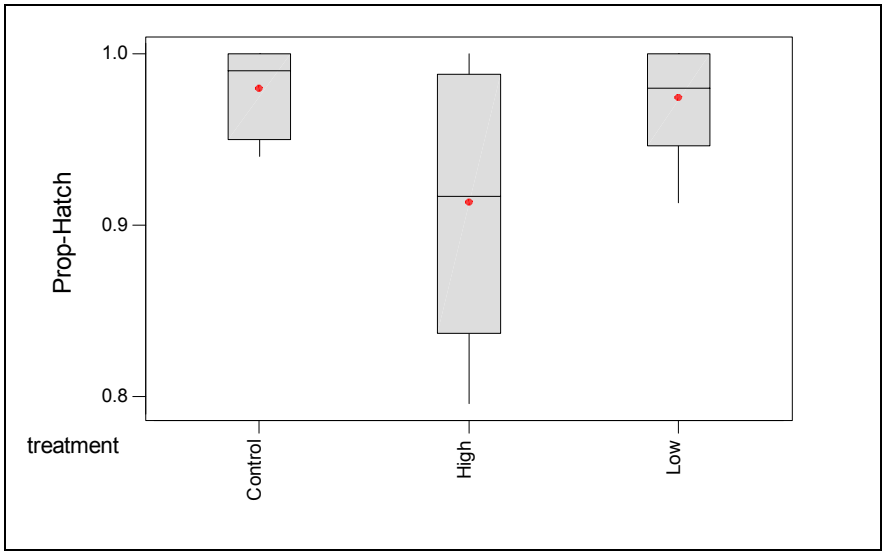

Figure 5.18. Boxplot of the proportion of fertile eggs that hatched by treatment for the EPA 14-Day Flutamide assay. The box represents the interquartile range, whiskers represent the data range, the horizontal line is the median value, and the circle is the mean value.

Eggs were collected during the pre-exposure period for the evaluation of larval development. The mean proportion of larvae that developed normally (i.e., that showed no morphological abnormalities) in the Control treatment was 1.00 (sd = 0.01). The mean proportion of normal larvae in the remaining treatments was 0.97 (sd = 0.02) in the Low concentration and 0.98 (sd = 0.02) in the High concentration. There were no significant differences among treatments in the proportion of eggs that hatched (Kruskal-Wallis, $H = 3.46$, $p = 0.178$, df = 2).

Eggs were collected during the EPA 14-Day Flutamide assay for the evaluation of larval development. The proportion of larvae that developed normally (i.e., that showed no morphological abnormalities) ranged from 0.96 to 1.00 for the Control treatment and ranged from 0.95 to 1.00 for the two test concentrations (Figure 5.19). There were no significant differences among treatments in the proportion of eggs that hatched (Kruskal-Wallis, $H = 3.38$, $p = 0.185$, df = 2). The achieved power for this endpoint was 12%, and the sample size required to detect a significant difference from the Control treatment at 80% power was 34 (Table 5.17).

Table 5.17. Summary statistics and power estimates for the proportion of normal larvae for the EPA 14-Day Flutamide assay.

Level	N	Mean	Stdev	CV	Achieved Power [1]	Sample Size Required [2]
control	4	0.98	0.02	2%	12%	34
low	5	0.99	0.01	1%		
high	5	0.97	0.02	2%		

[1] Calculated from arcsine square-root transformed data; with sample size = 4.
[2] To detect a significant difference from control treatment based on maximum achieved absolute difference; calculated on arcsine square-root transformed data.

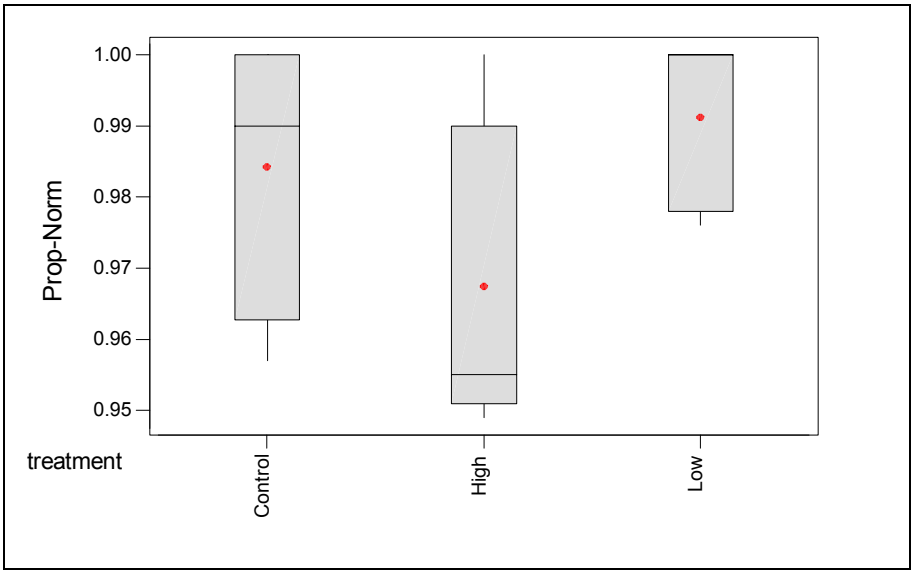

Figure 5.19. Boxplot of the proportion of normal larvae by treatment for the EPA 14-Day Flutamide assay. The box represents the interquartile range, whiskers represent the data range, the horizontal line is the median value, and the circle is the mean value.

5.1.11 Body Weight

The body weight of females used in the EPA 14-Day Flutamide assay ranged from 0.9 g to 3.1 g. There were no significant differences in mean body weight among treatments, although the probability was only slighter greater than the critical limit of 0.05 (Kruskal-Wallis, H =5.72, p = 0.057, df = 2). Females from the Control treatment tended to be larger than those form the other two treatments. The body weight of males used in the EPA 14-Day Flutamide assay ranged from 2.8 g to 5.6 g. There were no significant differences in mean body weight among treatments (Kruskal-Wallis, H = 2.72, p = 0.257, df = 2).

5.2 EPA 21-Day Assay for Flutamide

The EPA 21-Day Flutamide assay was conducted from February 11, 2003, to February 25, 2003 (pre-exposure assay), and from February 25, 2003, to March 18, 2003 (exposure assay).

5.2.1 Survival

All males and females in all treatments survived the EPA 21-Day Flutamide assay.

5.2.2 Vitellogenin

Vitellogenin concentrations in Control treatment females used during the EPA 21-Day Flutamide assay ranged from 252,800 ng/mL to 9,071,500 ng/mL (Figure 5.20). Among females exposed to the two flutamide concentrations, vitellogenin concentrations ranged from 3,006,000 ng/mL to 9,830,000 ng/mL. No significant differences in the mean vitellogenin concentration per treatment (Table 5.18) were detected (Kruskal-Wallis, H = 5.08, p = 0.079, df = 2). The achieved power for this endpoint was 44%, and the sample size required to detect a significant difference from the Control treatment at 80% power was 31 (Table 5.18).

Table 5.18. Summary statistics and power estimates for female vitellogenin concentrations (ng/mL) for the EPA 21-Day Flutamide assay.

Level	N	Mean	Stdev	CV	Achieved Power [1]	Sample Size Required [2]
control	16	4,917,456	2,135,773	43%	44%	31
low	15	6,182,160	1,860,122	30%		
high	14	6,483,000	1,538,230	24%		

[1] Calculated from natural log transformed data; with sample size = 14.
[2] To detect a significant difference from control treatment based on maximum achieved absolute difference; calculated on natural log transformed data.

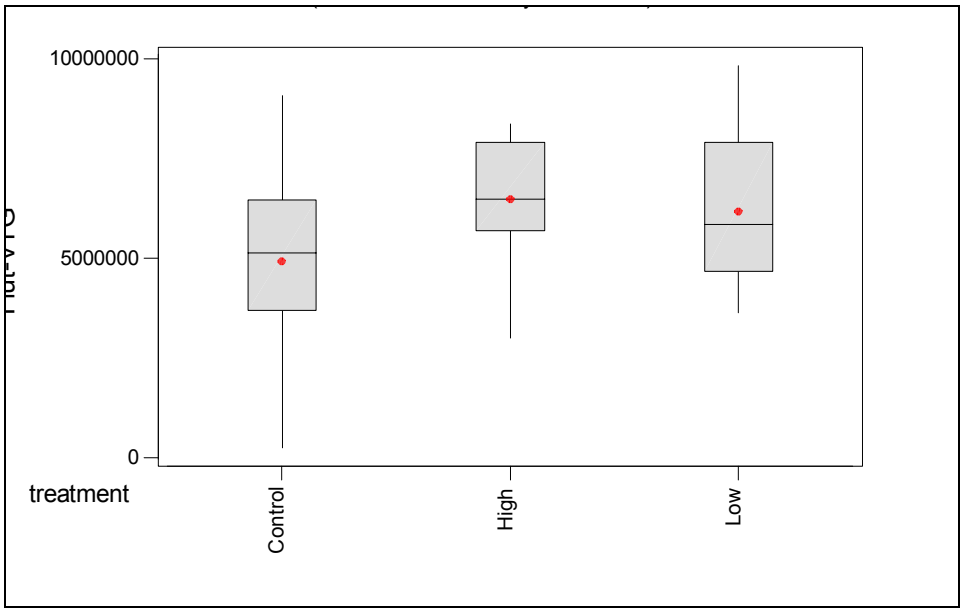

Figure 5.20. Boxplot of female vitellogenin concentration (ng/mL) by treatment for the EPA 21-Day Flutamide assay. The box represents the interquartile range, whiskers represent the data range, the horizontal line is the median value, and the circle is the mean value.

Vitellogenin concentrations in Control treatment males used during the EPA 21-Day Flutamide assay ranged from 51 ng/mL to 13,485 ng/mL (Figure 5.21). Among males exposed to the two flutamide concentrations, vitellogenin concentrations ranged from 0 ng/mL (not detected) to 35,355 ng/mL. No significant differences in the mean vitellogenin concentration per treatment (Table 5.19) were detected (Kruskal-Wallis, $H = 2.95$, $p = 0.229$, df = 2). The achieved power for this endpoint was 13%, and the sample size required to detect a significant difference from the Control treatment at 80% power was 70 (Table 5.19).

Table 5.19. Summary statistics and power estimates for male vitellogenin concentrations (ng/mL) for the EPA 21-Day Flutamide assay.

Level	N	Mean	Stdev	CV	Achieved Power [1]	Sample Size Required [2]
control	8	2,418	4,547	188%	13%	70
low	8	9,681	13,513	140%		
high	8	12,497	14,887	119%		

[1] Calculated from natural log transformed data; with sample size = 8.
[2] To detect a significant difference from control treatment based on maximum achieved absolute difference; calculated on natural log transformed data.

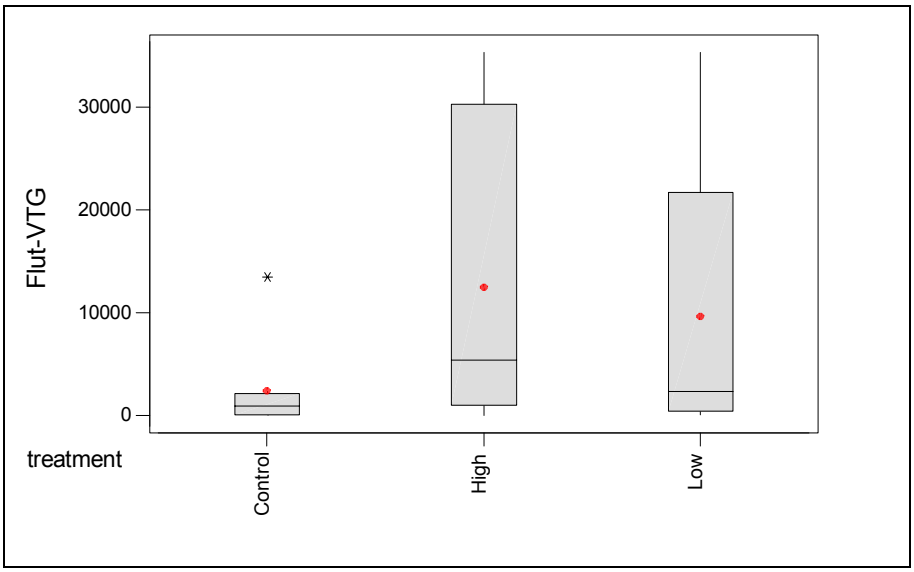

Figure 5.21. Boxplot of male vitellogenin concentration (ng/mL) by treatment for the EPA 21-Day Flutamide assay. The box represents the interquartile range, whiskers represent the data range, the horizontal line is the median value, the circle is the mean value, ant the asterisk represents a probable outlier.

5.2.3 Appearance / Secondary Sex Characteristics

All of the females used during the EPA 21-Day Flutamide assay exhibited typical female morphology (no fat pad, no tubercles, ovipositor present) except that one female from the High concentration that showed the dark vertical banding typically found in males.

Most males during the EPA 21-Day Flutamide assay had typical male morphological features. One male from the Low concentration lacked tubercles and a dorsal fat pad. Tubercles were present in all other males. Fat pads were present or pronounced in all other males from all treatments. Vertical banding was present or pronounced in all males.

5.2.4 Gonadosomatic Index

The range of GSI values calculated for females in the Control treatment varied from 2.1 to 14.3 (Figure 5.22), whereas the GSI values for the other treatments varied from 8.2 to 15.7. The overall variability within the treatment was moderate (CVs = 18%–35%; Table 5.20). No significant differences in the mean GSI value per treatment (Table 5.20) were detected (Kruskal-Wallis, $H = 3.00$, $p = 0.223$, df = 2). The achieved power for this endpoint was 40%, and the sample size required to detect a significant difference from the Control treatment at 80% power was 39 (Table 5.20).

Table 5.20. Summary statistics and power estimates for female gonadosomatic index data for the EPA 21-Day Flutamide assay.

Level	N	Mean	Stdev	CV	Achieved Power [1]	Sample Size Required [2]
control	16	10.0	3.5	35%	40%	39
low	16	11.8	2.2	19%		
high	16	10.7	2.0	18%		

[1] Calculated from arcsine square-root transformed data; with sample size = 16.
[2] To detect a significant difference from control treatment based on maximum achieved absolute difference; calculated on arcsine square-root transformed data.

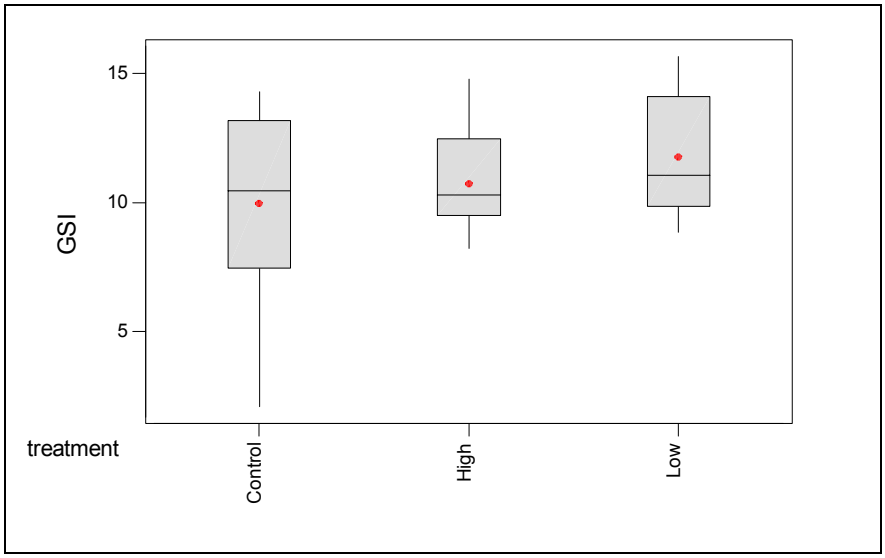

Figure 5.22. Boxplot of female GSI by treatment for the EPA 21-Day Flutamide assay. The box represents the interquartile range, whiskers represent the data range, the horizontal line is the median value, and the circle is the mean value.

The range of most GSI values calculated for males during the EPA 21-Day Flutamide assay, was small, ranging from 0.6 to 1.7 (Figure 5.23), which approximates the typical range for reproductively-active male fathead minnows. There were no significant differences in mean GSI values (Table 5.21) among treatments (Kruskal-Wallis, $H = 1.06$, $p = 0.590$, df = 2). The achieved power for this endpoint was 17%, and the sample size required to detect a significant difference from the Control treatment at 80% power was 48 (Table 5.21).

Table 5.21. Summary statistics and power estimates for male gonadosomatic index data for the EPA 21-Day Flutamide assay.

Level	N	Mean	Stdev	CV	Achieved Power [1]	Sample Size Required [2]
control	8	1.0	0.3	29%	17%	48
low	8	1.1	0.3	31%		
high	8	1.2	0.3	26%		

[1] Calculated from arcsine square-root transformed data; with sample size = 8.
[2] To detect a significant difference from control treatment based on maximum achieved absolute difference; calculated on arcsine square-root transformed data.

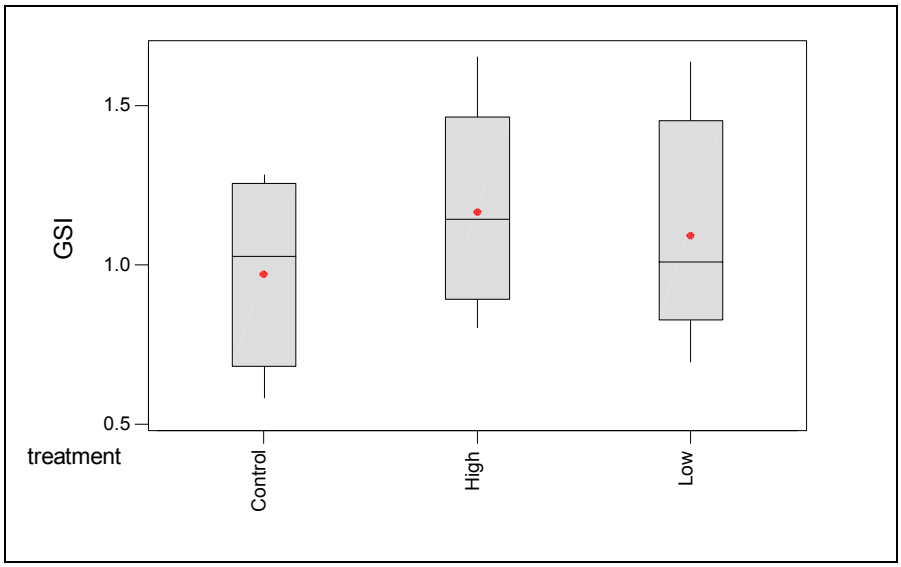

Figure 5.23. Boxplot of male GSI by treatment for the EPA 21-Day Flutamide assay. The box represents the interquartile range, whiskers represent the data range, the horizontal line is the median value, and the circle is the mean value.

5.2.5 Female Gonad Histology

Histological analyses were conducted on the ovaries of 48 females exposed to flutamide during the EPA 21-Day Assay.

General Ovary Staging—Statistical analysis of the mean ovarian staging from 12 microscopic fields per female in the EPA 21-Day Flutamide assay revealed no significant differences among treatments (Kruskal-Wallis, $H = 2.10$, $p = 0.350$, df = 2).

Quantitative Ovarian Staging—One hundred cells in each of three sections per female were examined to quantitatively determine the developmental stage of the ovaries. Ova from fish in all treatments ranged from Stage 1a to Stage 5 (see Methods for a description of the stages (Figure 5.24). Variability within treatments for each stage, particularly for Stage 5, was very high as indicated by CVs that ranged as high as 400% (Table 5.22). Statistical analyses showed that within each of the five developmental stages there were no significant differences among treatments in the proportion of cells occurring in the stage (Table 5.22). Therefore, flutamide dose had no effect on ovarian developmental stage.

Table 5.22. Descriptive statistics of the proportion of ovarian cells in each developmental stage for females from the EPA 21-Day Flutamide assay and results of the Kruskal-Wallis Test (df = 2) comparing treatments.

	Control (n = 16)			Low (n = 16)			High (n = 16)			Kruskal-Wallis	
Stage	Mean	Stdev	CV	Mean	Stdev	CV	Value	Stdev	CV	*H*	*p*
1a	0.079	0.028	35%	0.082	0.040	49%	0.080	0.035	44%	0.21	0.900
1b	0.303	0.083	27%	0.270	0.078	29%	0.270	0.057	21%	1.70	0.427
2	0.182	0.027	15%	0.204	0.051	25%	0.193	0.046	24%	0.68	0.714
3	0.193	0.037	19%	0.204	0.053	26%	0.184	0.075	40%	1.88	0.391
4	0.197	0.105	53%	0.218	0.119	55%	0.206	0.110	53%	0.65	0.722
5	0.015	0.043	294%	0.010	0.038	400%	0.014	0.034	246%	1.89	0.389

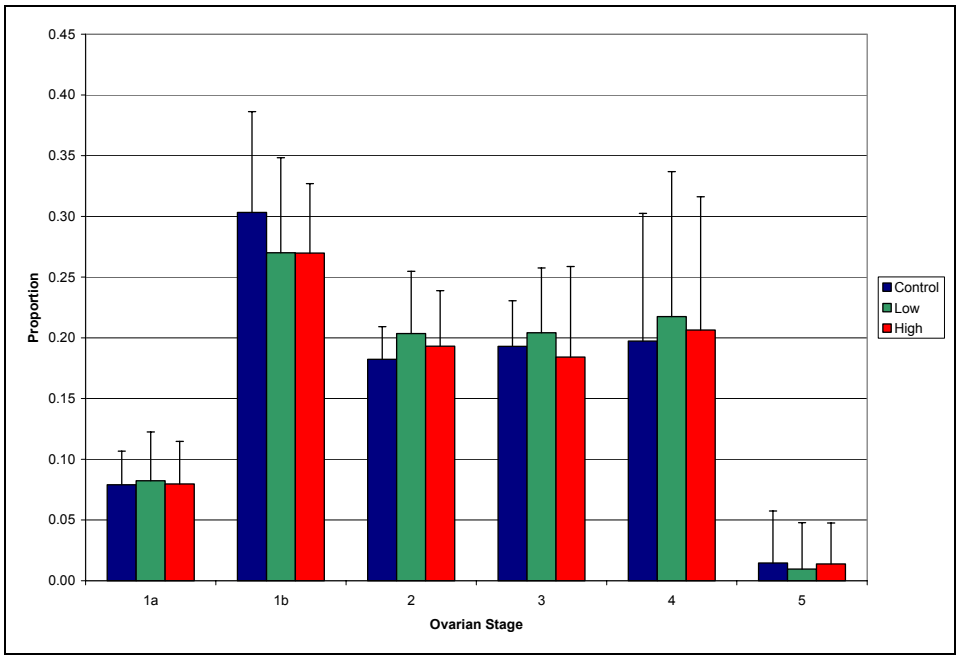

Figure 5.24. Frequency histogram showing the quantitative developmental staging of ovaries for each treatment of the EPA 21-Day Flutamide assay. For each treatment, the columns represent the grand mean proportion of cells in each stage and the bars represent the standard deviation.

Atretic Follicles—The mean proportion of atretic follicles per 300 follicles (counted per fish) ranged from 0.002 follicles for females in the Low concentration to 0.019 follicles for females in the Control treatment (Figure 5.25). One fish from the Control treatment had a very high proportion of atretic follicles (0.27). A significant difference in the proportions of atretic follicles among treatments was detected (Kruskal-Wallis, $H = 9.92$, $p = 0.007$, df = 2). The majority of the observations from the High concentration ranked higher than those of the remaining treatments.

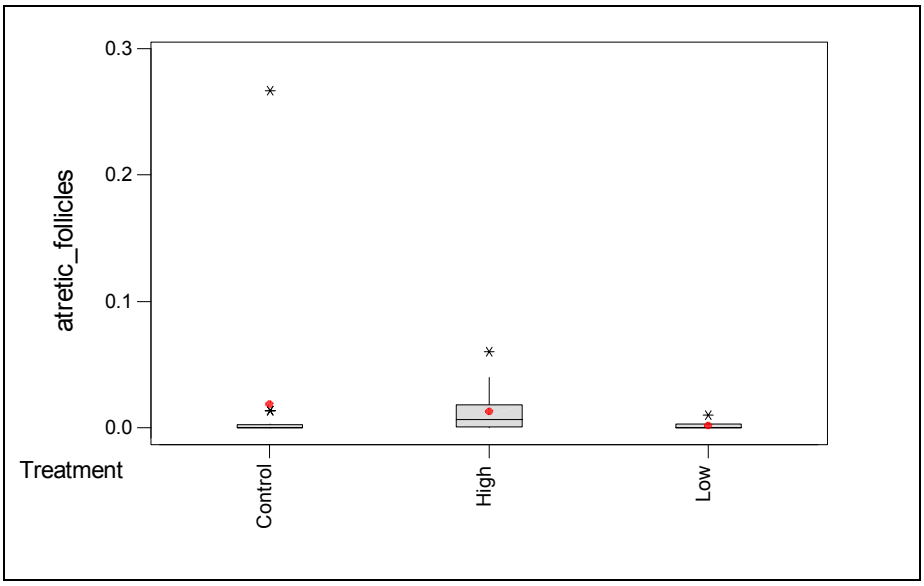

Figure 5.25. Boxplot of the proportion of atretic follicles per 300 follicles by treatment for the EPA 21-Day Flutamide assay. The box represents the interquartile range, whiskers represent the data range, the horizontal line is the median value, the circle is the mean value, and asterisks represent probable outliers.

Corpora Lutea—The mean proportion of corpora lutea per 300 follicles (counted per fish) ranged from 0.011 for females in the Low concentration to 0.040 for females in the High concentration (Figure 5.26). Variability within each treatment was very high, ranging from 150% for the High concentration to 236% for the Low concentration. There were no significant differences in the mean proportion of corpora lutea among treatments (Kruskal-Wallis, $H = 3.90$, $p = 0.142$, df = 2).

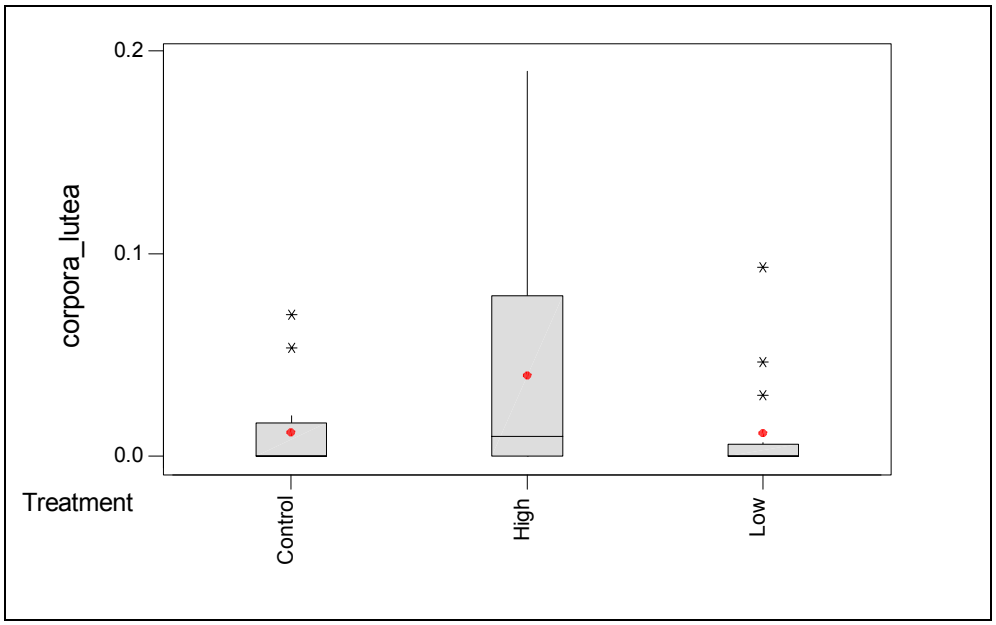

Figure 5.26. Boxplot of the proportion of corpora lutea per 300 follicles by treatment for the EPA 21-Day Flutamide assay. The box represents the interquartile range, whiskers represent the data range, the horizontal line is the median value, the circle is the mean value, and asterisks represent probable outliers.

Observations—Two fish from the Control treatment were observed to have very few eggs in the ovaries. Two fish from the Low concentration were observed to have very few eggs in the ovaries. One of these two fish was also noted to have very small yolk particles in some cortical alveoli cells. One fish from the High concentration were observed to have very few eggs in the ovaries. One other fish from the High concentration had extensive epithelial and ovarian debris in the ovary and one had debris from disintegrated vitellogenic ova in the ovary.

5.2.6 Male Gonad Histology

General Testes Staging—Testes from 24 males exposed to flutamide during the EPA 21-Day Flutamide assay were examined to determine the general developmental condition. Males in all treatments had well-developed testes with most showing Stage 4 and Stage 5 development (see Methods for description of developmental stages). All of the 96 microscopic fields examined in the 8 Control treatment males showed Stage 4 (76 fields) or Stage 5 (20 fields) development. Ninety-four of the 96 microscopic fields examined in the 8 Low-concentration treatment males showed Stage 4 (87 fields) or Stage 5 (7 fields) development. Two microscopic fields showed Stage 3 development. All of the 96 microscopic fields examined in the 8 High-concentration treatment males showed Stage 4 (68 fields) or Stage 5 (28 fields) development. Statistical analysis of the mean staging from 12 microscopic fields per fish revealed no significant differences among treatments (Kruskal-Wallis, $H = 4.21$, $p = 0.122$, df = 2).

Quantitative Testicular Staging—One hundred cells in each of three sections per male were examined to quantitatively determine the developmental condition of the testes. The developmental stage all treatment testes ranged from Stage 2a to Stage 5 (Figure 5.27). Variability within treatments for each stage was very high as indicated by CVs that ranged as high as 129% (Table 5.23). Although statistical analyses showed that there were no significant differences among treatments in the proportion of cells in any of the developmental stages, the probability value calculated for Stage 3b was slightly above the

critical limit of 0.050 (Table 5.23). The largest difference in the mean proportion of cells in this developmental stage was between the Low- and High-concentration treatments. Therefore, there was no effect on testicular developmental stage associated with flutamide dose.

Table 5.23. Descriptive statistics of the proportion of testes cells in each developmental stage for males from the EPA 21-Day Flutamide assay and results of the Kruskal-Wallis Test (df = 2) comparing treatments.

	Control (n = 8)			Low (n = 8)			High (n = 8)			Kruskal-Wallis	
Stage	Mean	Stdev	CV	Mean	Stdev	CV	Value	Stdev	CV	*H*	*p*
1	0	0	–	0	0	–	0	0	–	–	–
2a	0.003	0.004	129%	0.003	0.003	93%	0.006	0.005	79%	2.21	0.331
2b	0.009	0.009	101%	0.012	0.013	112%	0.010	0.009	96%	0.04	0.980
3a	0.123	0.096	78%	0.094	0.048	51%	0.128	0.081	64%	0.60	0.740
3b	0.205	0.118	57%	0.325	0.102	31%	0.211	0.064	30%	5.95	0.051
4	0.118	0.073	62%	0.152	0.086	56%	0.151	0.064	42%	0.60	0.740
5	0.543	0.271	50%	0.415	0.132	32%	0.495	0.168	34%	1.03	0.598

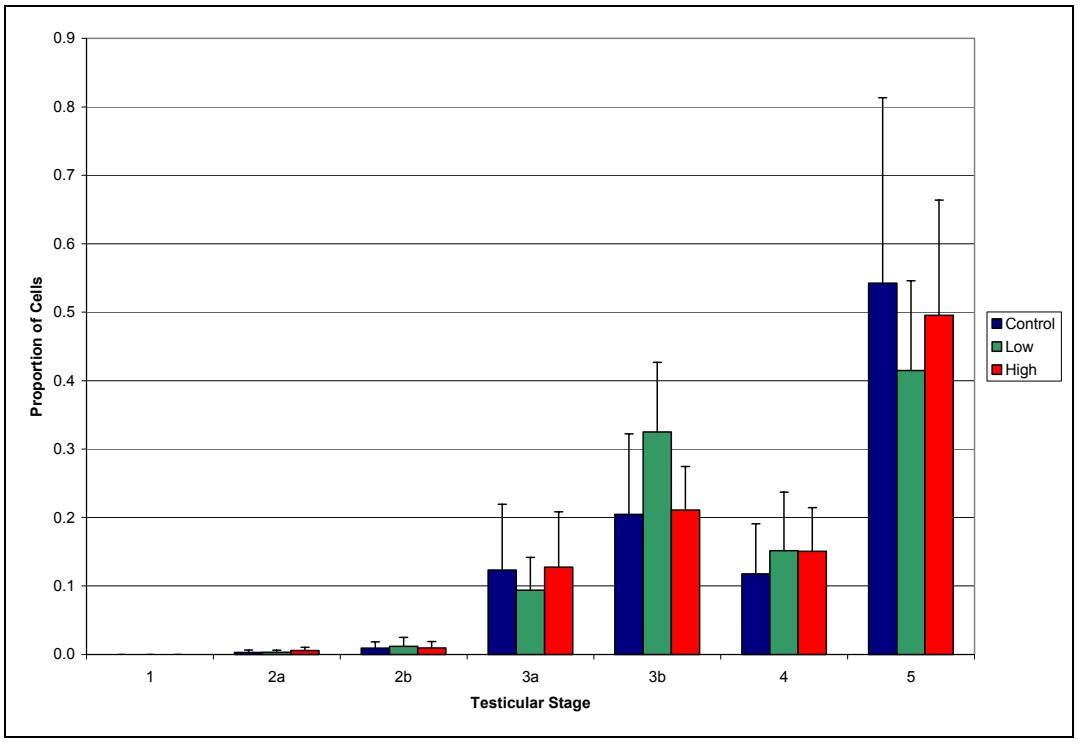

Figure 5.27. Frequency histogram showing the quantitative developmental staging of testes for each treatment of the EPA 21-Day Flutamide assay. For each treatment, the columns represent the grand mean proportion of cells in each stage and the bars represent the standard deviation.

Tubule Diameter—The average diameter of the seminiferous tubules of males from the Control treatment ranged from 112.5 µm to 165.6 µm (Figure 5.28). Tubule diameters of males from the two test concentrations ranged from 96.7 µm to 148.9 µm. No significant differences in the mean tubule diameter

per treatment (Table 5.24) were detected (Kruskal-Wallis, $H = 5.57$, $p = 0.062$, df = 2). The achieved power for this endpoint was 63%, and the sample size required to detect a significant difference from the Control treatment at 80% power was 12 (Table 5.24).

Table 5.24. Summary statistics and power estimates for male seminiferous tubule diameter (μm) data for the EPA 21-Day Flutamide assay.

Level	N	Mean	Stdev	CV	Achieved Power [1]	Sample Size Required [2]
control	8	137.4	18.3	13%	63%	12
low	8	116.0	16.5	14%		
high	8	132.0	11.7	9%		

[1] Calculated from natural log transformed data; with sample size = 8.
[2] To detect a significant difference from control treatment based on maximum achieved absolute difference; calculated on natural log transformed data.

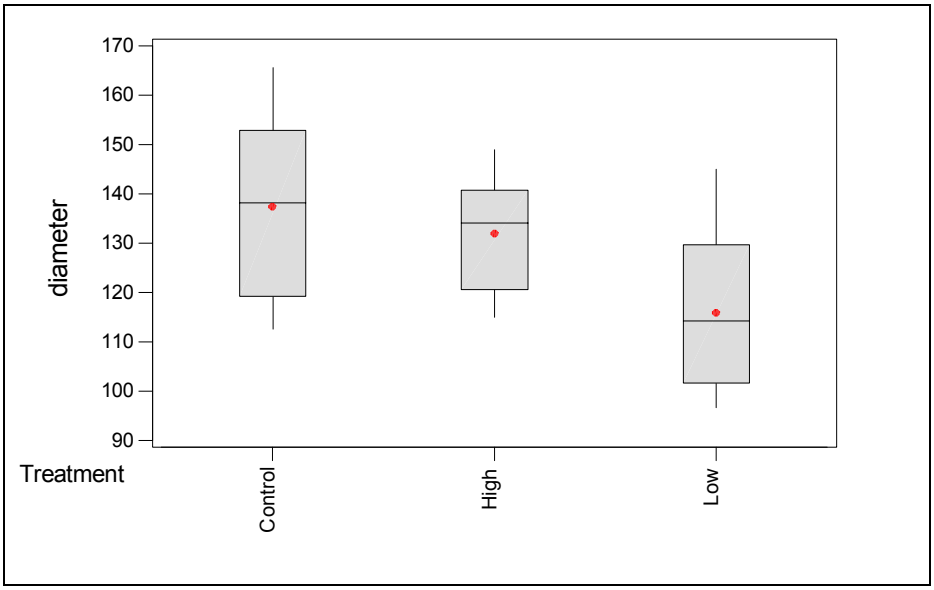

Figure 5.28. Boxplot of male seminiferous tubule diameter (μm) by treatment for the EPA 21-Day Flutamide assay. The box represents the interquartile range, whiskers represent the data range, the horizontal line is the median value, the circle is the mean value.

Observations—One male in the High-concentration treatment showed multifocal proliferation of Sertoli cells and Leydig cells. One male from the Control treatment and two males from the Low concentration showed focal proliferation of Leydig cells. No testicular atrophy was recorded and no ovatestes were observed for any treatment.

5.2.7 Plasma Steroid Concentrations

Estradiol—Estradiol concentrations in Control-treatment females used during the EPA 21-Day Flutamide assay ranged from 0 pg/mL (not detected)to 3,854 pg/mL (Figure 5.29). Among females exposed to the two flutamide concentrations, estradiol concentrations ranged from 0 pg/mL (not detected) to

12,367 pg/mL. No significant differences in the mean estradiol concentration per treatment (Table 5.25) were detected (Kruskal-Wallis, $H = 3.12$, $p = 0.210$, df = 2). The achieved power for this endpoint was 22%, and the sample size required to detect a significant difference from the Control treatment at 80% power was 65 (Table 5.25).

Table 5.25. Summary statistics and power estimates for female estradiol concentrations (pg/mL) for the EPA 21-Day Flutamide assay.

Level	N	Mean	Stdev	CV	Achieved Power [1]	Sample Size Required [2]
control	15	1,225	1,260	103%	22%	65
low	14	1,570	1,172	75%		
high	16	2,477	3,074	124%		

[1] Calculated from natural log transformed data; with sample size = 14.
[2] To detect a significant difference from control treatment based on maximum achieved absolute difference; calculated on natural log transformed data.

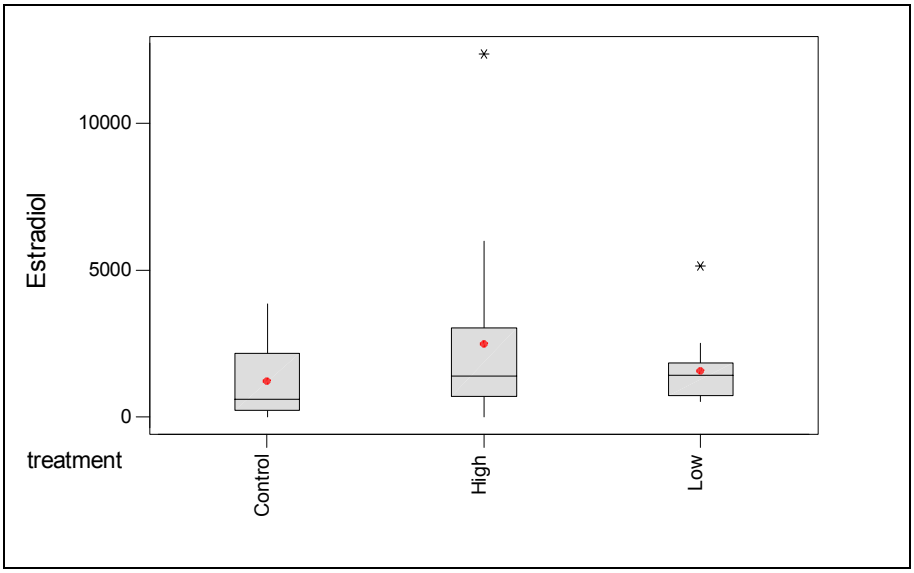

Figure 5.29. Boxplot of female estradiol concentration (pg/mL) by treatment for the EPA 21-Day Flutamide assay. The box represents the interquartile range, whiskers represent the data range, the horizontal line is the median value, the circle is the mean value, and the asterisks represent probable outliers.

Estradiol concentrations in Control treatment males used during the EPA 21-Day Flutamide assay ranged from 0 pg/mL (not detected) to 298 pg/mL (Figure 5.30). Among males exposed to the two flutamide concentrations, estradiol concentrations ranged from 0 pg/mL (not detected) to 467 pg/mL. Significant differences in the mean estradiol concentration per treatment (Table 5.26) were detected (Kruskal-Wallis, $H = 9.63$, $p = 0.008$, df = 2). The mean concentration of estradiol in males from the High concentration was less than those of males from the Low concentration and the Control treatment. The achieved power for this endpoint was 79%, and the sample size required to detect a significant difference from the Control treatment at 80% power was 8 (Table 5.26).

Table 5.26. Summary statistics and power estimates for male estradiol concentrations (pg/mL) for the EPA 21-Day Flutamide assay.

Level	N	Mean	Stdev	CV	Achieved Power [1]	Sample Size Required [2]
control	8	197	87	44%	79%	8
low	7	301	86	28%		
high	8	68	125	185%		

[1] Calculated from natural log transformed data; with sample size = 7.
[2] To detect a significant difference from control treatment based on maximum achieved absolute difference; calculated on natural log transformed data.

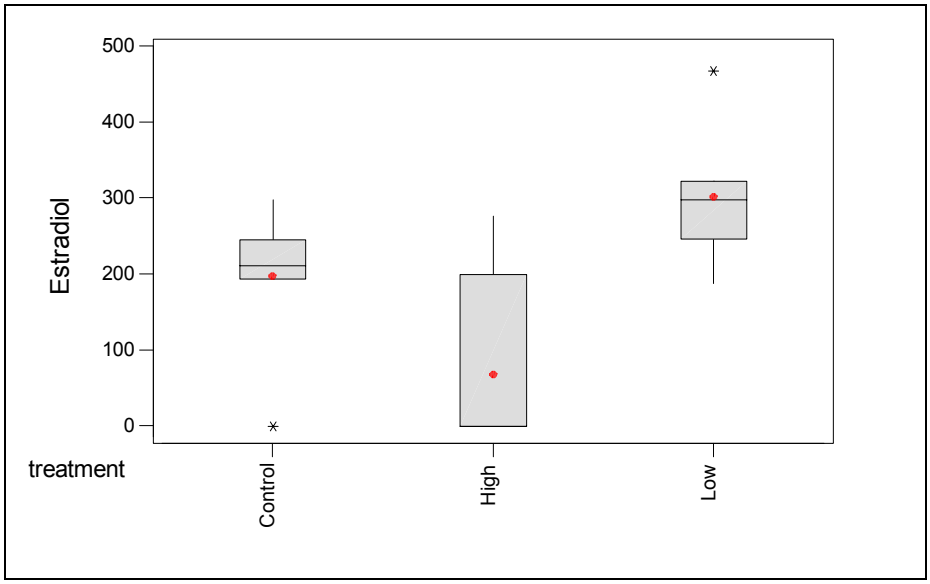

Figure 5.30. Boxplot of male estradiol concentration (pg/mL) by treatment for the EPA 21-Day Flutamide assay. The box represents the interquartile range, whiskers represent the data range, the horizontal line is the median value, the circle is the mean value, and the asterisks represent probable outliers.

Testosterone—Testosterone concentrations in Control-treatment females used during the EPA 21-Day Flutamide assay ranged from 0 pg/mL (not detected) to 3,475 pg/mL (Figure 5.31). Among females exposed to the two flutamide concentrations, testosterone concentrations ranged from 340 pg/mL to 4,612 pg/mL. Significant differences in the mean testosterone concentration per treatment (Table 5.27) were detected (Kruskal-Wallis, $H = 6.68$, $p = 0.035$, df = 2). The mean testosterone concentration in females from the High concentration was greater than that of females from the Low concentration and the Control treatment. The achieved power for this endpoint was 45%, and the sample size required to detect a significant difference from the Control treatment at 80% power was 21 (Table 5.27).

Table 5.27. Summary statistics and power estimates for female testosterone concentrations (pg/mL) for the EPA 21-Day Flutamide assay.

Level	N	Mean	Stdev	CV	Achieved Power [1]	Sample Size Required [2]
control	10	1,329	1,062	80%	45%	21
low	11	1,430	886	62%		
high	12	2,692	1,420	53%		

[1] Calculated from natural log transformed data; with sample size = 10.
[2] To detect a significant difference from control treatment based on maximum achieved absolute difference; calculated on natural log transformed data.

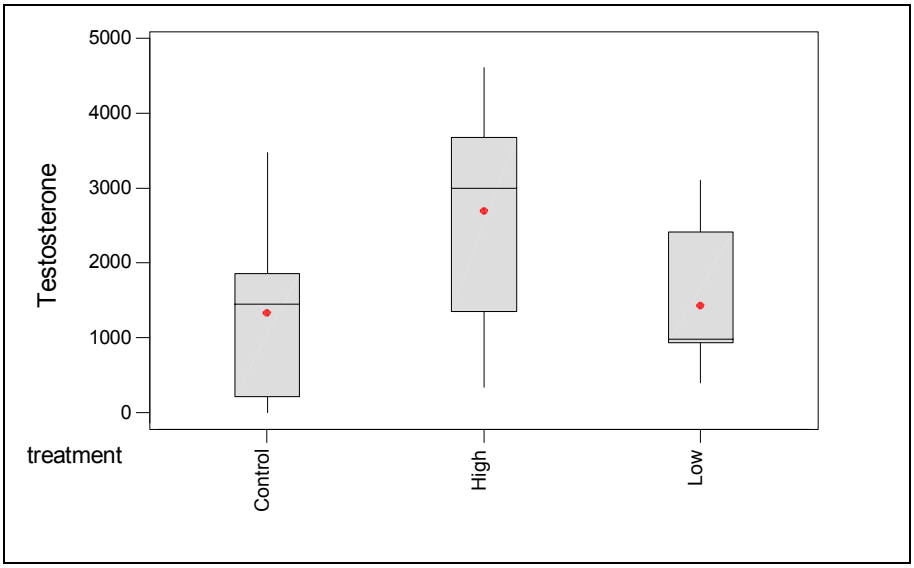

Figure 5.31. Boxplot of female testosterone concentration (pg/mL) by treatment for the EPA 21-Day Flutamide assay. The box represents the interquartile range, whiskers represent the data range, the horizontal line is the median value, and the circle is the mean value.

Testosterone concentrations in Control treatment males used during the EPA 21-Day Flutamide assay ranged from 1,044 pg/mL to 4,437 pg/mL (Figure 5.32). Among males exposed to the two flutamide concentrations, testosterone concentrations ranged from 390 pg/mL to 6,131 pg/mL. A significant difference in the mean testosterone concentration per treatment (Table 5.28) was detected (Kruskal-Wallis, $H = 7.26$, $p = 0.027$, df = 2). The mean testosterone concentrations in males from the Low concentration and the Control treatment was less than that of males from the High concentration. The achieved power for this endpoint was 48%, and the sample size required to detect a significant difference from the Control treatment at 80% power was 16 (Table 5.28).

Table 5.28. Summary statistics and power estimates for male testosterone concentrations (pg/mL) for the EPA 21-Day Flutamide assay.

Level	N	Mean	Stdev	CV	Achieved Power [1]	Sample Size Required [2]
control	8	2,218	1,168	53%	48%	16
low	8	2,278	1,751	77%		
high	8	4,344	1,220	28%		

[1] Calculated from natural log transformed data; with sample size = 8.
[2] To detect a significant difference from control treatment based on maximum achieved absolute difference; calculated on natural log transformed data.

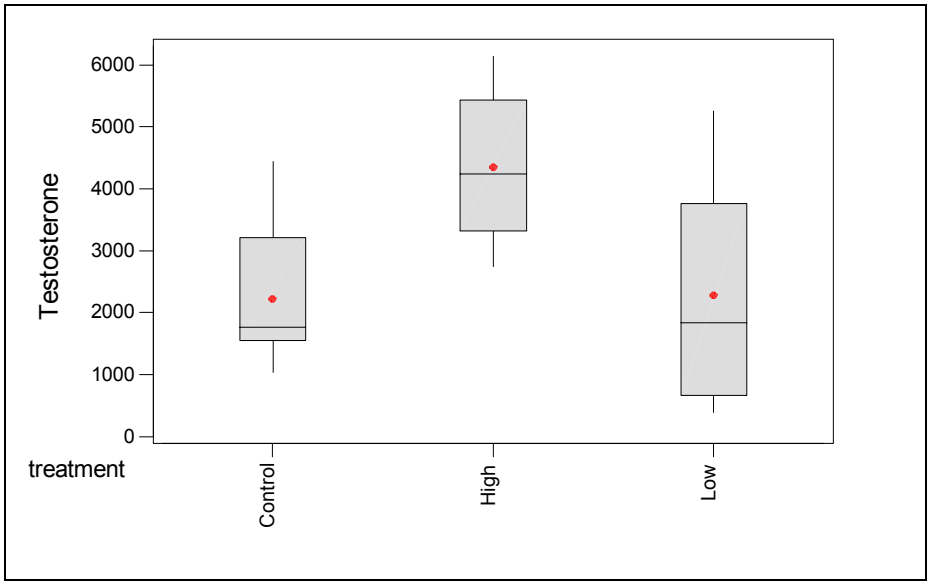

Figure 5.32. Boxplot of male testosterone concentration (pg/mL) by treatment for the EPA 21-Day Flutamide assay. The box represents the interquartile range, whiskers represent the data range, the horizontal line is the median value, and the circle is the mean value.

11-ketotestosterone—11-ketotesosterone was not detected in females from the Control treatment (six individuals) or from the Low concentration (one individual). No females from the High concentration were available for 11-ketotestosteron analyses exposed during the EPA 21-Day Flutamide assay.

11-ketotesosterone concentrations in Control treatment males used during the EPA 21-Day Flutamide assay ranged from 8,555 pg/mL to 31,716 pg/mL (Figure 5.33). Among males exposed to the two flutamide concentrations, 11-ketotesosterone concentrations ranged from 1,814 pg/mL to 95,944 pg/mL. Significant differences in the mean 11-ketotesosterone concentration per treatment (Table 5.29) were detected (Kruskal-Wallis, $H = 9.38$, $p = 0.009$, df = 2). The mean 11-ketotesosterone concentration in males from the High concentration was greater than those of males from the Low concentration and the Control treatment. The achieved power for this endpoint was 58%, and the sample size required to detect a significant difference from the Control treatment at 80% power was 13 (Table 5.29).

Table 5.29. Summary statistics and power estimates for male 11-ketotesosterone concentrations (pg/mL) for the EPA 21-Day Flutamide assay.

Level	N	Mean	Stdev	CV	Achieved Power [1]	Sample Size Required [2]
control	8	16,888	8,477	50%	58%	13
low	8	23,603	28,698	122%		
high	8	53,209	24,556	46%		

[1] Calculated from natural log transformed data; with sample size = 8.
[2] To detect a significant difference from control treatment based on maximum achieved absolute difference; calculated on natural log transformed data.

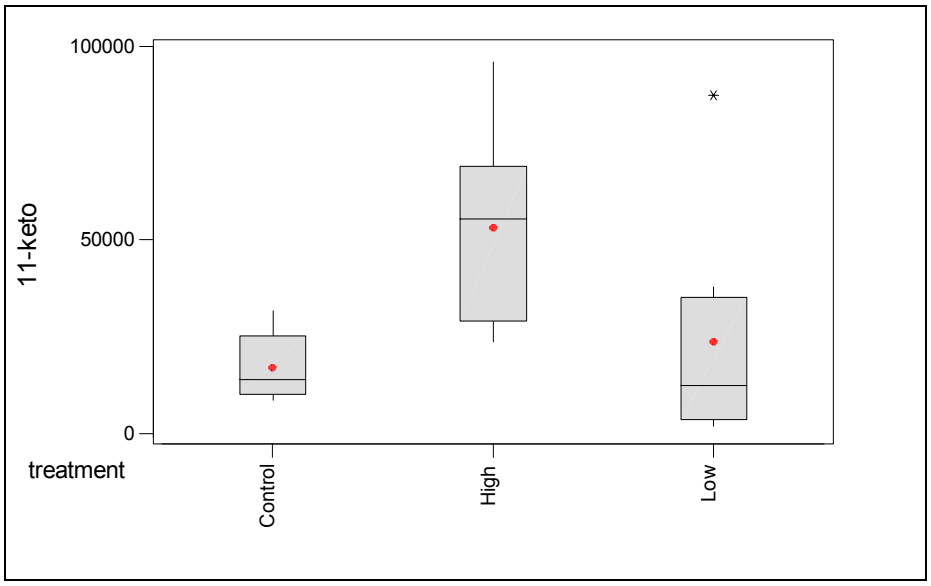

Figure 5.33. Boxplot of male 11-ketotesosterone concentration (pg/mL) by treatment for the EPA 21-Day Flutamide assay. The box represents the interquartile range, whiskers represent the data range, the horizontal line is the median value, the circle is the mean value, and the asterisk represents a probable outlier.

5.2.8 Fecundity

Total Fecundity—A 14-day pre-exposure evaluation of total egg production was performed. Total 14-day counts among the tanks that were eventually used for the three treatments in the exposure assay (individual tank values summed for each treatment) ranged from about 12,000 eggs to 13,000 eggs (Figure 5.34). No significant differences in the mean 14-day egg production among the groups of replicates eventually used in the flutamide exposure assay were detected (Kruskal-Wallis, $H = 0.15$, $p = 0.926$, df = 2).

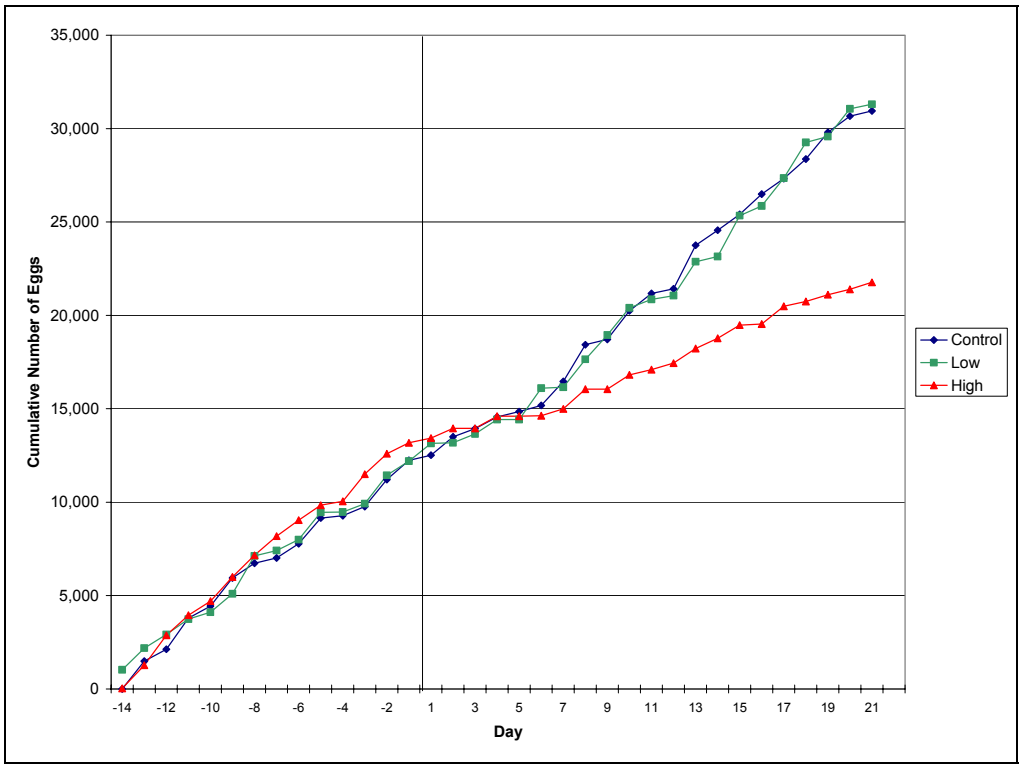

Figure 5.34. Total egg production per treatment for the EPA 21-Day Flutamide assay. The vertical line denotes the initiation of the exposure assay.

During the EPA 21-Day Flutamide assay, total counts in the Control treatment were varied almost 1.5 fold among replicates, varying from 3,804 eggs to 5,391 eggs (Figure 5.35). Variability in total egg production among Low concentration replicates was somewhat less, ranging from 4,003 eggs to 5,275 eggs. Total counts among the High concentration replicates varied about four-fold, ranging from 874 eggs to 3,647 eggs. Statistical analysis of square-root transformed egg counts showed significant among-treatment differences (Kruskal-Wallis, $H = 7.38$, $p = 0.025$, df = 2) in mean total numbers of eggs produced (Table 5.30). The total fecundity of females in the High-concentration treatment was significantly less than it was for females in the Low concentration or the Control treatment. The achieved power for this assay was 83%, and the sample size required to detect a significant difference from the Control treatment at 80% power was 4 (Table 5.30).

Table 5.30. Summary statistics and power estimates for total fecundity data for the EPA 21-Day Flutamide assay.

Level	N	Mean	Stdev	CV	Achieved Power [1]	Sample Size Required [2]
control	4	4677	772	17%	83%	4
low	4	4777	552	12%		
high	4	2147	1192	56%		

[1] Calculated from square-root transformed data; with sample size = 4.
[2] To detect a significant difference from control treatment based on maximum achieved absolute difference; calculated on square-root transformed data.

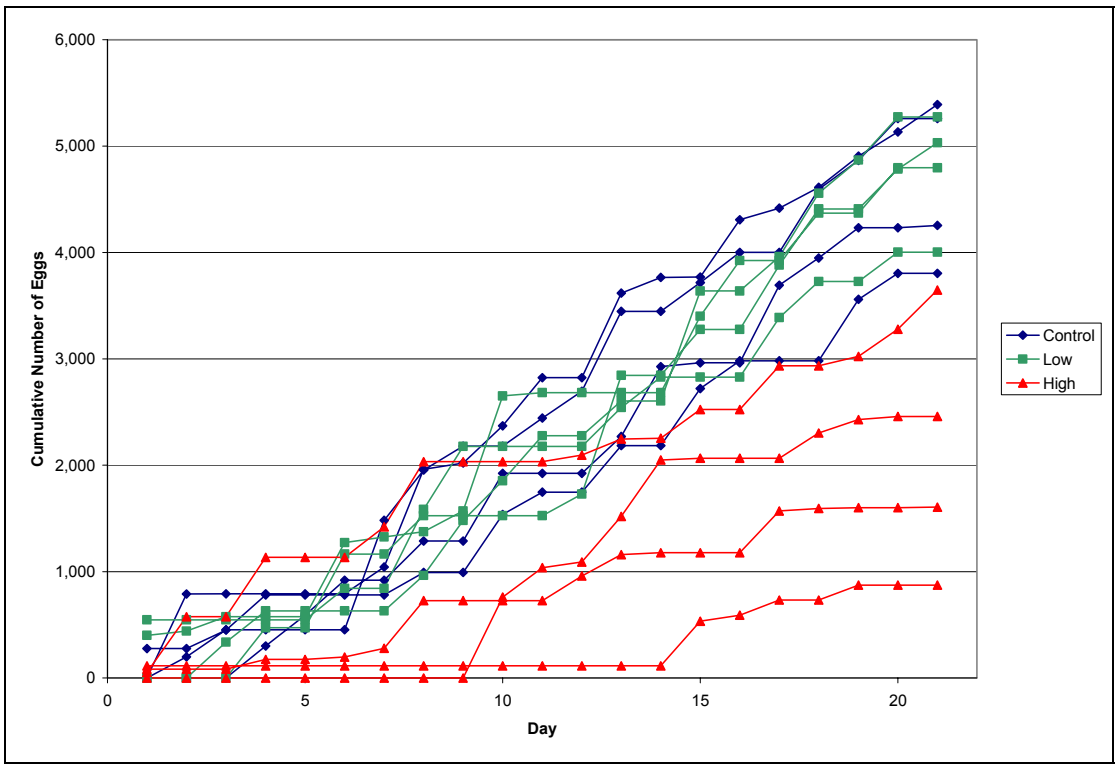

Figure 5.35. Total egg production by replicate per treatment for the EPA 21-Day Flutamide assay.

Fecundity per Female Reproductive Day—During the 14-Day pre-exposure evaluation, the mean number of eggs produced per female reproductive day ranged from 54.4 eggs/day for the tanks that would be used for the Low-concentration treatment to 58.8 eggs/day for the tanks that would be used for the High concentration during the 21-day exposure assay. There were no significant differences among treatments in the numbers of eggs produced per reproductive day during the pre-exposure period (Kruskal-Wallis, $H = 0.11$, $p = 0.990$, df = 2).

During the EPA 21-Day Flutamide assay, the maximum number of female reproductive days was achieved for all treatments (Table 5.31). The number of eggs produced per female reproductive day varied from 45.3 eggs to 64.2 eggs in the Control treatment and from 47.7 to 62.8 in the Low concentration (Figure 5.36). For the High concentration, the number of eggs produced per female reproductive day ranged from 10.4 eggs to 43.4 eggs, although fish in two of the replicates yielded fewer than 20 eggs per day (10.4, 19.1). Because no fish died during the assay, the statistical results reported here are the same as those reported for total fecundity. A significant difference in the mean number of eggs laid per day per treatment (Table 5.31) occurred. The number of eggs laid per day in the High-concentration treatment was significantly less than it was for females in the Low concentration or the Control treatment. The achieved power for this assay was 73%, and the sample size required to detect a significant difference from the Control treatment at 80% power was 5 (Table 5.31).

Table 5.31. Summary statistics and power estimates for fecundity per female reproductive day for the EPA 21-Day Flutamide assay.

Level	Mean Number of Reproductive Days [1]	N	Mean	Stdev	CV	Achieved Power [2]	Sample Size Required [3]
control	84	4	55.7	9.2	17%	73%	5
low	84	4	56.9	6.6	12%		
high	84	4	25.6	14.2	56%		

[1] Maximum number = 84.
[2] Calculated from natural log transformed data; with sample size = 4.
[3] To detect a significant difference from control treatment based on maximum achieved absolute difference; calculated on natural log transformed data.

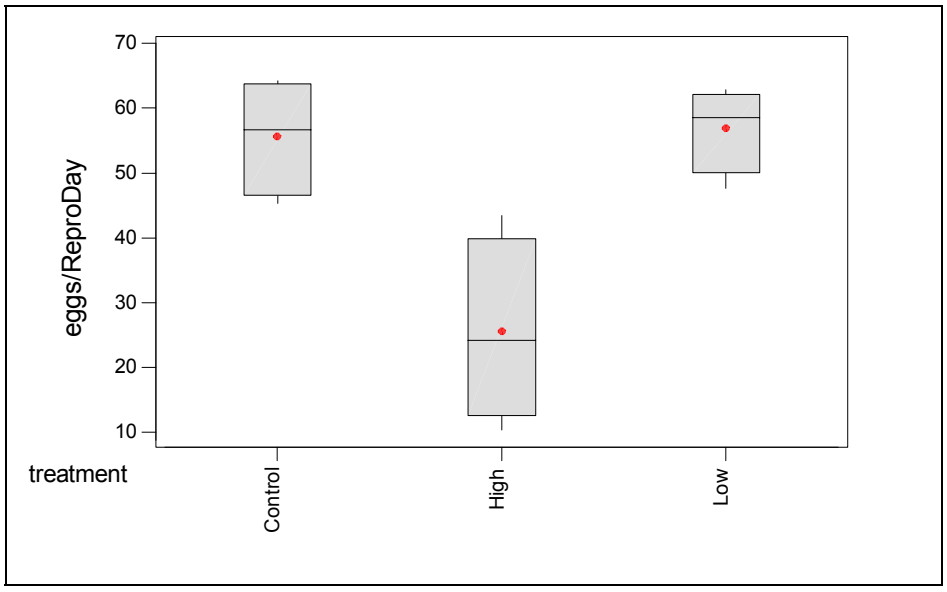

Figure 5.36. Boxplot of the number of eggs produced per female reproductive day by treatment for the EPA 21-Day Flutamide assay. The box represents the interquartile range, whiskers represent the data range, the horizontal line is the median value, and the circle is the mean value.

Eggs on Tiles/Dishes—The mean number of eggs laid on the tiles during the 14-day pre-exposure assay varied from 1,944 eggs for the tanks that would be used for the Low concentration to 2,355 eggs for the tanks that would be used for the Control treatment. The mean number of eggs on dishes ranged from 704 eggs for the Control treatment to 1,223 eggs for the High concentration. Because of the variability in the total number of eggs laid per treatment, the proportional difference in the number of eggs on dishes versus those on tiles [1–(# eggs on dishes ÷ # eggs on tiles)] was calculated. There were no significant differences in the mean proportional difference among treatments during the 14-day pre-exposure assay (Kruskal-Wallis, $H = 5.73$, $p = 0.126$, df = 3).

The mean number of eggs laid on the tiles among the treatments during the EPA 21-Day Flutamide assay varied from 1,343 eggs for the High concentration to 3,463 eggs for the Low-concentration treatment (Appendix E, Table 3.7). The number of eggs on dishes ranged from 207 eggs for the Control treatment to 350 eggs for the High concentration. The proportional difference ranged from 0.16 (one High

concentration replicate) to 0.87 (one Control-treatment replicate) (Appendix E, Figure 3.6). There was a significant difference in the mean proportional difference among treatments (Kruskal-Wallis, $H = 8.35$, $p = 0.015$, df = 2). The mean proportional difference in the number of eggs on dishes versus those on tiles for the High concentration was significantly lower than those for the Low concentration and the Control treatment.

5.2.9 Fertilization Success

Total Fertilization—Eggs were collected during the 14-day pre-exposure period for the evaluation of fertilization-success rate. The mean proportion of eggs fertilized in the Control treatment was 0.972 [standard deviation (sd) = 0.053], 0.999 (sd = 0.004) in the Low concentration, and 0.999 (sd = 0.005) in the High concentration. The mean proportion of eggs fertilized in the replicates that were not used in the 21-day validation assay was 0.997 (sd = 0.004). There were no significant differences among treatments in the proportion of eggs that hatched (Kruskal-Wallis, $H = 5.47$, $p = 0.140$, df = 3).

The total (tiles + dishes) fertilization-success rates for most treatment replicates during the EPA 21-Day Flutamide assay were high, ranging from 0.994 (one Low-concentration replicate) to 1.00 (two Low-concentration replicates) (Figure 5.37). One High-concentration replicate had a relatively low proportion of eggs fertilized (0.857%). No significant differences in mean fertilization-success rates (Table 5.32) among treatments were detected (Kruskal-Wallis, $H = 4.32$, $p = 0.115$, df = 2). The achieved power for this assay was 20%, and the sample size required to detect a significant difference from the Control treatment at 80% power was 17 (Table 5.32).

Table 5.32. Summary statistics and power estimates for the proportion of eggs fertilized for the EPA 21-Day Flutamide assay.

Level	N	Mean	Stdev	CV	Achieved Power [1]	Sample Size Required [2]
control	4	0.999	0.001	0.1%	20%	17
low	4	0.998	0.003	0.3%		
high	4	0.961	0.070	7%		

[1] Calculated from arcsine square-root transformed data; with sample size = 4.
[2] To detect a significant difference from control treatment based on maximum achieved absolute difference; calculated on arcsine square-root transformed data.

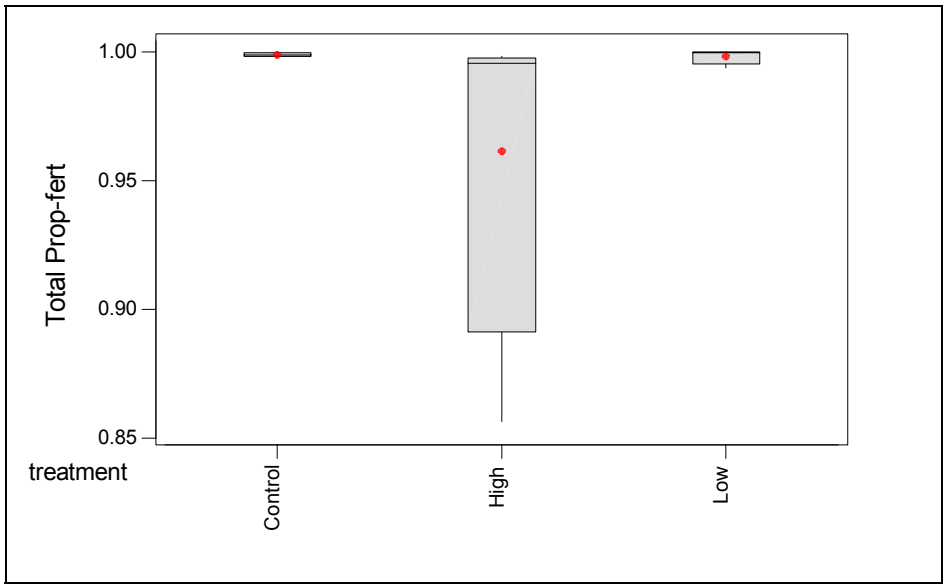

Figure 5.37. Boxplot of the proportion of eggs fertilized by treatment for the EPA 21-Day Flutamide assay. The box represents the interquartile range, whiskers represent the data range, the horizontal line is the median value, and the circle is the mean value.

Fertilization of Eggs on Tiles and Dishes—During the 14-Day pre-validation assay, there were no significant differences in the fertilization-success rates among treatments for eggs laid in tiles (Kruskal-Wallis, $H = 7.44$, $p = 0.059$, df = 3) or on dishes (Kruskal-Wallis, $H = 4.85$, $p = 0.183$, df = 3). The fertilization-success rates for all treatment replicates for eggs laid on tiles during the EPA 21-Day Flutamide assay were high, ranging from 0.993 (one Low-concentration replicate) to 1.00 (three Low- and one High-concentration replicates) (Appendix E, Figure 3.7). No significant differences in mean fertilization-success rates for eggs laid on tiles (Appendix E, Table 3.8) among treatments were detected (Kruskal-Wallis, $H = 1.33$, $p = 0.516$, df = 2). The fertilization-success rates for most treatment replicates for eggs laid on dishes during the assay were high, ranging from 0.972 (one High concentration replicate) to 1.00 (several replicates; including all treatments) (Appendix E, Figure 3.8). One High-concentration replicate had a relatively low proportion of eggs (laid on tiles) that were fertilized (0.693%). No significant differences in mean fertilization-success rates (Appendix E, Table 3.8) among treatments were detected (Kruskal-Wallis, $H = 4.91$, $p = 0.086$, df = 2).

5.2.10 Hatchability and Larval Development

Eggs were collected during the 14-day pre-exposure period for the evaluation of hatchability. The mean proportion of fertilized eggs that hatched in the Control treatment was 0.98 [standard deviation (sd) = 0.03], 1.00 (sd = 0.01) in the Low concentration, and 1.00 (sd = 0.01) in the High concentration. There were no significant differences among treatments in the proportion of eggs that hatched (Kruskal-Wallis, $H = 1.04$, $p = 0.594$, df = 2).

Eggs were collected during the EPA 21-Day Flutamide assay for the evaluation of hatchability. The proportion of fertilized eggs that hatched ranged from 0.92 to 1.00 in the Control treatment and from 0.33 to 1.00 for the two test concentrations (Figure 5.38). There were no significant differences among treatments in the proportion of eggs that hatched (Kruskal-Wallis, $H = 1.15$, $p = 0.563$, df = 2). The achieved power for this endpoint was 18%, and the sample size required to detect a significant difference from the Control treatment at 80% power was 44 (Table 5.33).

Table 5.33. Summary statistics and power estimates for the proportion of fertile eggs that hatched for the EPA 21-Day Flutamide assay.

Level	N	Mean	Stdev	CV	Achieved Power [1]	Sample Size Required [2]
control	9	0.98	0.03	3%	18%	44
low	9	0.97	0.04	4%		
high	8	0.89	0.23	26%		

[1] Calculated from arcsine square-root transformed data; with sample size = 8.
[2] To detect a significant difference from control treatment based on maximum achieved absolute difference; calculated on arcsine square-root transformed data.

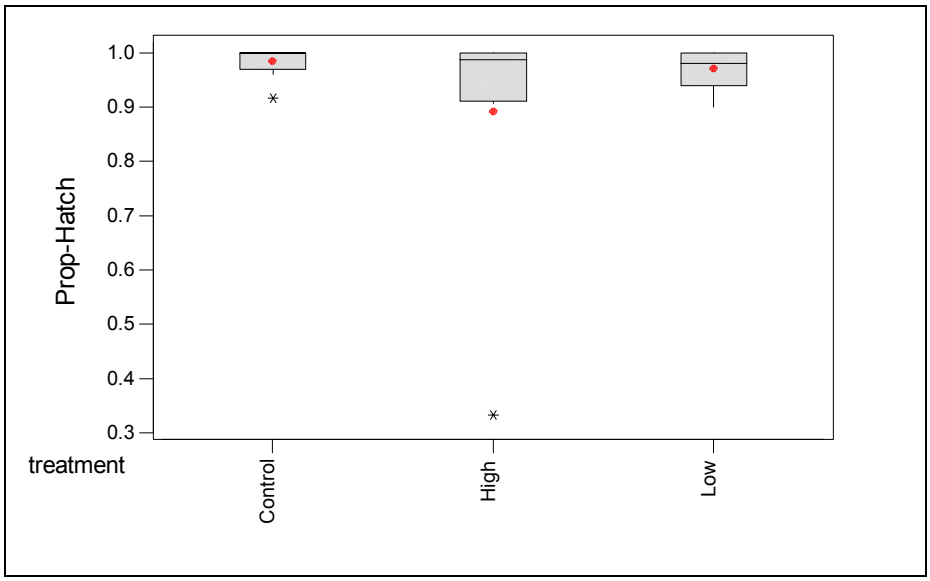

Figure 5.38. Boxplot of the proportion of fertile eggs that hatched by treatment for the EPA 21-Day Flutamide assay. The box represents the interquartile range, whiskers represent the data range, the horizontal line is the median value, the circle is the mean value, and the asterisk represents a probable outlier.

Eggs were collected during the pre-exposure period for the evaluation of larval development. The mean proportion of larvae that developed normally (i.e., that showed no morphological abnormalities) in the Control treatment was 0.99 (sd = 0.01). The mean proportion of normal larvae in the remaining treatments was 0.98 (sd = 0.03) in the Low concentration and 0.98 (sd = 0.02) in the High concentration. There were no significant differences among treatments in the proportion of normal larvae (Kruskal-Wallis, $H = 0.71$, $p = 0.703$, df = 2).

Eggs were collected during the EPA 21-Day Flutamide assay for the evaluation of larval development. The proportion of larvae that developed normally (i.e., that showed no morphological abnormalities) ranged from 0.84 to 1.00 in the Control treatment and from 0.70 to 1.00 for the two test concentrations (Figure 5.39). There was a significant difference among treatments in the proportion of normal larvae (Kruskal-Wallis, $H = 8.17$, $p = 0.017$, df = 2). The Mean proportion of larvae that developed normally

was significantly less in the High concentration than it was in the Control treatment. The achieved power for this endpoint was 75%, and the sample size required to detect a significant difference from the Control treatment at 80% power was 9 (Table 5.34).

Table 5.34. Summary statistics and power estimates for the proportion of normal larvae for the EPA 21-Day Flutamide assay.

Level	N	Mean	Stdev	CV	Achieved Power [1]	Sample Size Required [2]
control	9	0.97	0.05	5%	75%	9
low	9	0.96	0.04	4%		
high	8	0.89	0.11	12%		

[1] Calculated from arcsine square-root transformed data; with sample size = 8.
[2] To detect a significant difference from control treatment based on maximum achieved absolute difference; calculated on arcsine square-root transformed data.

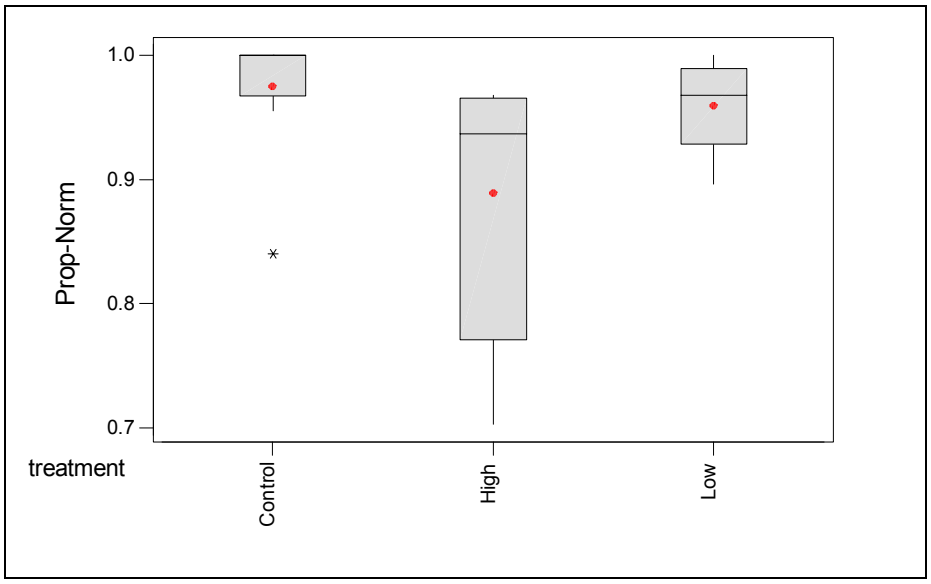

Figure 5.39. Boxplot of the proportion of normal larvae by treatment for the EPA 21-Day Flutamide assay. The box represents the interquartile range, whiskers represent the data range, the horizontal line is the median value, the circle is the mean value, and the asterisk represents a probable outlier.

5.2.11 Body Weight

The body weight of females used in the EPA 21-Day Flutamide assay ranged from 1.1 g to 2.8 g. There were no significant differences in mean body weight among treatments (Kruskal-Wallis, $H = 5.19$, $p = 0.075$, df = 2). The body weight of males used in the EPA 21-Day Flutamide assay ranged from 3.1 g to 5.6 g. There were no significant differences in mean body weight among treatments (Kruskal-Wallis, $H = 3.80$, $p = 0.150$, df = 2).

5.3 Non-spawning Adult 14-Day Assay for Flutamide

The Non-spawning Adult 14-Day Flutamide assay was conducted from March 10, 2003 to March 24, 2003 (exposure assay).

5.3.1 Survival

All males and females in all treatments survived the Non-spawning Adult 14-Day Flutamide assay.

5.3.2 Vitellogenin

Vitellogenin concentrations in Control treatment females used during the Non-spawning Adult 14-Day Flutamide assay ranged from 1,252,000 ng/mL to 5,983,000 ng/mL (Figure 5.40). Among females exposed to the two flutamide concentrations, vitellogenin concentrations ranged from 3,325,000 ng/mL to 10,905,000 ng/mL. Significant differences in the mean vitellogenin concentration among treatments (Table 5.35) were detected (Kruskal-Wallis, $H = 10.88$, $p = 0.012$, df = 2). Mean vitellogenin concentrations among females exposed to the High and Medium flutamide concentrations were greater than the mean concentration for Control treatment females. The achieved power for this endpoint was 82%, and the sample size required to detect a significant difference from the Control treatment at 80% power was 10 (Table 5.35).

Table 5.35. Summary statistics and power estimates for female vitellogenin concentrations (ng/mL) for the Non-spawning Adult 14-Day Flutamide assay.

Level	N	Mean	Stdev	CV	Achieved Power [1]	Sample Size Required [2]
control	10	4,189,350	1,516,107	36%	82%	10
low	10	6,147,250	2,219,643	36%		
Medium	10	7,342,100	2,699,666	37%		
High	10	7,546,150	2,137,789	28%		

[1] Calculated from natural log transformed data; with sample size = 10.
[2] To detect a significant difference from control treatment based on maximum achieved absolute difference; calculated on natural log transformed data.

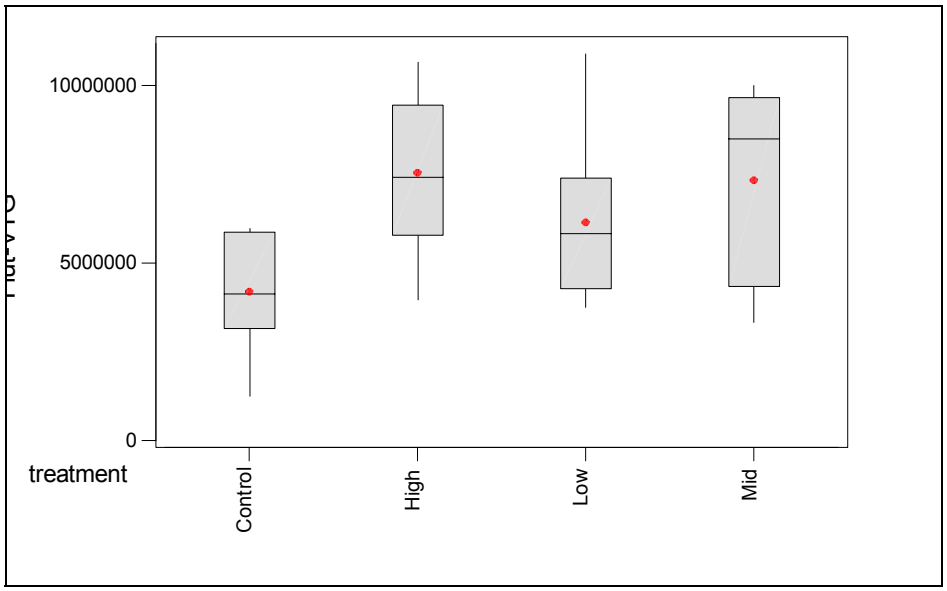

Figure 5.40. Boxplot of female vitellogenin concentration (ng/mL) by treatment for the Non-spawning Adult 14-Day Flutamide assay. The box represents the interquartile range, whiskers represent the data range, the horizontal line is the median value, and the circle is the mean value.

Vitellogenin concentrations in Control treatment males used during the Non-spawning Adult 14-Day Flutamide assay ranged from 0 ng/mL (not detected) to 1,523 ng/mL (Figure 5.41). Among most males exposed to the two flutamide concentrations, vitellogenin concentrations ranged from 0 ng/mL (not detected) to 3,285 ng/mL. One male exposed to the Low-flutamide concentration had a vitellogenin concentration of 10,575 ng/mL. No significant differences in the mean vitellogenin concentration per treatment (Table 5.36) were detected (Kruskal-Wallis, $H = 3.66$, $p = 0.300$, df = 2). The achieved power for this endpoint was 12%, and the sample size required to detect a significant difference from the Control treatment at 80% power was 94 (Table 5.36).

Table 5.36. Summary statistics and power estimates for male vitellogenin concentrations (ng/mL) for the Non-spawning Adult 14-Day Flutamide assay.

Level	N	Mean	Stdev	CV	Achieved Power [1]	Sample Size Required [2]
control	10	243	461	190%	12%	94
low	10	1,337	3,342	250%		
Medium	10	631	1,325	210%		
High	10	76	89	117%		

[1] Calculated from natural log transformed data; with sample size = 10.
[2] To detect a significant difference from control treatment based on maximum achieved absolute difference; calculated on natural log transformed data.

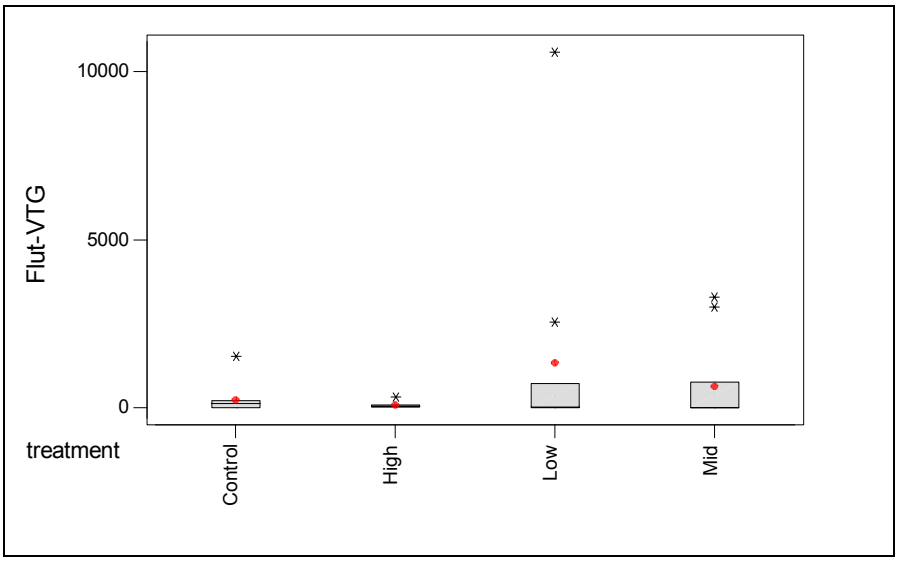

Figure 5.41. Boxplot of male vitellogenin concentration (ng/mL) by treatment for the Non-spawning Adult 14-Day Flutamide assay. The box represents the interquartile range, whiskers represent the data range, the horizontal line is the median value, the circle is the mean value, and asterisks represent probable outliers.

5.3.3 Appearance / Secondary Sex Characteristics

All females used during the Non-spawning Adult 14-Day Flutamide assay showed normal female morphology.

Morphological development among males used during the flutamide Non-spawning Adult 14-day assay varied among treatments (Figure 5.42). One male exposed to the Medium concentration exhibited a female body shape. Fourteen of the 40 males used during the assay lacked tubercles. Most of these males also lacked a fat pad and vertical banding. There was no consistent dose-related pattern to these variations in morphology.

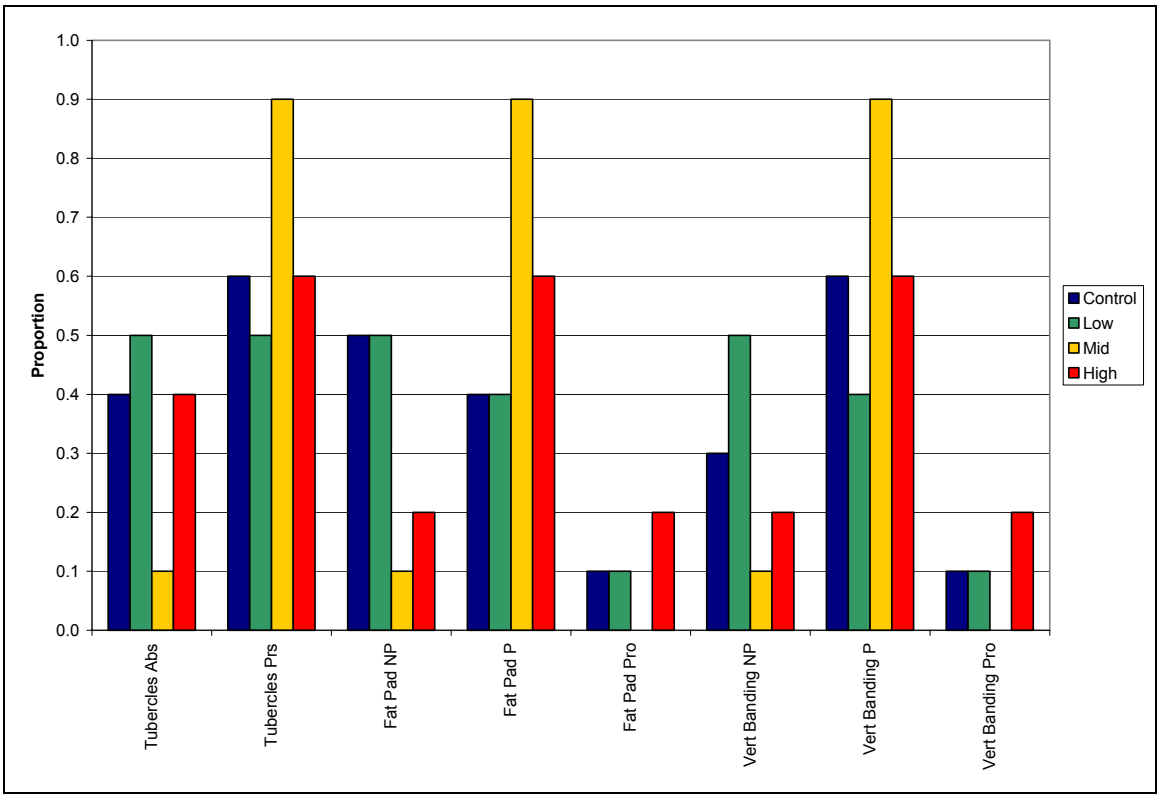

Figure 5.42. Secondary sex characteristics of males used during the EPA Non-spawning Adult 14-Day Flutamide assay.

5.3.4 Gonadosomatic Index

The range of GSI values calculated for females in the Control treatments varied from 5.5 to 24.8 (Figure 5.43), whereas the GSI values for the other treatments varied from 3.8 to 19.7. The overall within-treatment variability was moderate (CVs = 29%–43%; Table 5.37). There were no significant differences in mean GSI values (Table 5.37) among treatments (Kruskal-Wallis, $H = 2.78$, $p = 0.426$, df = 3). The achieved power for this endpoint was 18%, and the sample size required to detect a significant difference from the Control treatment at 80% power was 55 (Table 5.37).

Table 5.37. Summary statistics and power estimates for female gonadosomatic index data for the Non-spawning Adult 14-Day Flutamide assay.

Level	N	Mean	Stdev	CV	Achieved Power [1]	Sample Size Required [2]
control	10	13.7	5.6	41%	18%	55
low	10	11.7	4.5	38%		
medium	10	13.0	5.6	43%		
high	10	10.4	3.0	29%		

[1] Calculated from arcsine square-root transformed data; with sample size = 10.
[2] To detect a significant difference from control treatment based on maximum achieved absolute difference; calculated on arcsine square-root transformed data.

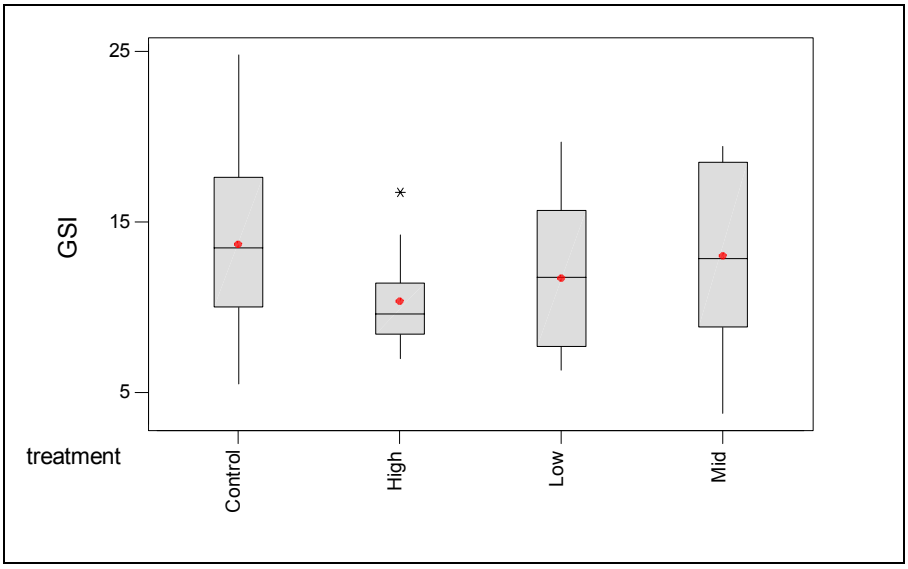

Figure 5.43. Boxplot of female GSI by treatment for the Non-spawning Adult 14-Day Flutamide assay. The box represents the interquartile range, whiskers represent the data range, the horizontal line is the median value, the circle is the mean value, and the asterisk represents a probable outlier.

The range of most GSI values calculated for most males during the Non-spawning Adult 14-Day Flutamide assay, was large, varying from 0.3 to 2.7 (Figure 5.44), which approximates the typical range for reproductively-active male fathead minnows. One male from the Low concentration had an exceptionally high GSI value of 21.3. This male was small (3.2 g) and had a hypertrophied gonad (0.68 g) that was much larger than the average gonad weight (0.068 g, sd = 0.031) for the other males used during this test. This male was excluded from all analyses. Overall within-treatment variability was moderate to high (CVs = 31%–50%; Table 5.38). The highest, excluding the male mentioned above, and lowest male GSI values were 2.6–2.7 (for two fish in the High concentration) and 0.3–0.4 (three fish, one from each treatment except the Medium concentration), respectively. However, there were no significant differences in mean GSI values (Table 5.38) among treatments (Kruskal-Wallis, $H = 1.23$, $p = 0.746$, df = 3). The achieved power for this endpoint was 10%, and the sample size required to detect a significant difference from the Control treatment at 80% power was 108 (Table 5.38).

Table 5.38. Summary statistics and power estimates for male gonadosomatic index data for the Non-spawning Adult 14-Day Flutamide assay.

Level	N	Mean	Stdev	CV	Achieved Power [1]	Sample Size Required [2]
control	10	1.3	0.5	39%	10%	108
low	9	1.4	0.7	50%		
medium	10	1.3	0.4	31%		
high	10	1.6	0.8	47%		

[1] Calculated from arcsine square-root transformed data; with sample size = 9.
[2] To detect a significant difference from control treatment based on maximum achieved absolute difference; calculated on arcsine square-root transformed data.

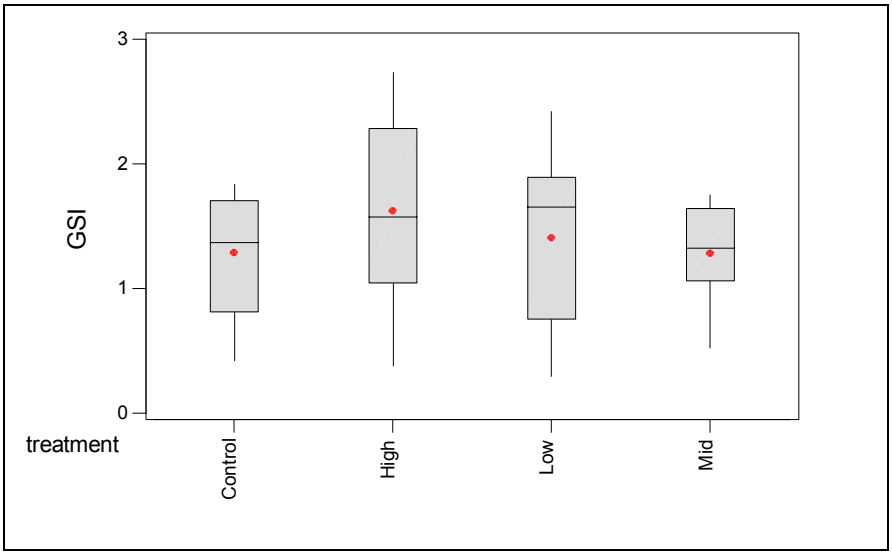

Figure 5.44. Boxplot of male GSI by treatment for the Non-spawning Adult 14-Day Flutamide assay. The box represents the interquartile range, whiskers represent the data range, the horizontal line is the median value, and the circle is the mean value.

5.3.5 Female Gonad Histology

Histological analyses were conducted on the ovaries of 40 females exposed to flutamide during the Non-Spawning Adult 14-Day Assay.

General Ovary Staging—Statistical analysis of the mean ovarian staging from 12 microscope fields per fish in the Non-spawning Adult 14-Day Flutamide assay revealed no significant differences among treatments (Kruskal-Wallis, $H = 1.08$, $p = 0.782$, df = 3).

Quantitative Ovarian Staging—One hundred cells in each of three sections per female were examined to quantitatively determine the developmental condition of the ovaries. Ova from fish in the three exposure treatments ranged from Stage 1a to Stage 5 (see Methods for a description of the stages), whereas ova from females from the Control treatment showed Stage 1a to Stage 4 development (Figure 5.45). Variability within treatments for each stage was very high as indicated by CVs that ranged as high as 316% (Table 5.39). Although statistical analyses showed that there was a significant difference among treatments in the proportion of cells in developmental Stages 1b and 3, there were no significant differences among treatments in the proportion of cells in the developmental Stages 1a, 2, 4, and 5 (Table 5.39). The proportion of cells in developmental Stages 1b and 3 in the High concentration were significantly greater than those in the Low-concentration treatments. There was no consistent pattern of significant difference associated with flutamide dose.

Table 5.39. Descriptive statistics of the proportion of ovarian cells in each developmental stage for females from the Non-spawning Adult 14-Day Flutamide assay and results of the Kruskal-Wallis Test (df = 2) comparing treatments.

	Control (n = 10)			Low (n = 10)			Medium (n = 10)			High (n = 10)			Kruskal-Wallis	
Stage	Mean	Stdev	CV	Mean	Stdev	CV	Mean	Stdev	CV	Mean	Stdev	CV	*H*	*p*
1a	0.087	0.028	32%	0.072	0.030	42%	0.062	0.022	36%	0.091	0.021	23%	6.79	0.079
1b	0.256	0.072	28%	0.229	0.075	33%	0.265	0.073	27%	0.326	0.058	18%	8.45	0.038 *
2	0.183	0.049	27%	0.195	0.055	28%	0.209	0.071	34%	0.213	0.049	23%	2.23	0.526
3	0.136	0.045	33%	0.171	0.054	31%	0.141	0.039	28%	0.103	0.046	45%	8.25	0.041 *
4	0.287	0.115	40%	0.226	0.082	36%	0.238	0.103	43%	0.168	0.071	42%	6.56	0.087
5	0	0	–	0.010	0.033	316%	0.001	0.003	316%	0.016	0.052	316%	1.06	0.787

* $p < 0.05$

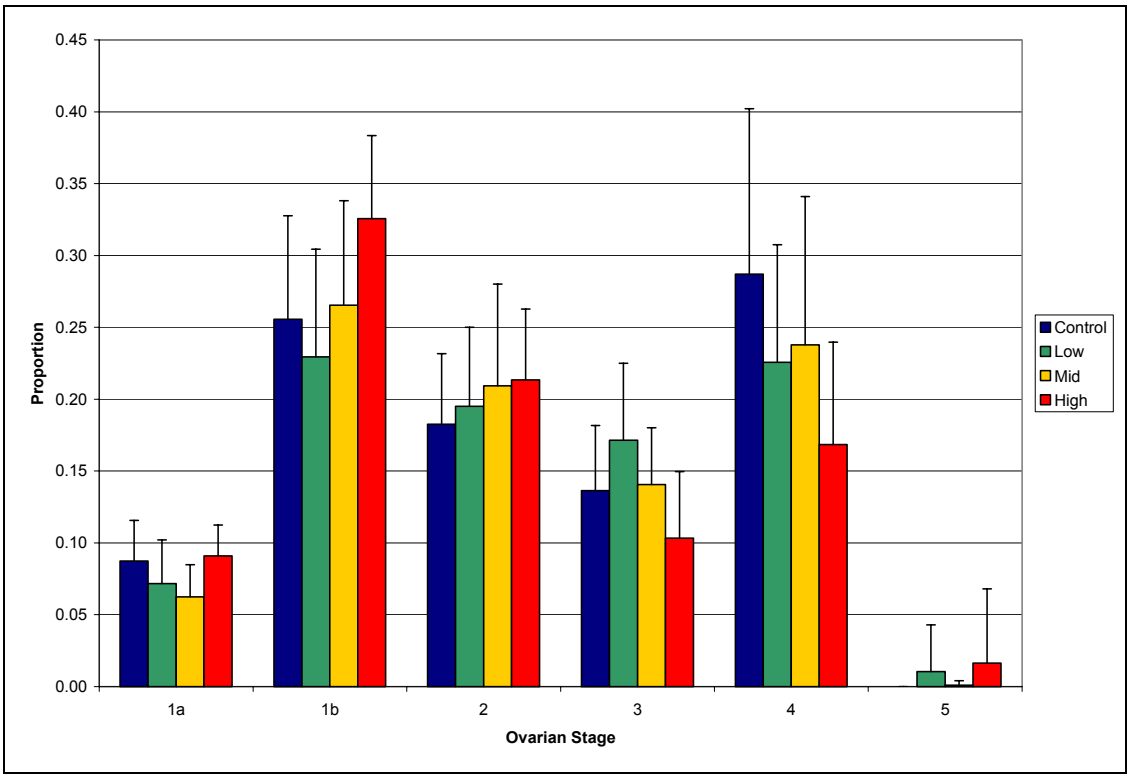

Figure 5.45. Frequency histogram showing the quantitative developmental staging of ovaries for each treatment of the Non-spawning Adult 14-Day Flutamide assay. For each treatment, the columns represent the grand mean proportion of cells in each stage and the bars represent the standard deviation.

Atretic Follicles—The mean proportion of atretic follicles per 300 follicles (counted per fish) ranged from 0.012 follicles for females in the High concentration to 0.030 follicles for females in the Medium concentration treatment (Figure 5.46). There were no significant differences in the mean proportion of atretic follicles among treatments (Kruskal-Wallis, $H = 2.83$, $p = 0.419$, df = 3).

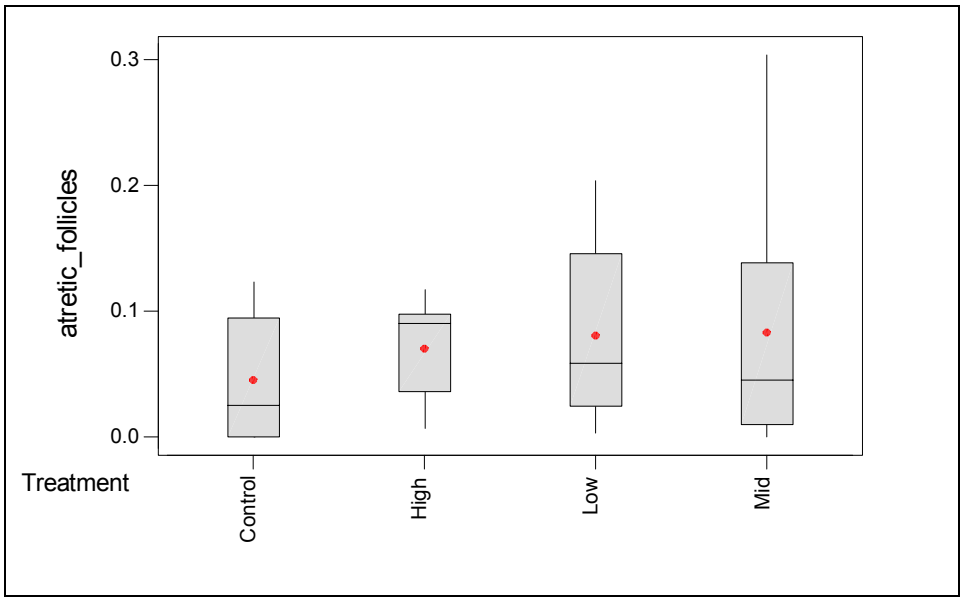

Figure 5.46. Boxplot of the proportion of atretic follicles per 300 follicles by treatment for the Non-spawning Adult 14-Day Flutamide assay. The box represents the interquartile range, whiskers represent the data range, the horizontal line is the median value, the circle is the mean value, asterisks represent probable outliers.

Corpora Lutea—The mean proportion of corpora lutea per 300 follicles (counted per fish) ranged from 0.001 for females in the Medium concentration to 0.016 for females in the Low concentration (Figure 5.47). There was a significant differences in the mean proportion of corpora lutea among treatments (Kruskal-Wallis, $H = 10.81$, $p = 0.013$, df = 3). The mean proportion of corpora lutea in ovaries from females in the High concentration was greater than those for females from the Medium and Low concentrations, but did not differ from that for females from the Control treatment.

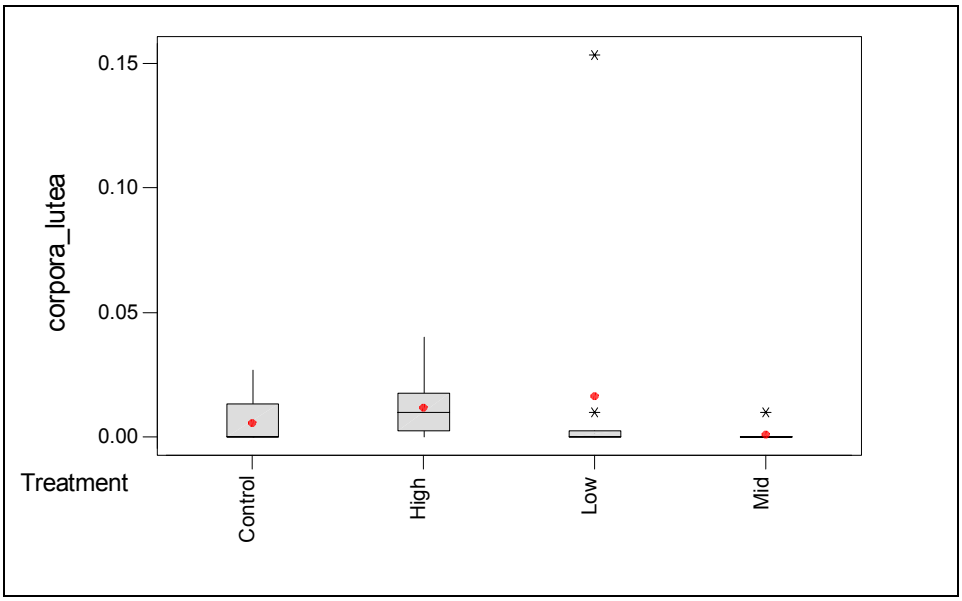

Figure 5.47. Boxplot of the proportion of corpora lutea per 300 follicles by treatment for the Non-spawning Adult 14-Day Flutamide assay. The box represents the interquartile range, whiskers represent the data range, the horizontal line is the median value, the circle is the mean value, and asterisks represent probable outliers.

Observations—One fish from the Control treatment was observed to have interstitial inflammation. The ovary of one fish exposed to the Low-flutamide concentration had a zone of only Stage 5 eggs and another zone with developing ova, containing mix of all stages, including Stage 5. The Stage 5 eggs were abnormally sequestered in the ovary of this fish. One fish exposed to the High-flutamide concentration had Stage 5 eggs segregated in the ovary.

5.3.6 Male Gonad Histology

General Testes Staging—Testes from 39 males exposed to flutamide during the Non-spawning Adult 14-Day Flutamide assay were examined to determine the general developmental condition. Males in all treatments had well-developed testes with most showing Stage 4 and Stage 5 development (see Methods for description of developmental stages). All except 1 of the 120 microscopic fields examined in the 10 Control treatment males showed Stage 4 (45 fields) or Stage 5 (74 fields) development. Most of the 120 microscopic fields examined in the 9 Low-concentration treatment males showed Stage 4 (49 fields) or Stage 5 (47 fields) development. Twelve microscopic fields showed Stage 1 development. In the 10 Medium concentration males available for examination, 112 of the 120 microscopic fields showed Stage 4 (64 fields) or Stage 5 (48 fields) development. In the 10 High concentration males available for examination, 108 of the 120 microscopic fields showed Stage 4 (53 fields) or Stage 5 (55 fields) development. Statistical analysis of the mean staging from 12 microscopic fields per fish revealed that no significant differences among treatments (Kruskal-Wallis, $H = 3.35$, $p = 0.341$, df = 3).
Quantitative Testicular Staging—One hundred cells in each of three sections per male were examined to quantitatively determine the developmental condition of the testes. The developmental stage all treatment testes ranged from Stage 2a to Stage 5 (Figure 5.48). Variability within treatments for each stage was very high as indicated by CVs that ranged as high as 216% (Table 5.40). There were no significant differences among treatments in the proportion of cells in any of the developmental stages (Table 5.40). Therefore, there was no effect on testicular developmental stage associated with flutamide dose.

Table 5.40. Descriptive statistics of the proportion of testes cells in each developmental stage for males from the Non-spawning Adult 14-Day Flutamide assay and results of the Kruskal-Wallis Test (df = 2) comparing treatments.

	Control (n = 10)			Low (n = 8)			Medium (n = 10)			High (n = 10)			Kruskal-Wallis	
Stage	Mean	Stdev	CV	Mean	Stdev	CV	Mean	Stdev	CV	Mean	Stdev	CV	*H*	*p*
1	0	0	–	0	0	–	0	0	–	0	0	–	–	–
2a	0.002	0.004	211%	0.003	0.004	142%	0.001	0.003	211%	0.001	0.002	216%	1.41	0.703
2b	0.008	0.010	131%	0.013	0.015	112%	0.034	0.048	140%	0.015	0.030	200%	5.83	0.120
3a	0.074	0.112	152%	0.043	0.030	72%	0.069	0.098	141%	0.107	0.203	190%	0.01	1.000
3b	0.104	0.134	129%	0.124	0.066	54%	0.110	0.117	106%	0.115	0.064	56%	3.01	0.390
4	0.057	0.068	120%	0.077	0.072	94%	0.099	0.099	100%	0.059	0.057	96%	1.54	0.673
5	0.755	0.250	33%	0.741	0.148	20%	0.686	0.280	41%	0.703	0.272	39%	1.34	0.720

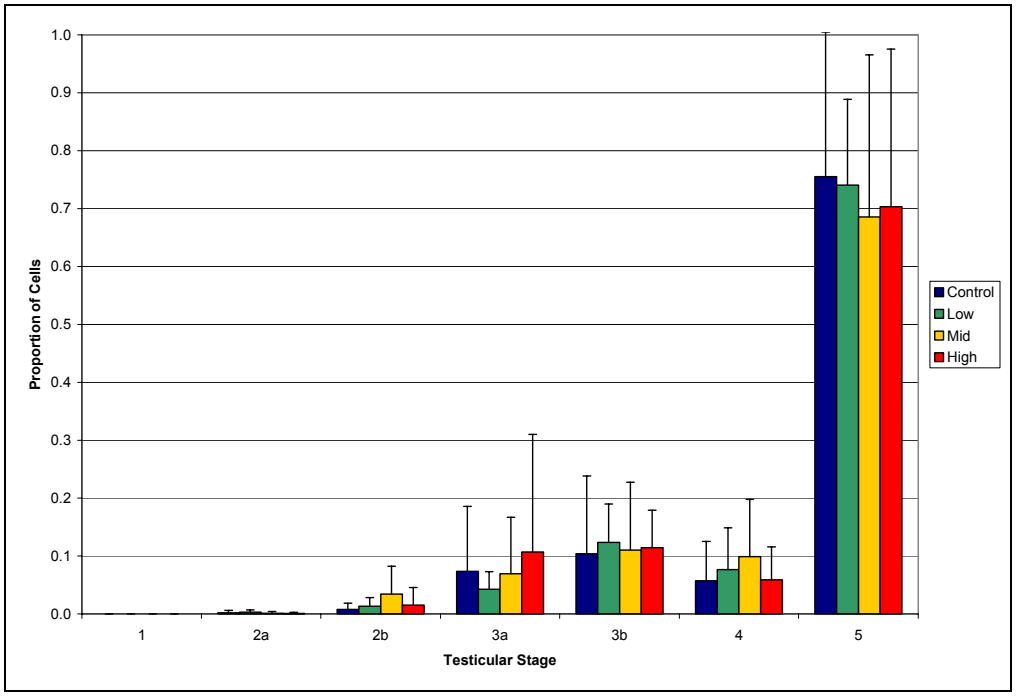

Figure 5.48. Frequency histogram showing the quantitative developmental staging of testes for each treatment of the Non-spawning Adult 14-Day Flutamide assay. For each treatment, the columns represent the grand mean proportion of cells in each stage and the bars represent the standard deviation.

Tubule Diameter—The diameter of the seminiferous tubules of males from the Control treatment ranged from 92.2 µm to 194.4 µm (Figure 5.49). Tubule diameters of males from the three test concentrations ranged from 52.5 µm to 254.4 µm. No significant differences in the mean tubule diameter per treatment (Table 5.41) were detected (Kruskal-Wallis, $H = 0.22$, $p = 0.974$, df = 3). The achieved power for this endpoint was 5%, and the sample size required to detect a significant difference from the Control treatment at 80% power was 1,361 (Table 5.41).

Table 5.41. Summary statistics and power estimates for male seminiferous tubule diameter data for the Non-spawning Adult 14-Day Flutamide assay.

Level	N	Mean	Stdev	CV	Achieved Power [1]	Sample Size Required [2]
control	10	147.1	37.0	25%	5%	1,361
low	9	144.8	45.4	31%		
medium	10	146.6	37.6	26%		
high	10	153.1	51.3	34%		

[1] Calculated from natural log transformed data; with sample size = 9.
[2] To detect a significant difference from control treatment based on maximum achieved absolute difference; calculated on natural log transformed data.

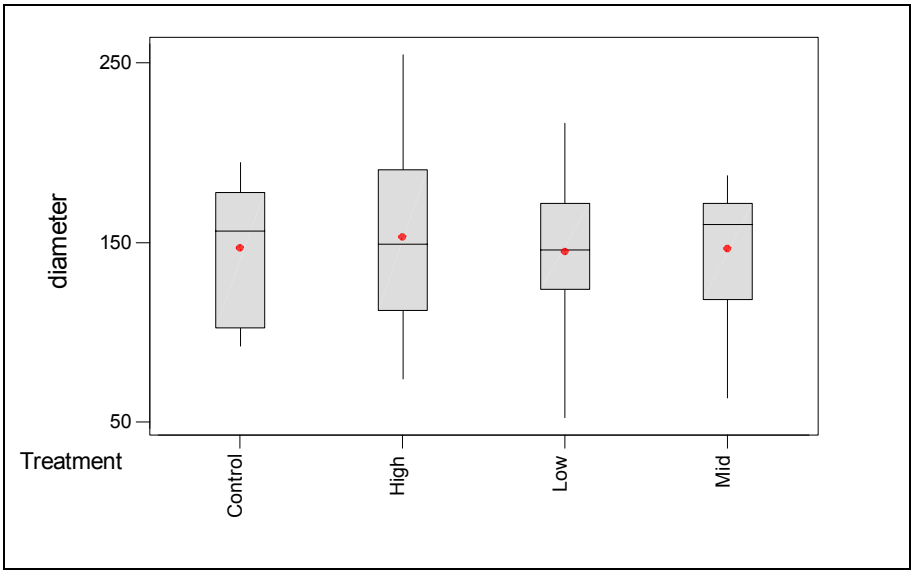

Figure 5.49. Boxplot of seminiferous tubule diameter (μm) by treatment for the Non-spawning Adult 14-Day Flutamide assay. The box represents the interquartile range, whiskers represent the data range, the horizontal line is the median value, and the circle is the mean value.

Observations—No Sertoli cell proliferation was observed for any treatment. One male exposed to the Medium flutamide concentration showed multifocal Leydig cell proliferation. Three males exposed to the High concentration were found to have focal or multifocal Leydig cell proliferation. One male from the Medium flutamide concentration had an egg embedded in the testes. Testicular atrophy was observed for one of the High concentration males (Fish ID 218632 in Table 5.42) that showed multifocal Leydig cell proliferation. Several males exposed to flutamide had abnormal testicular conditions (Table 5.42).

Table 5.42. Histological observations for males exposed to concentrations of flutamide during the Non-spawning Adult 14-D assay.

Fish ID	Concentration	Leydig Cell Proliferation	Observations
218604	Low	None	Premature release of secondary spermatocytes (stage 3b) in lumina. High concentration of spermatogonia in mature tubules.
218620	Medium	None	Premature release of secondary spermatocytes (stage 3b) in lumina. Some released stage 3b cells necrotic. Lumina generally occluded.
218623	Medium	None	Multifocal necrotic primary and secondary spermatocytes (3a and 3b) in crypts of tubules
218624	Medium	None	Premature release of secondary spermatocytes (stage 3b) in lumina. Multifocal necrosis of secondary spermatocytes (stage 3b).
218625	Medium	Multifocal	Basophilic nodules in testicular duct.
218626	Medium	None	Focal area proliferation and sequestration of sperm cells.
218627	Medium	None	Nodules in spermatic ducts and some tubules.
218628	High	Focal	Multifocal but not extensive necrosis of secondary spermatocytes (stage 3b).
218632	High	Multifocal	Multifocal tubules with encysted structures that were possibly abnormal oocytes. Multifocal but sparse necrotic spermatogonia. One ova in section, probably an artifact.
218635	High	Multifocal	Tubule lumina occluded. Multifocal necrosis of secondary spermatocytes (stage 3b).
218636	High	None	Multifocal necrosis of secondary spermatocytes (stage 3b).

5.3.7 Plasma Steroid Concentrations

Estradiol—Estradiol concentrations in Control-treatment females used during the Non-Spawning Adult 14-Day Flutamide assay ranged from 105 pg/mL to 2,990 pg/mL (Figure 5.50). Among females exposed to the three flutamide concentrations, estradiol concentrations ranged from 478 pg/mL to 5,591 pg/mL. No significant differences in the mean estradiol concentration per treatment (Table 5.43) were detected (Kruskal-Wallis, $H = 3.06$, $p = 0.383$, df = 2). The achieved power for this endpoint was 8%, and the sample size required to detect a significant difference from the Control treatment at 80% power was 196 (Table 5.43).

Table 5.43. Summary statistics and power estimates for female estradiol concentrations (pg/mL) for the Non-Spawning Adult 14-Day Flutamide assay.

Level	N	Mean	Stdev	CV	Achieved Power [1]	Sample Size Required [2]
control	10	1,638	850	52%	8%	196
low	10	1,371	842	61%		
medium	10	1,909	1,423	75%		
high	10	1,180	400	34%		

[1] Calculated from natural log transformed data; with sample size = 10.
[2] To detect a significant difference from control treatment based on maximum achieved absolute difference; calculated on natural log transformed data.

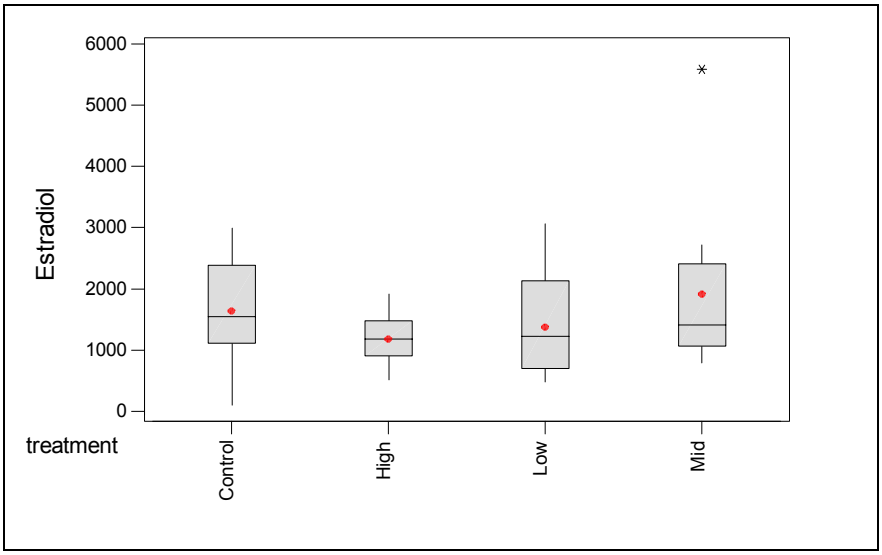

Figure 5.50. Boxplot of female estradiol concentration (pg/mL) by treatment for the Non-Spawning Adult 14-Day Flutamide assay. The box represents the interquartile range, whiskers represent the data range, the horizontal line is the median value, the circle is the mean value, and the asterisk represents a probable outlier.

Estradiol was not detected in the 10 Control-treatment males used during the Non-Spawning Adult 14-Day Flutamide assay (Figure 5.51). Among males exposed to the three flutamide concentrations, estradiol was not detected in the 9 Low-concentration males or the 10 Medium concentration males (Table 5.44). Estradiol was detected in two of the eight males from the High concentration that were analyzed.

Table 5.44. Summary statistics and power estimates for male estradiol concentrations (pg/mL) for the Non-Spawning Adult 14-Day Flutamide assay.

Level	N	Mean	Stdev	CV	Achieved Power [1]	Sample Size Required [1]
control	10	0	0	–	–	–
low	9	0	0	–		
medium	10	0	0	–		
high	8	65	124	191%		

[1] Not calculated.

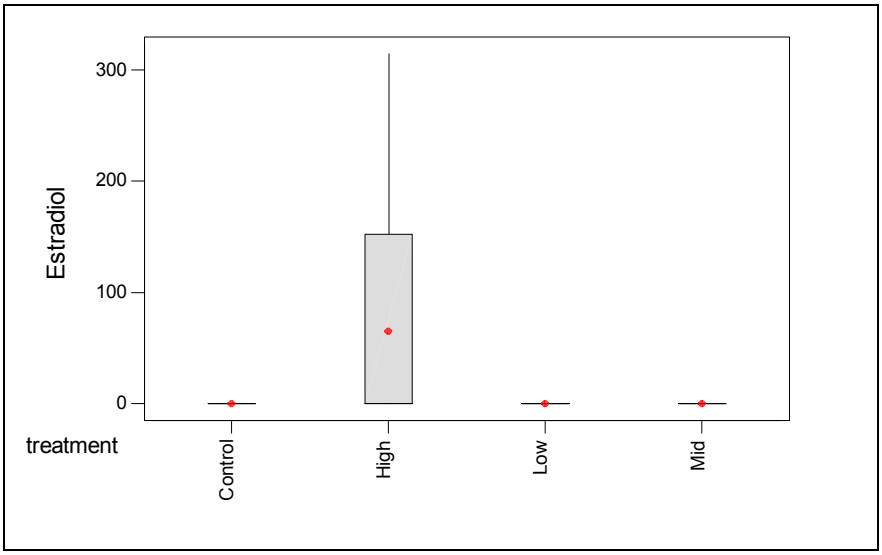

Figure 5.51. Boxplot of male estradiol concentration (pg/mL) by treatment for the Non-Spawning Adult 14-Day Flutamide assay. The box represents the interquartile range, whiskers represent the data range, the horizontal line is the median value, and the circle is the mean value.

Testosterone—Testosterone concentrations in Control-treatment females used during the Non-Spawning Adult 14-Day Flutamide assay ranged from 771 pg/mL to 3,053 pg/mL (Figure 5.52). Among females exposed to the three flutamide concentrations, testosterone concentrations ranged from 145 pg/mL to 4,057 pg/mL. No significant differences in the mean testosterone concentration per treatment (Table 5.45) were detected (Kruskal-Wallis, $H = 5.51$, $p = 0.138$, df = 2). The achieved power for this endpoint was 23%, and the sample size required to detect a significant difference from the Control treatment at 80% power was 32 (Table 5.45).

Table 5.45. Summary statistics and power estimates for female testosterone concentrations (pg/mL) for the Non-Spawning Adult 14-Day Flutamide assay.

Level	N	Mean	Stdev	CV	Achieved Power [1]	Sample Size Required [2]
control	8	1,565	857	55%	23%	32
low	8	880	475	54%		
medium	9	2,340	1,478	63%		
high	10	1,235	816	66%		

[1] Calculated from natural log transformed data; with sample size = 8.
[2] To detect a significant difference from control treatment based on maximum achieved absolute difference; calculated on natural log transformed data.

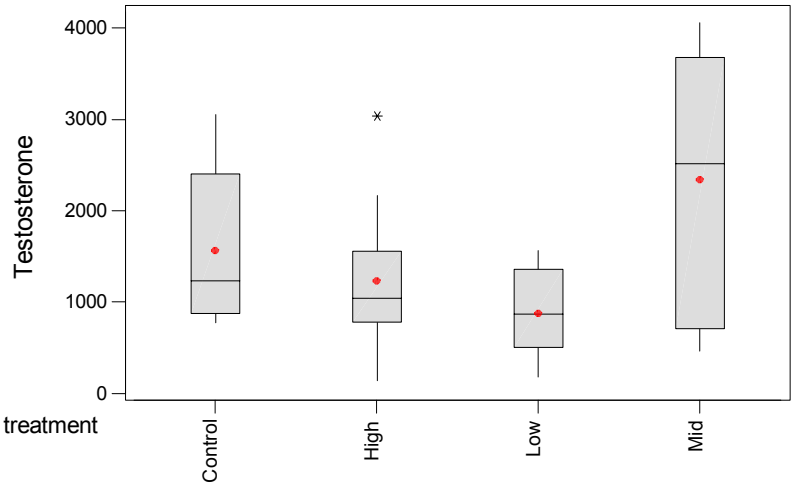

Figure 5.52. Boxplot of female testosterone concentration (pg/mL) by treatment for the Non-Spawning Adult 14-Day Flutamide assay. The box represents the interquartile range, whiskers represent the data range, the horizontal line is the median value, the circle is the mean value, and the asterisk represents a probable outlier.

Testosterone concentrations in Control treatment males used during the Non-Spawning Adult 14-Day Flutamide assay ranged from 607 pg/mL to 4,044 pg/mL (Figure 5.53). Among males exposed to the three flutamide concentrations, testosterone concentrations ranged from 513 pg/mL to 6,506 pg/mL. No significant differences in the mean testosterone concentration per treatment (Table 5.46) were detected (Kruskal-Wallis, $H = 5.00$, $p = 0.172$, df = 2). The achieved power for this endpoint was 28%, and the sample size required to detect a significant difference from the Control treatment at 80% power was 26 (Table 5.46).

Table 5.46. Summary statistics and power estimates for male testosterone concentrations (pg/mL) for the Non-Spawning Adult 14-Day Flutamide assay.

Level	N	Mean	Stdev	CV	Achieved Power [1]	Sample Size Required [2]
control	10	2,096	1,180	56%	28%	26
low	10	1,737	1,787	103%		
medium	10	1,045	405	39%		
high	8	2,042	1,596	78%		

[1] Calculated from natural log transformed data; with sample size = 8.
[2] To detect a significant difference from control treatment based on maximum achieved absolute difference; calculated on natural log transformed data.

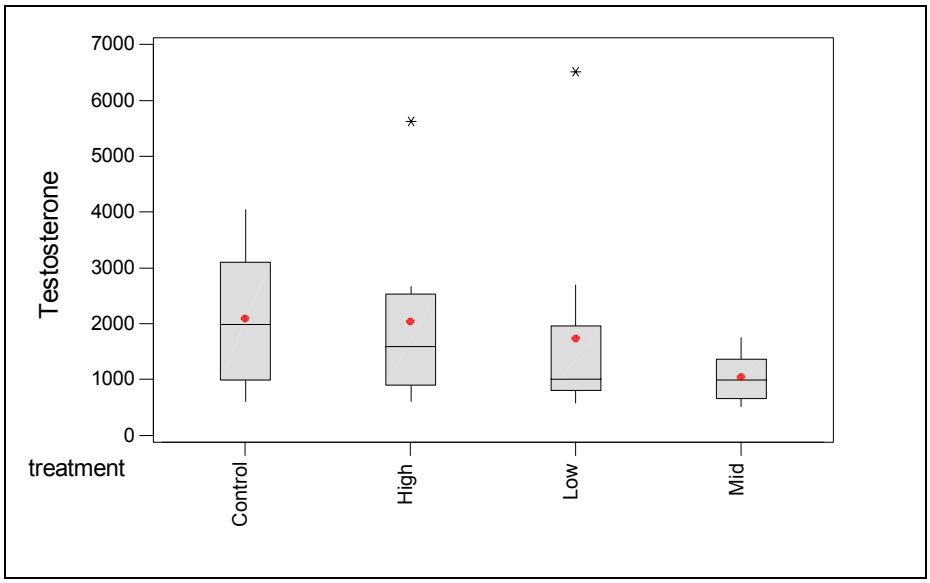

Figure 5.53. Boxplot of male testosterone concentration (pg/mL) by treatment for the Non-Spawning Adult 14-Day Flutamide assay. The box represents the interquartile range, whiskers represent the data range, the horizontal line is the median value, the circle is the mean value, and asterisks represent probable outliers.

11-ketotestosterone—11-ketotesosterone was detected in one of seven Control-treatment females and in one of five females from the Low concentration during the Non-Spawning Adult 14-Day Flutamide assay (Figure 5.54). 11-ketotesosterone was not detected in females from the Medium concentration (six individuals) and the High concentration (five individuals) exposed during the Non-Spawning Adult 14-Day Flutamide assay. No significant differences in the mean 11-ketotesosterone concentration per treatment (Table 5.47) were detected (Kruskal-Wallis, $H = 1.98$, $p = 0.577$, df = 2). The achieved power for this endpoint was 8%, and the sample size required to detect a significant difference from the Control treatment at 80% power was 91 (Table 5.47).

Table 5.47. Summary statistics and power estimates for female 11-ketotesosterone concentrations (pg/mL) for the Non-Spawning Adult 14-Day Flutamide assay.

Level	N	Mean	Stdev	CV	Achieved Power [1]	Sample Size Required [2]
control	7	70	184	265%	8%	91
low	5	86	192	223%		
medium	6	0	0	–		
high	5	0	0	–		

[1] Calculated from natural log transformed data; with sample size = 5.
[2] To detect a significant difference from control treatment based on maximum achieved absolute difference; calculated on natural log transformed data.

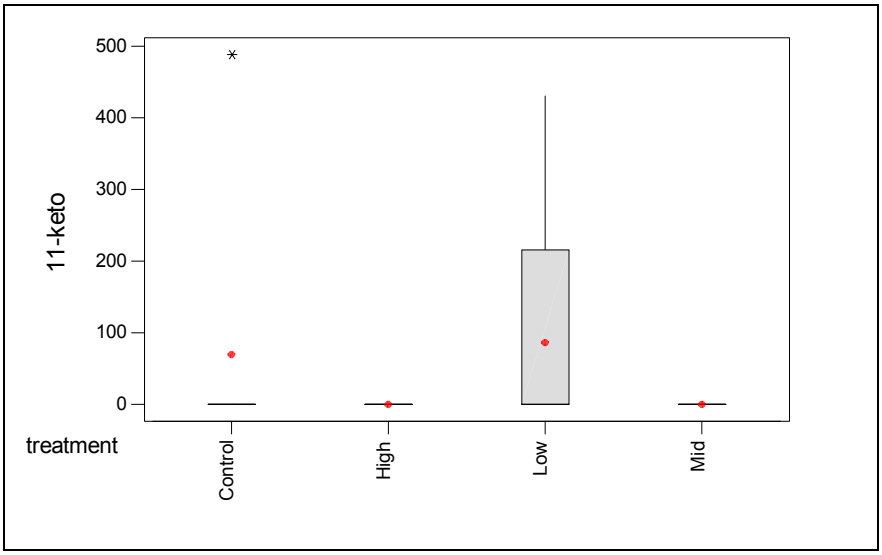

Figure 5.54. Boxplot of female 11-ketotesosterone concentration (pg/mL) by treatment for the Non-Spawning Adult 14-Day Flutamide assay. The box represents the interquartile range, whiskers represent the data range, the horizontal line is the median value, the circle is the mean value, and the asterisk represents a probable outlier.

11-ketotesosterone concentrations in Control treatment males used during the Non-Spawning Adult 14-Day Flutamide assay ranged from 2,467 pg/mL to 29,425 pg/mL (Figure 5.55). Among males exposed to the three flutamide concentrations, 11-ketotesosterone concentrations ranged from 1,816 pg/mL to 89,227 pg/mL. No significant differences in the mean 11-ketotesosterone concentration per treatment (Table 5.48) were detected (Kruskal-Wallis, $H = 0.67$, $p = 0.879$, df = 2). The achieved power for this endpoint was 6%, and the sample size required to detect a significant difference from the Control treatment at 80% power was 295 (Table 5.48).

Table 5.48. Summary statistics and power estimates for male 11-ketotesosterone concentrations (pg/mL) for the Non-Spawning Adult 14-Day Flutamide assay.

Level	N	Mean	Stdev	CV	Achieved Power [1]	Sample Size Required [2]
control	8	10,190	8,876	87%	6%	295
low	10	19,906	27,524	138%		
medium	10	6,502	2,949	45%		
high	7	13,341	12,900	97%		

[1] Calculated from natural log transformed data; with sample size = 7.
[2] To detect a significant difference from control treatment based on maximum achieved absolute difference; calculated on natural log transformed data.

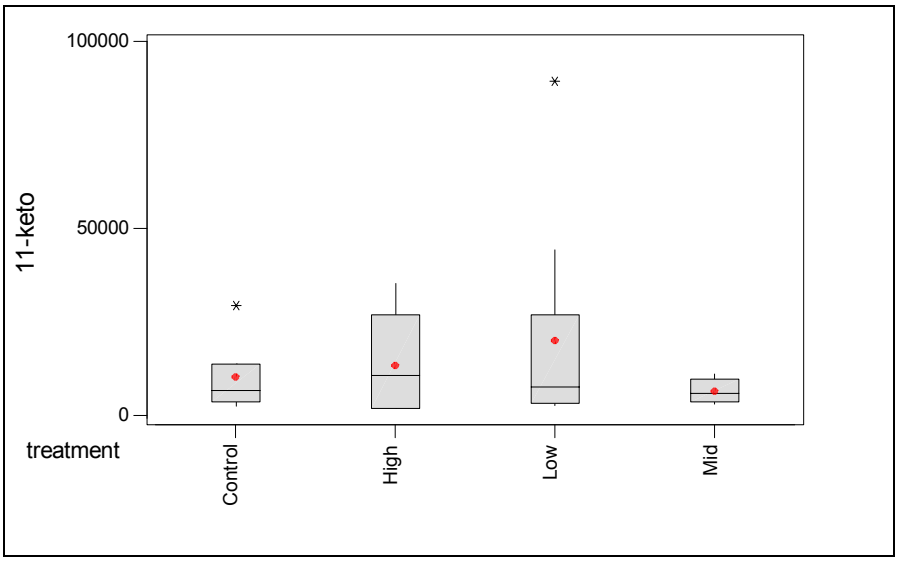

Figure 5.55. Boxplot of male 11-ketotesosterone concentration (pg/mL) by treatment for the Non-Spawning Adult 14-Day Flutamide assay. The box represents the interquartile range, whiskers represent the data range, the horizontal line is the median value, the circle is the mean value, and asterisk represent probable outliers.

5.3.8 Body Weight and Length

The body weight of females used in the Non-spawning Adult 14-Day Flutamide assay ranged from 1.9 g to 3.7 g (Figure 5.56). There were no significant differences in mean body weight (natural log transformed) among treatments (Kruskal-Wallis, $H = 3.90$, $p = 0.272$, df = 3). The achieved power for this endpoint was 36%, and the sample size required to detect a significant difference from the Control treatment at 80% power was 26 (Table 5.49).

The body (fork) length of females used in the Non-spawning Adult 14-Day Flutamide assay ranged from 46 mm to 61 mm (Figure 5.57). There were no significant differences in mean body length (natural log transformed) among treatments (Kruskal-Wallis, $H = 1.74$, $p = 0.627$, df = 3). The achieved power for

this endpoint was 10%, and the sample size required to detect a significant difference from the Control treatment at 80% power was 123 (Table 5.50).

Table 5.49. Summary statistics and power estimates for female body weight (g) data for the Non-spawning Adult 14-Day Flutamide assay.

Level	N	Mean	Stdev	CV	Achieved Power [1]	Sample Size Required [2]
control	10	2.9	0.5	19%	36%	26
low	10	2.6	0.4	15%		
medium	10	2.5	0.3	13%		
high	10	2.5	0.4	15%		

[1] Calculated from natural log transformed data; with sample size = 10.
[2] To detect a significant difference from control treatment based on maximum achieved absolute difference; calculated on natural log transformed data.

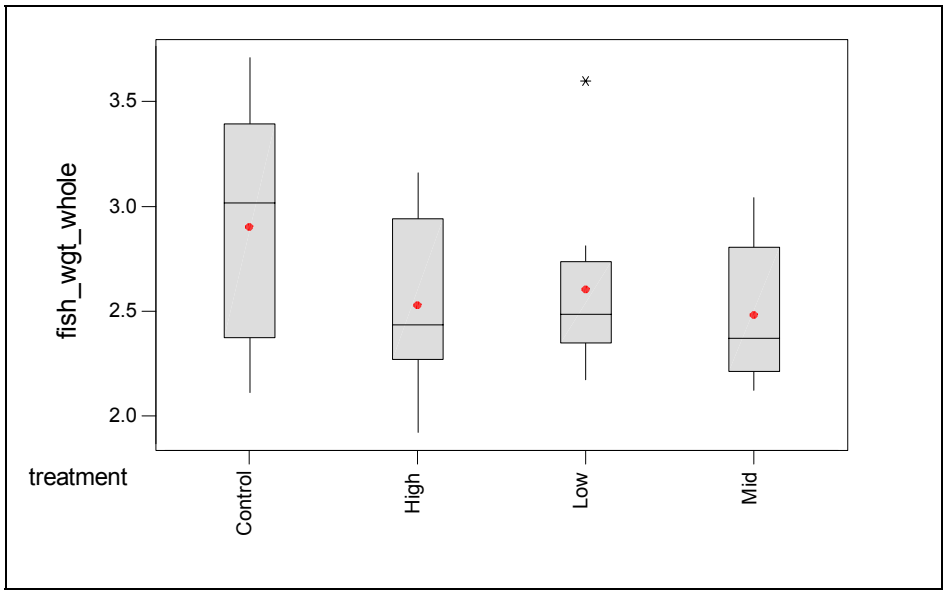

Figure 5.56. Boxplot of female body weight (g) by treatment for the Non-spawning Adult 14-Day Flutamide assay. The box represents the interquartile range, whiskers represent the data range, the horizontal line is the median value, the circle is the mean value, and the asterisk represents a probable outlier.

Table 5.50. Summary statistics and power estimates for female body length (mm) data for the Non-spawning Adult 14-Day Flutamide assay.

Level	N	Mean	Stdev	CV	Achieved Power [1]	Sample Size Required [2]
control	10	53.6	5.3	10%	10%	123
low	10	53.4	3.3	6%		
medium	10	53.1	2.7	5%		
high	10	55.0	2.8	5%		

[1] Calculated from natural log transformed data; with sample size = 10.
[2] To detect a significant difference from control treatment based on maximum achieved absolute difference; calculated on natural log transformed data.

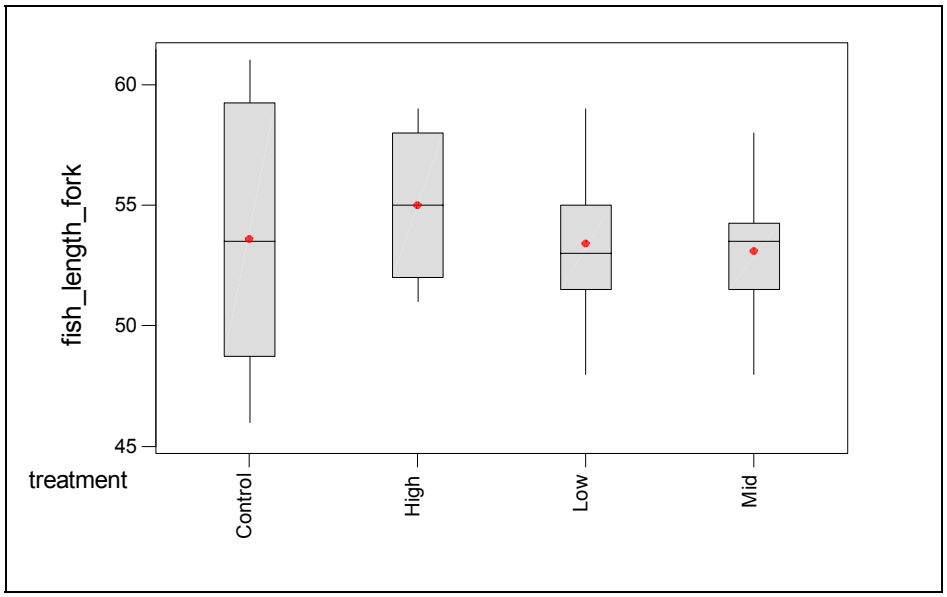

Figure 5.57. Boxplot of female body length (mm) by treatment for the Non-spawning Adult 14-Day Flutamide assay. The box represents the interquartile range, whiskers represent the data range, the horizontal line is the median value, and the circle is the mean value.

The body weight of males used in the Non-spawning Adult 14-Day Flutamide assay ranged from 2.8 g to 6.9 g (Figure 5.58). There were no significant differences in mean body weight among treatments (Kruskal-Wallis, $H = 7.08$, $p = 0.069$, df = 3). The achieved power for this endpoint was 30%, and the sample size required to detect a significant difference from the Control treatment at 80% power was 28 (Table 5.51).

The body (fork) length of males used in the Non-spawning Adult 14-Day Flutamide assay ranged from 54 mm to 75 mm (Figure 5.59). There were no significant differences in mean body length (natural log transformed) among treatments (Kruskal-Wallis, $H = 5.42$, $p = 0.143$, df = 3). The achieved power for this endpoint was 17%, and the sample size required to detect a significant difference from the Control treatment at 80% power was 52 (Table 5.52).

Table 5.51. Summary statistics and power estimates for male body weight (g) data for the Non-spawning Adult 14-Day Flutamide assay.

Level	N	Mean	Stdev	CV	Achieved Power [1]	Sample Size Required [2]
control	10	5.0	0.5	10%	30%	28
low	9	4.9	0.9	18%		
medium	10	5.3	0.8	16%		
high	10	4.3	0.7	16%		

[1] Calculated from natural log transformed data; with sample size = 9.
[2] To detect a significant difference from control treatment based on maximum achieved absolute difference; calculated on natural log transformed data.

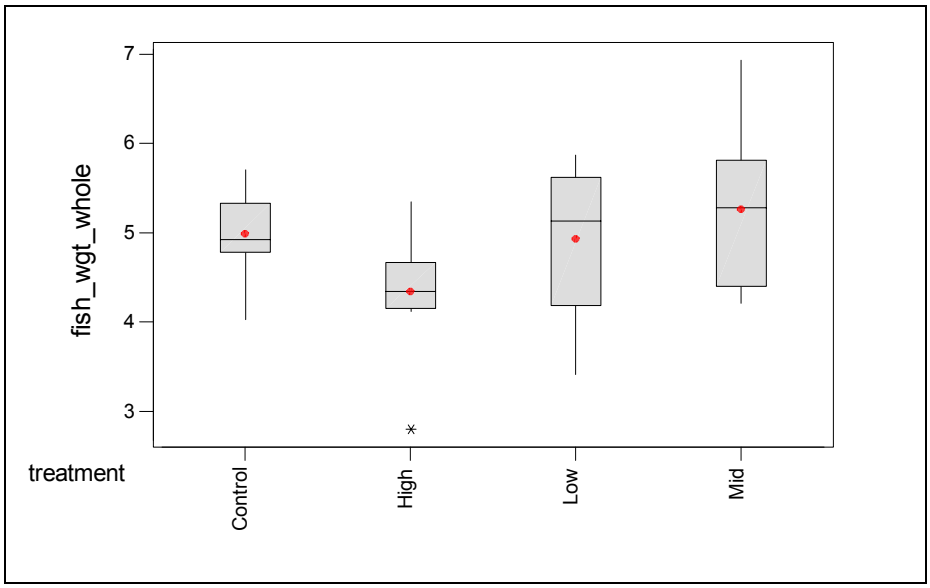

Figure 5.58. Boxplot of male body weight (g) by treatment for the Non-spawning Adult 14-Day Flutamide assay. The box represents the interquartile range, whiskers represent the data range, the horizontal line is the median value, and the circle is the mean value.

Table 5.52. Summary statistics and power estimates for male body length (mm) data for the Non-spawning Adult 14-Day Flutamide assay.

Level	N	Mean	Stdev	CV	Achieved Power [1]	Sample Size Required [2]
control	10	65.4	1.8	3%	17%	52
low	9	68.0	4.0	6%		
medium	10	68.2	4.6	7%		
high	10	64.2	5.0	8%		

[1] Calculated from natural log transformed data; with sample size = 9.
[2] To detect a significant difference from control treatment based on maximum achieved absolute difference; calculated on natural log transformed data.

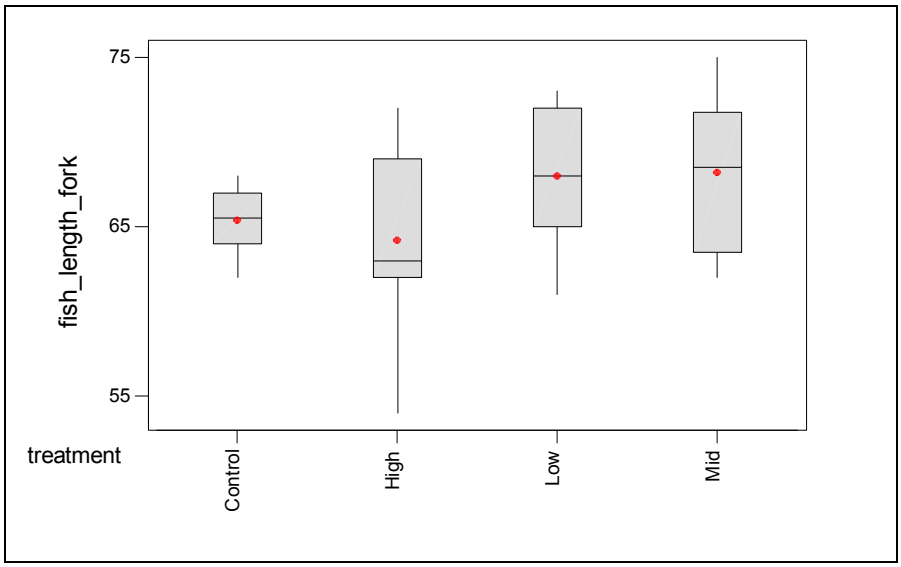

Figure 5.59. Boxplot of male body length (mm) by treatment for the Non-spawning Adult 14-Day Flutamide assay. The box represents the interquartile range, whiskers represent the data range, the horizontal line is the median value, and the circle is the mean value.