6.0 RESULTS: FADROZOLE

6.1 EPA 14-Day Assay for Fadrozole

The EPA 14-Day Fadrozole assay was conducted from March 17, 2003, to March 27, 2003 (pre-exposure assay), and from March 27, 2003, to April 10, 2003 (exposure assay).

6.1.1 Survival

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

6.1.2 Vitellogenin

Vitellogenin concentrations in Control treatment females used during the EPA 14-Day fadrozole assay ranged from 3,426,500 ng/mL to 6,920,500 ng/mL (Figure 6.1). Among females exposed to the two fadrozole concentrations, vitellogenin concentrations ranged from 0 ng/mL (not detected) to 2,798,000 ng/mL. Significant differences in the mean vitellogenin concentration per treatment (Table 6.1) were detected (Kruskal-Wallis, $H = 41.80$, $p = <0.001$, df = 2). Vitellogenin concentrations in Control-treatment females were significantly greater than those in females exposed to the Low and High fadrozole concentrations. Additionally, vitellogenin concentrations in females from the Low concentration were greater than those in females from the High concentration. The achieved power for this endpoint was 100%, and the sample size required to detect a significant difference from the Control treatment at 80% power was 2 (Table 6.1).

Table 6.1. Summary statistics and power estimates for female vitellogenin concentrations (ng/mL) for the EPA 14-Day Fadrozole assay.

Level	N	Mean	Stdev	CV	Achieved Power [1]	Sample Size Required [2]
control	16	5,364,000	1,059,237	20%	100%	2
low	16	1,148,553	703,249	61%		
high	16	1,693	1,986	117%		

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

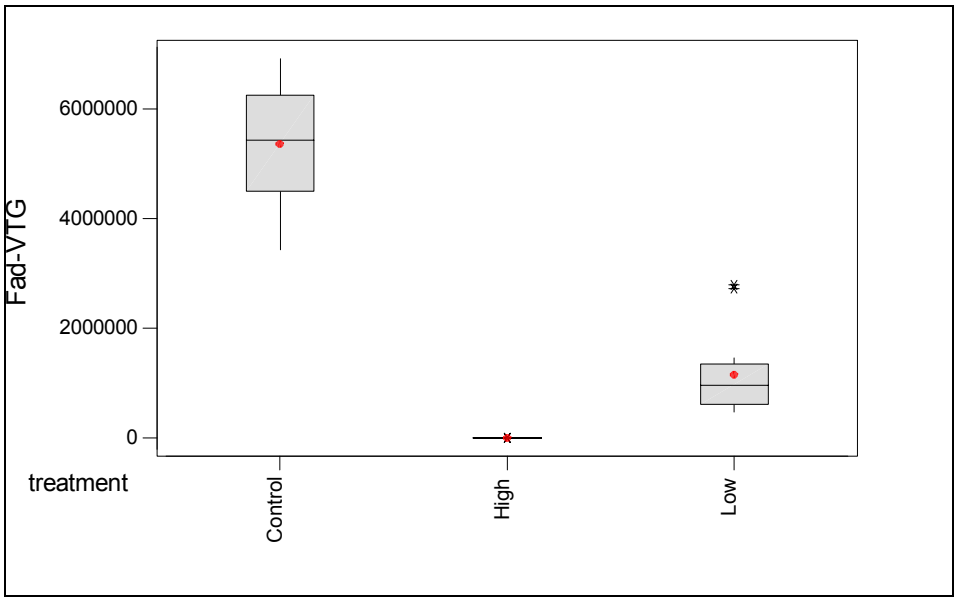

Figure 6.1. Boxplot of female vitellogenin concentration (ng/mL) by treatment for the EPA 14-Day Fadrozole assay. The box represents the interquartile range, whiskers represent the data range, the horizontal line is the median value, the circle is the mean value, and asterisks represent probable outliers.

Vitellogenin concentrations in Control treatment males used during the EPA 14-Day fadrozole assay ranged from 0 ng/mL (not detected) to 7,123 ng/mL (Figure 6.2). Among most males exposed to the two fadrozole concentrations, vitellogenin concentrations ranged from 0 ng/mL (not detected) to 5,604 ng/mL. One male exposed to the Low fadrozole concentration treatment had a vitellogenin concentration of 15,191 ng/mL. No significant differences in the mean vitellogenin concentration per treatment (Table 6.2) were detected (Kruskal-Wallis, $H = 0.67$, $p = 0.716$, 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 528 (Table 6.2).

Table 6.2. Summary statistics and power estimates for male vitellogenin concentrations (ng/mL) for the EPA 14-Day Fadrozole assay.

Level	N	Mean	Stdev	CV	Achieved Power [1]	Sample Size Required [2]
control	8	2,150	3,088	144%	6%	528
low	8	2,856	5,111	179%		
high	8	1,056	1,914	181%		

[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.

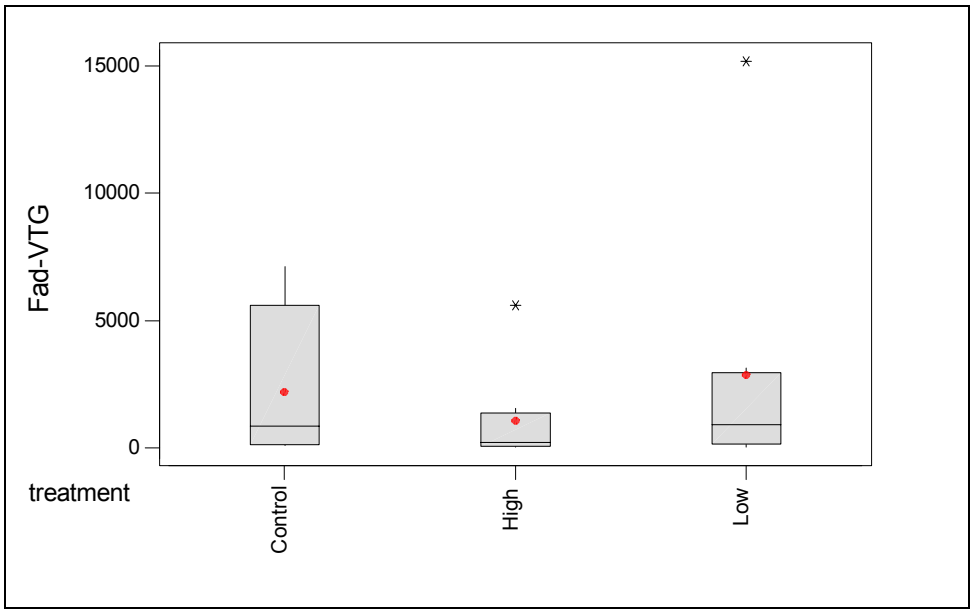

Figure 6.2. Boxplot of male vitellogenin concentration (ng/mL) by treatment for the EPA 14-Day Fadrozole assay. The box represents the interquartile range, whiskers represent the data range, the horizontal line is the median value, the circle is the mean value, and asterisks represent probable outliers.

6.1.3 Appearance / Secondary Sex Characteristics

All of the females used during the EPA 14-Day Fadrozole assay exhibited typical female morphology (no fat pad, no tubercles, no vertical banding, ovipositor present).

Most of the males used during the EPA 14-Day Fadrozole assay exhibited typical male morphology (fat pads, tubercles, vertical banding, no ovipositor present). One male from the Control treatment lacked a fat pad and vertical banding.

6.1.4 Gonadosomatic Index

The range of GSI values calculated for females in the all treatments varied about two-fold (Figure 6.3), and the overall variability within the treatment was moderate (CVs = 22%–24%; Table E14FAD GSI-1). The highest female GSI values were about 21–23 (two fish each in the Low and High concentrations). Significant differences in mean GSI values (Table 6.3) among treatments were detected (Kruskal-Wallis, $H = 13.54$, $p = 0.001$, df = 2). The mean GSI values for females exposed to the Low and High concentrations were significantly greater than GSI values for females from the Control treatment. The achieved power for this endpoint was 92%, and the sample size required to detect a significant difference from the Control treatment at 80% power was 12 (Table 6.3).

Table 6.3. Summary statistics and power estimates for female gonadosomatic index data for the EPA 14-Day Fadrozole assay.

Level	N	Mean	Stdev	CV	Achieved Power [1]	Sample Size Required [2]
control	16	11.0	2.4	22%	92%	12
low	16	15.1	3.5	23%		
high	16	14.8	3.5	24%		

[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.

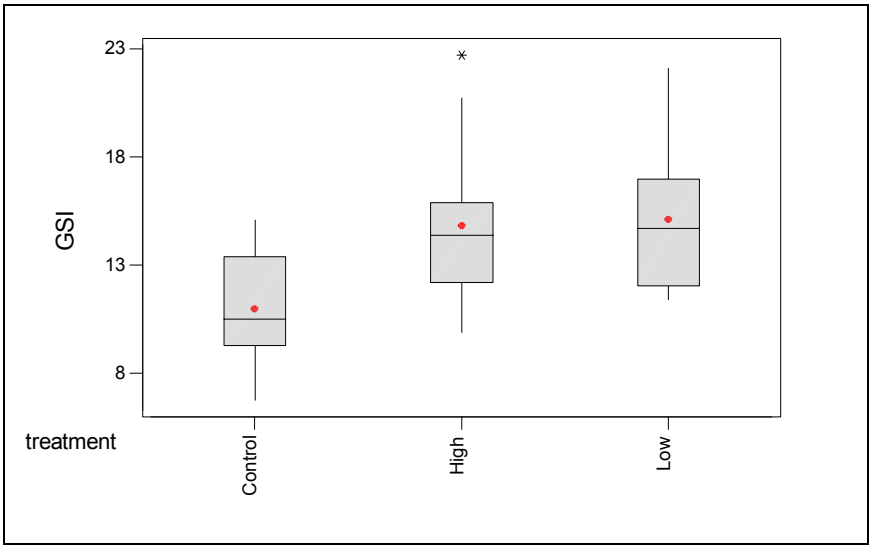

Figure 6.3. Boxplot of female GSI by treatment for the EPA 14-Day Fadrozole assay. The box represents the interquartile range, whiskers represent the data range, the horizontal line is the median value, the circle is the mean value, and the asterisk represents a probable outlier.

The range of most GSI values calculated for males during the EPA 14-Day Fadrozole assay, was small, ranging from 0.7 to 1.6 (Figure 6.4), which approximates the typical range for reproductively-active male fathead minnows. However, five fish had GSI values ≥2.0 (at least one fish from each treatment). The highest male GSI value was 3.6 for a small fish (2.5 g body weight) exposed to the Low fadrozole concentration that had a relatively large gonad (0.09 g). There were no significant differences in mean GSI values (Table 6.4) among treatments (Kruskal-Wallis, $H = 4.12$, $p = 0.128$, df = 2). The achieved power for this endpoint was 24%, and the sample size required to detect a significant difference from the Control treatment at 80% power was 32 (Table 6.4).

Table 6.4. Summary statistics and power estimates for male gonadosomatic index data for the EPA 14-Day Fadrozole assay.

Level	N	Mean	Stdev	CV	Achieved Power [1]	Sample Size Required [2]
control	8	1.23	0.42	34%	24%	32
low	8	1.45	0.87	60%		
high	8	1.66	0.45	27%		

[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.

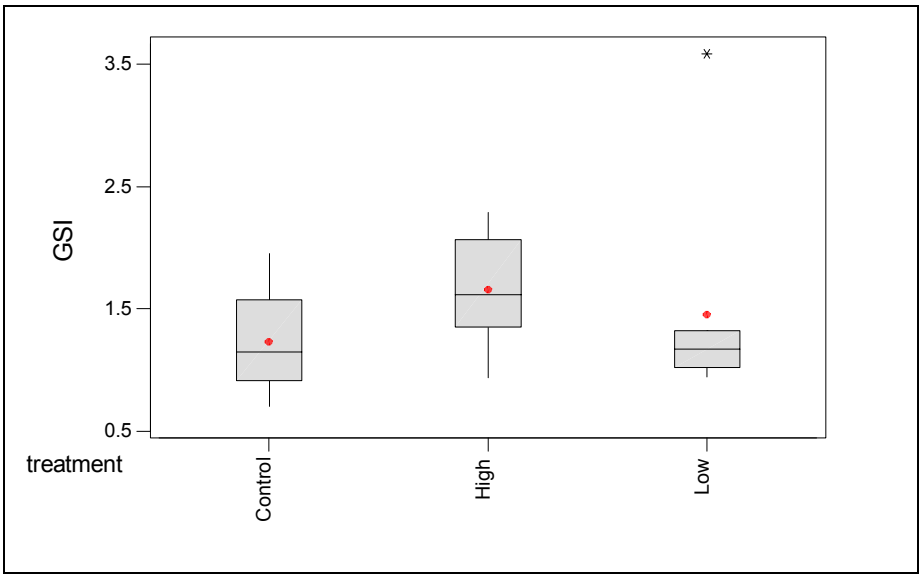

Figure 6.4. Boxplot of male GSI by treatment for the EPA 14-Day Fadrozole assay. The box represents the interquartile range, whiskers represent the data range, the horizontal line is the median value, the circle is the mean value, and the asterisk represents a probable outlier.

6.1.5 Female Gonad Histology

Histological analyses were conducted on the ovaries of 48 females exposed to fadrozole 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 fadrozole assay revealed significant differences among treatments (Kruskal-Wallis, $H = 8.78$, $p = 0.012$, df = 2). The mean ovarian stage of females from the High concentration was significantly less than those of females from the Control treatment and the Low concentration.

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 Low concentration ranged from Stage 1a to Stage 5 (see Methods for a description of the stages), whereas ovaries in females from the High-concentration treatment showed Stage 1a to Stage 4

development (Figure 6.5). Variability within treatments for each stage was very high as indicated by CVs that ranged as high as 400% (Table 6.5). Statistical analyses revealed significant difference among treatments in the proportion of cells in three of the five developmental stages (Table 6.5). The proportion of cells in developmental Stage 1a was greater in females from the Control treatment than in females from the High and Low concentrations. Also, the proportion of cells in developmental Stage 1b was greater in females from the Control treatment than in females from the High concentration. The proportion of cells in developmental Stage 3 was greater in females from the High concentration than in females from the Control treatment and the Low concentration. The proportion of cells in developmental Stage 4 was less in females from the High concentration than in females from the Control treatment and the Low concentration.

Table 6.5. Descriptive statistics of the proportion of ovarian cells in each developmental stage for females from the EPA 14-Day Fadrozole 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	0.085	0.028	33%	0.058	0.019	32%	0.050	0.025	50%	14.19	0.001*
1b	0.277	0.067	24%	0.220	0.057	26%	0.205	0.067	33%	8.83	0.012**
2	0.200	0.048	24%	0.198	0.032	16%	0.189	0.073	39%	1.21	0.545
3	0.192	0.046	24%	0.228	0.060	26%	0.351	0.100	28%	20.70	<0.001***
4	0.206	0.107	52%	0.272	0.080	29%	0.103	0.065	63%	20.79	<0.001***
5	0.015	0.045	308%	0.000	0.002	400%	0	0	–	2.18	0.337

* $p < 0.01$
** $p < 0.05$
*** $p < 0.001$

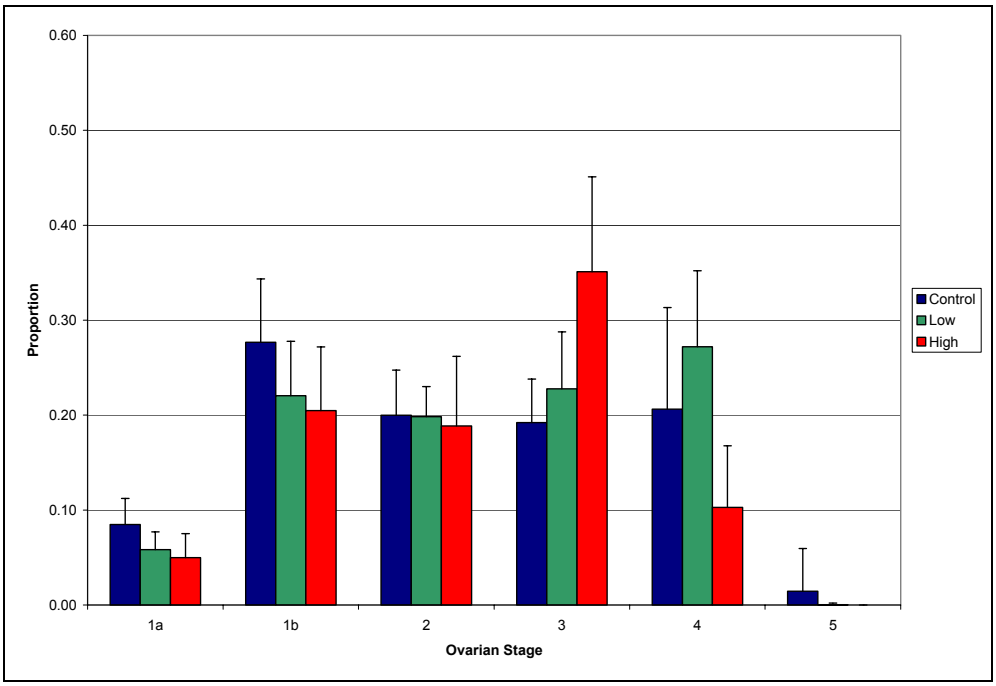

Figure 6.5. Frequency histogram showing the quantitative developmental staging of ovaries for each treatment of the EPA 14-Day Fadrozole assay. For each treatment, the columns represent the grand mean proportion of cells in each stage and the bars represent the standard deviation.

Atretic Follicles—The mean proportion of atretic follicles per 300 follicles (counted per fish) ranged from 0.009 follicles for females in the Control treatment to 0.102 follicles for females in the High concentration (Figure 6.6). There was a significant difference in the mean proportions of atretic follicles among treatments (Kruskal-Wallis, $H = 14.26$, $p = 0.001$, df = 2). The proportion of atretic follicles was greater for females from the High concentration than for those from the Control treatment or the Low concentration.

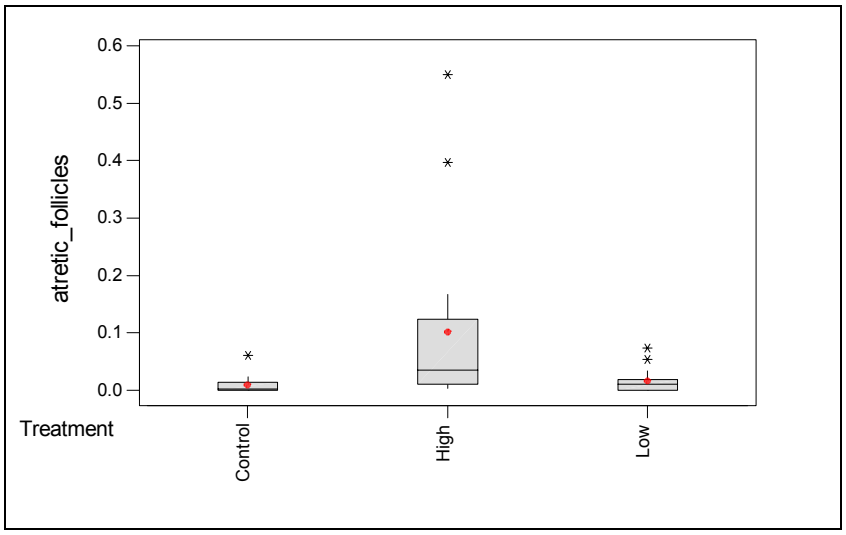

Figure 6.6. Boxplot of atretic follicles per 300 follicles by treatment for the EPA 14-Day Fadrozole assay. The box represents the interquartile range, whiskers represent the data range, the horizontal line is the median value, the circle is the mean value, and asterisks represent probable outliers.

Corpora Lutea—The mean proportion of corpora lutea per 300 follicles (counted per fish) ranged from 0.001 for females in the High concentration to 0.012 for females in the Control treatment (Figure 6.7). There were significant differences in the mean proportion of corpora lutea among treatments (Kruskal-Wallis, $H = 11.24$, $p = 0.004$, df = 2). The value for the Control treatment was significantly greater than those of the other two treatments.

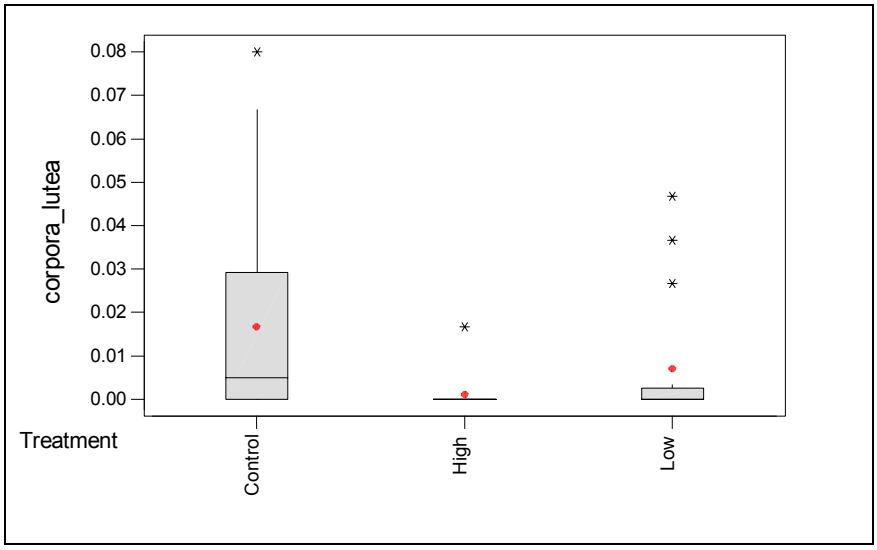

Figure 6.7. Boxplot of corpora lutea per 300 follicles by treatment for the EPA 14-Day Fadrozole assay. The box represents the interquartile range, whiskers represent the data range, the horizontal line is the median value, the circle is the mean value, and asterisks represent probable outliers.

Observations—One female from the Control treatment was observed to have abnormal-appearing Stage 2 ova that had crenulated surfaces. Six females from the High concentration had vacuoles in the nuclei of some Stage 3 and/or Stage 4 cells. One additional female from the High concentration had inflammatory cells in the ovary.

6.1.6 Male Gonad Histology

General Testes Staging—Testes from 24 males exposed to fadrozole during the EPA 14-Day Fadrozole assay were examined to determine the general developmental condition. Males in all treatments had well-developed testes, 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 (49 fields) or Stage 5 (47 fields) development. All of the 96 microscopic fields examined in the 8 Low-concentration treatment males showed Stage 4 (67 fields) or Stage 5 (29 fields) development. All of the 96 microscopic fields examined in the 8 High-concentration treatment males showed Stage 4 (40 fields) or Stage 5 (56 fields) development. Statistical analysis of the mean staging from 12 microscopic fields per fish revealed no significant differences among treatments (Kruskal-Wallis, $H = 3.15$, $p = 0.207$, 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 6.8). Variability within treatments for each stage was very high as indicated by CVs that ranged as high as 197% (Table 6.6). Statistical analyses showed that there were significant differences among treatments in the proportion of cells in developmental Stage 4. The proportion of cells showing Stage 4 development was greater in males from the Low concentration than in males from the Control treatment or the High concentration. The probability value calculated for Stage 5 was slightly above the critical limit of 0.050 (Table E14FAD MHist-1). The greatest difference was between the mean proportion of cells showing Stage 5 development in males from the High concentration (0.68) and the Low concentration (0.40). Therefore, there was no effect on testicular developmental stage associated with fadrozole dose.

Table 6.6. Descriptive statistics of the proportion of testes cells in each developmental stage for males from the EPA 14-Day Fadrozole 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	0.007	0.010	148%	0.003	0.006	197%	0.003	0.003	95%	0.93	0.629
2b	0.022	0.020	91%	0.026	0.013	48%	0.014	0.012	88%	4.27	0.118
3a	0.120	0.114	95%	0.208	0.131	63%	0.115	0.108	94%	3.40	0.183
3b	0.160	0.108	68%	0.154	0.092	60%	0.085	0.067	78%	2.89	0.235
4	0.098	0.058	59%	0.207	0.093	45%	0.102	0.011	11%	8.22	0.016*
5	0.592	0.252	43%	0.403	0.198	49%	0.681	0.158	23%	5.81	0.055

* $p < 0.05$

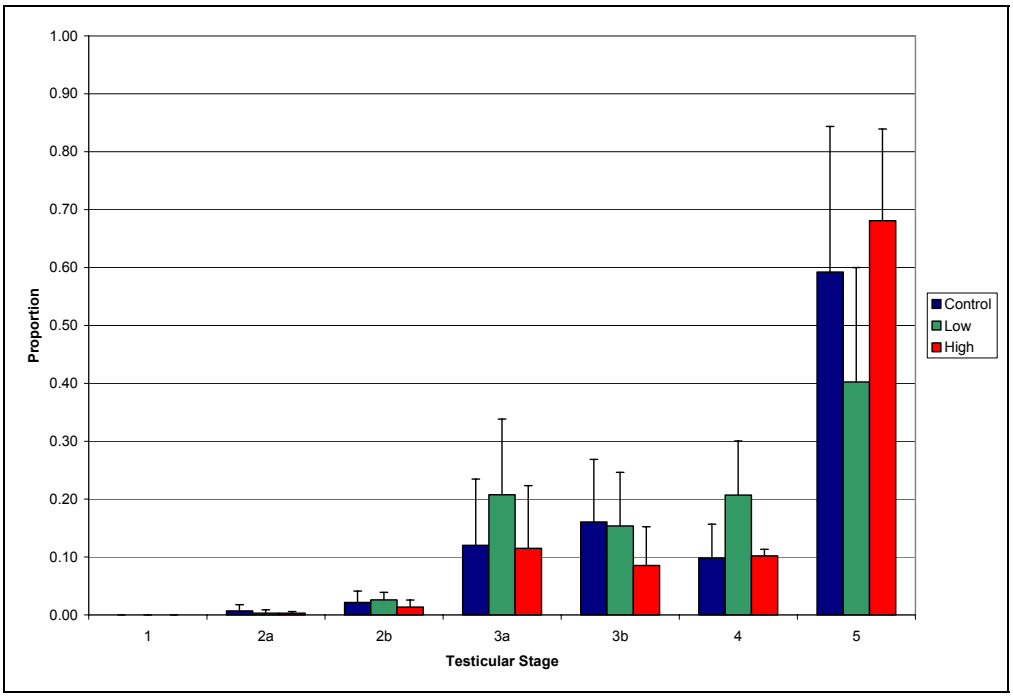

Figure 6.8. Frequency histogram showing the quantitative developmental staging of testes for each treatment of the EPA 14-Day Fadrozole assay. For each treatment, the columns represent the grand mean proportion of cells in each stage and the bars represent the standard deviation.

Tubule Diameter—The diameter of the seminiferous tubules of males from the Control treatment ranged from 145.0 µm to 248.3 µm (Figure 6.9). Tubule diameters of males from the two test concentrations ranged from 114.7 µm to 240.3 µm. No significant differences in the mean tubule diameter per treatment (Table 6.7) were detected (Kruskal-Wallis, $H = 4.07$, $p = 0.131$, 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 67 (Table 6.7).

Table 6.7. Summary statistics and power estimates for male seminiferous tubule diameter data for the EPA 14-Day Fadrozole assay.

Level	N	Mean	Stdev	CV	Achieved Power [1]	Sample Size Required [2]
control	8	167.9	35.8	21%	13%	67
low	8	151.2	23.0	15%		
high	8	177.2	34.5	19%		

[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.

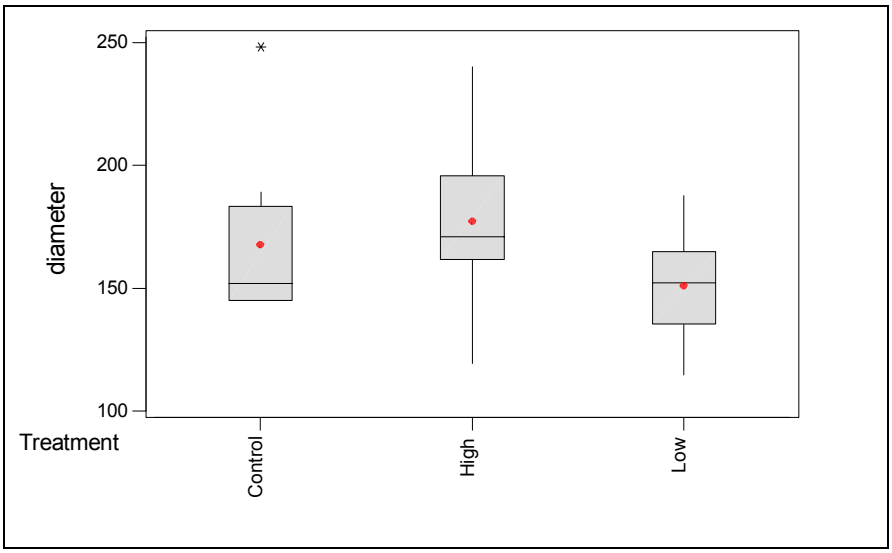

Figure 6.9. Boxplot of seminiferous tubule diameter (µm) by treatment for the EPA 14-Day Fadrozole assay. The box represents the interquartile range, whiskers represent the data range, the horizontal line is the median value, the circle is the mean value, and the asterisk represents a probable outlier.

Observations—One male from the Control treatment showed Sertoli cell proliferation with sperm phagocytosis. The same male also showed focal tubule disorganization, multifocal proliferation of secondary spermatogonia, and sperm clumping in the tubule lamina. One male from the Low concentration and three males from the High concentration were observed to have multifocal Leydig cell proliferation. One male from the Low concentration had an abnormal-appearing arrangement of components of spermatogenesis. Another male from the Low concentration showed tubules in developmental Stage 5 that appeared to have a smaller diameter than normal and had a lower than normal density of sperm in tubule lumina. Ovatestes, containing three Stage 3 ova, were detected in one male from the High concentration. One male of the males from the High concentration that had multifocal Leydig cell proliferation also showed focal disruption of tubule developmental sequence, with the proliferation of developmental Stage 2b cells in mature tubules containing developmental Stage 5 cells and had occasional foci of two or three necrotic Stage 3a or Stage 3b cells. Another male from the High concentration had multifocal areas of three or four abnormal developmental Stage 3b and Stage 4 cells with the Stage 4 cell clumps appearing to be inside cell wall of the stage 3b cells. No testicular atrophy was recorded and no ovatestes were observed for any treatment.

6.1.7 Plasma Steroid Concentrations

Estradiol—Estradiol concentrations in Control-treatment females used during the EPA 14-Day fadrozole assay ranged from 1,164 pg/mL to 5,523 pg/mL (Figure 6.10). Among females exposed to the two fadrozole concentrations, estradiol concentrations ranged from 0 pg/mL (not detected) to 2,158 pg/mL. A significant difference in the mean estradiol concentration per treatment (Table 6.8) was detected (Kruskal-Wallis, $H = 36.86$, $p < 0.001$, df = 2). The mean estradiol concentration in females from the High concentration was less than that in females from the Control treatment and the Low concentration. The achieved power for this endpoint was 100%, and the sample size required to detect a significant difference from the Control treatment at 80% power was 3 (Table 6.8).

Table 6.8. Summary statistics and power estimates for female estradiol concentrations (pg/mL) for the EPA 14-Day Fadrozole assay.

Level	N	Mean	Stdev	CV	Achieved Power [1]	Sample Size Required [2]
control	15	2,064	1,176	57%	100%	3
low	16	1,088	468	43%		
high	16	39	86	218%		

[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.

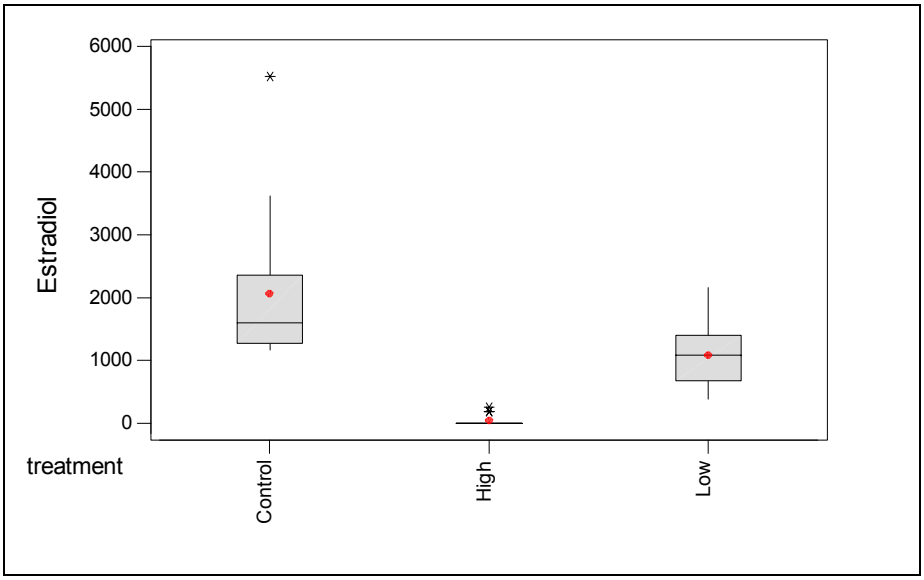

Figure 6.10. Boxplot of female estradiol concentration (pg/mL) by treatment for the EPA 14-Day Fadrozole assay. The box represents the interquartile range, whiskers represent the data range, the horizontal line is the median value, the circle is the mean value, and the asterisks represent probable outliers.

Estradiol was not detected in males from the Control treatment (8 individuals) or in those exposed to the Low- and High-fadrozole concentrations (8 individuals per treatment).

Testosterone—Testosterone concentrations in Control-treatment females used during the EPA 14-Day fadrozole assay ranged from 221 pg/mL to 2,644 pg/mL (Figure 6.11). Among females exposed to the two fadrozole concentrations, testosterone concentrations ranged from 732 pg/mL to 4,992 pg/mL. Significant differences in the mean testosterone concentration per treatment (Table 6.9) were detected (Kruskal-Wallis, $H = 14.91$, $p = 0.001$, df = 2). The mean testosterone concentration in females from the Control treatment was less than those in females from the Low and High concentrations. The achieved power for this endpoint was 96%, and the sample size required to detect a significant difference from the Control treatment at 80% power was 9 (Table 6.9).

Table 6.9. Summary statistics and power estimates for female testosterone concentrations (pg/mL) for the EPA 14-Day Fadrozole assay.

Level	N	Mean	Stdev	CV	Achieved Power [1]	Sample Size Required [2]
control	13	934	629	67%	96%	9
low	14	2,356	1,268	54%		
high	16	2,355	1,175	50%		

[1] Calculated from natural log transformed data; with sample size = 13.
[2] To detect a significant difference from control treatment based on maximum achieved absolute difference; calculated on natural log transformed data.

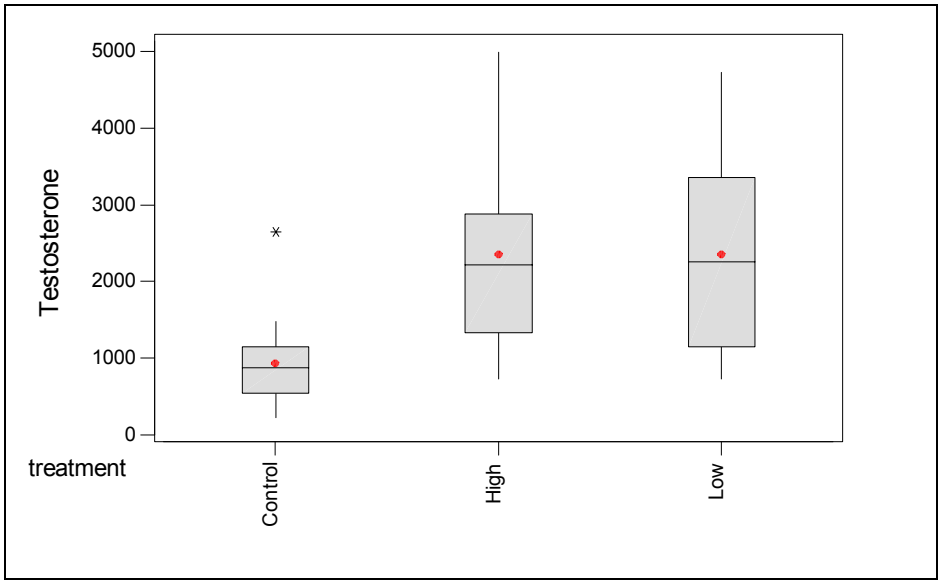

Figure 6.11. Boxplot of female testosterone concentration (pg/mL) by treatment for the EPA 14-Day Fadrozole assay. The box represents the interquartile range, whiskers represent the data range, the horizontal line is the median value, the circle is the mean value, and the asterisk represents a probable outlier.

Testosterone concentrations in Control treatment males used during the EPA 14-Day fadrozole assay ranged from 198 pg/mL to 3,569 pg/mL (Figure 6.12). Among males exposed to the two fadrozole concentrations, testosterone concentrations ranged from 1,182 pg/mL to 7,124 pg/mL. A significant difference in the mean testosterone concentration per treatment (Table 6.10) was detected (Kruskal-Wallis, $H = 7.12$, $p = 0.029$, df = 2). The mean testosterone concentration in males from the Control treatment was less than those in males from the High concentration. The achieved power for this endpoint was 68%, and the sample size required to detect a significant difference from the Control treatment at 80% power was 11 (Table 6.10).

Table 6.10. Summary statistics and power estimates for male testosterone concentrations (pg/mL) for the EPA 14-Day Fadrozole assay.

Level	N	Mean	Stdev	CV	Achieved Power [1]	Sample Size Required [2]
control	8	1,790	997	56%	68%	11
low	8	3,169	833	26%		
high	8	4,431	2,294	52%		

[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.

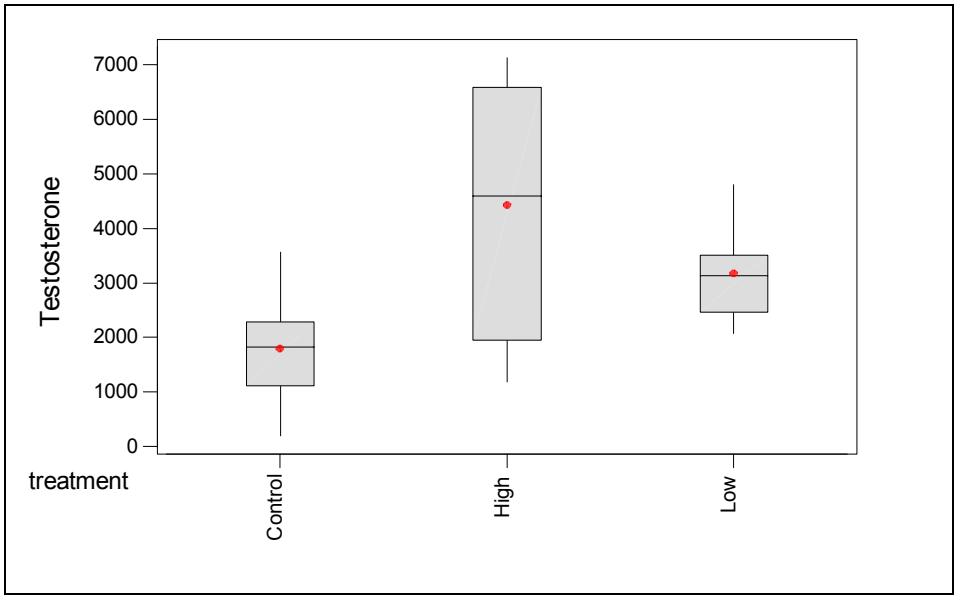

Figure 6.12. Boxplot of male testosterone concentration (pg/mL) by treatment for the EPA 14-Day Fadrozole assay. The box represents the interquartile range, whiskers represent the data range, the horizontal line is the median value, and the circle is the mean value.

11-ketotestosterone—11-ketotesosterone was detected in only 1 of 11 Control-treatment females used during the EPA 14-Day fadrozole assay (Figure 6.13). Among females exposed to the two fadrozole concentrations, testosterone concentrations ranged from 0 pg/mL (not detected) to 1,528 pg/mL. No significant differences in the mean 11-ketotestosterone concentration per treatment (Table 6.11) were detected (Kruskal-Wallis, $H = 5.69$, $p = 0.058$, df = 2). However, the calculated probability was slightly greater than the selected critical limit (0.050) for the test. The greatest difference in mean 11-ketotestosterone concentration was between females from the Control treatment (60 pg/mL) and those from the High concentration (426 pg/mL). The mean value for Control females was also much less than that for females from the Low concentration (400 pg/mL). The achieved power for this endpoint was 54%, and the sample size required to detect a significant difference from the Control treatment at 80% power was 19 (Table 6.11).

Table 6.11. Summary statistics and power estimates for female 11-ketotestosterone concentrations (pg/mL) for the EPA 14-Day Fadrozole assay.

Level	N	Mean	Stdev	CV	Achieved Power [1]	Sample Size Required [2]
control	11	60	197	332%	54%	19
low	11	400	516	129%		
high	14	426	430	101%		

[1] Calculated from natural log transformed data; with sample size = 11.
[2] To detect a significant difference from control treatment based on maximum achieved absolute difference; calculated on natural log transformed data.

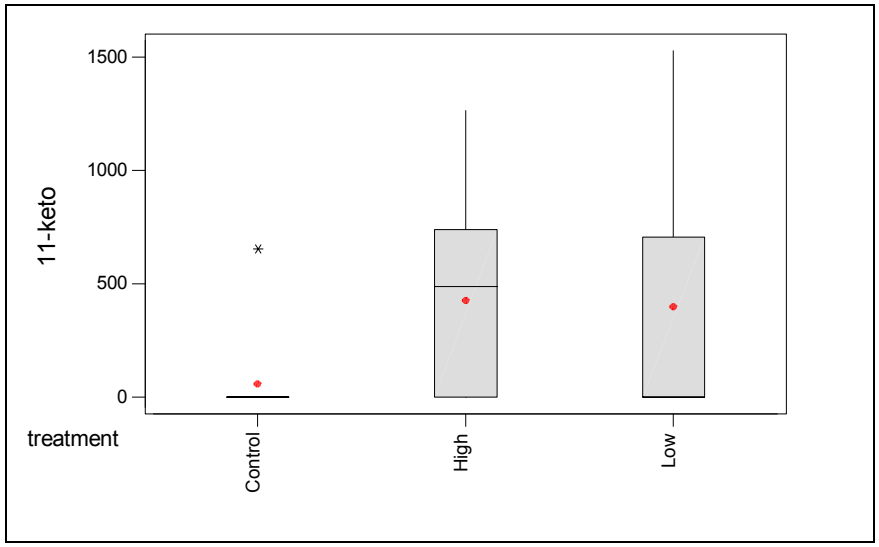

Figure 6.13. Boxplot of female 11-ketotestosterone concentration (pg/mL) by treatment for the EPA 14-Day Fadrozole assay. The box represents the interquartile range, whiskers represent the data range, the horizontal line is the median value, the circle is the mean value, and the asterisk represents a probable outlier.

11-ketotesosterone concentrations in Control treatment males used during the EPA 14-Day fadrozole assay ranged from 959pg/mL to 41,357 pg/mL (Figure 6.14g). Among males exposed to the two fadrozole concentrations, 11-ketotesosterone concentrations ranged from 1,665 pg/mL to 103,500 pg/mL. A significant difference in the mean 11-ketotesosterone concentration per treatment (Table 6.12) was detected (Kruskal-Wallis, $H = 6.54$, $p = 0.038$, df = 2). The mean 11-ketotestosterone concentration in males from the Control treatment was less than that in males from the Low concentration. The achieved power for this endpoint was 64%, and the sample size required to detect a significant difference from the Control treatment at 80% power was 11 (Table 6.12).

Table 6.12. Summary statistics and power estimates for male 11-ketotesosterone concentrations (pg/mL) for the EPA 14-Day Fadrozole assay.

Level	N	Mean	Stdev	CV	Achieved Power [1]	Sample Size Required [2]
control	8	14,376	13,061	91%	64%	11
low	8	56,077	31,055	55%		
high	8	41,690	36,973	89%		

[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.

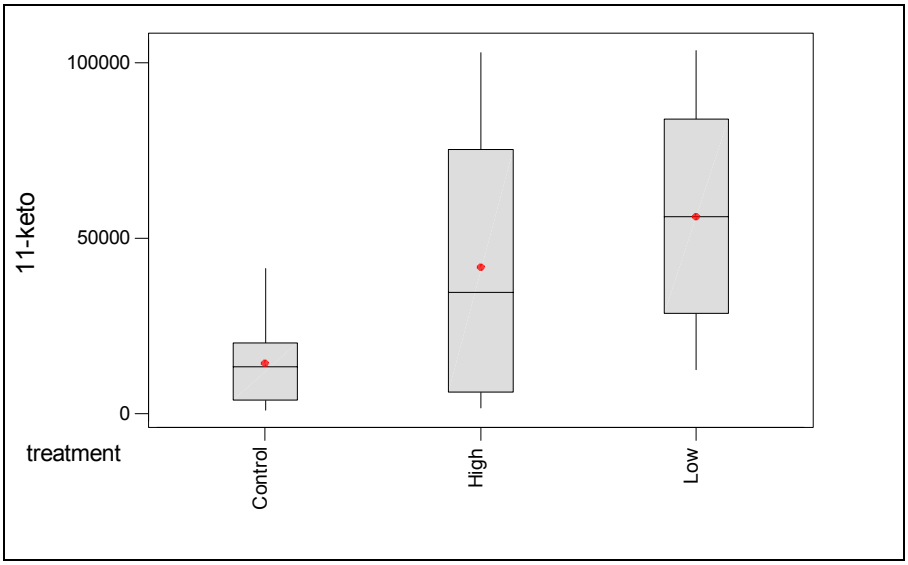

Figure 6.14. Boxplot of male 11-ketotesosterone concentration (pg/mL) by treatment for the EPA 14-Day Fadrozole assay. The box represents the interquartile range, whiskers represent the data range, the horizontal line is the median value, and the circle is the mean value.

6.1.8 Fecundity

Total Fecundity—Variability among treatments in the total number of eggs produced during the EPA 14-Day Fadrozole assay was moderate to high (Figure 6.15). Total counts in the Control treatment ranged from 3,063 eggs to 5,252 eggs and from 2,164 eggs to 3,408 eggs in the Low concentration. Total counts for three replicates of the High fadrozole concentration treatment were similar, ranging from 644 eggs to 1,041 eggs, whereas the total number of eggs produced in the fourth replicate was 32. Egg production by females in the High concentration stopped after Day 4 of the assay. Significant differences in the mean numbers of eggs (square-root transformed) produced per treatment (Table 6.13) were detected (Kruskal-Wallis, $H = 9.27$, $p = 0.010$, df = 2). The mean numbers of eggs produced by females in the Control treatment and the Low concentration were greater than the mean numbers of eggs laid by females in the High concentration. The achieved power for this assay was 100%, and the sample size required to detect a significant difference from the Control treatment at 80% power was 3 (Table 6.13).

Table 6.13. Summary statistics and power estimates for fecundity data for the EPA 14-Day Fadrozole assay.

Level	N	Mean	Stdev	CV	Achieved Power [1]	Sample Size Required [2]
control	4	4462	961	22%	100%	3
low	4	2517	602	24%		
high	4	609	422	69%		

[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.

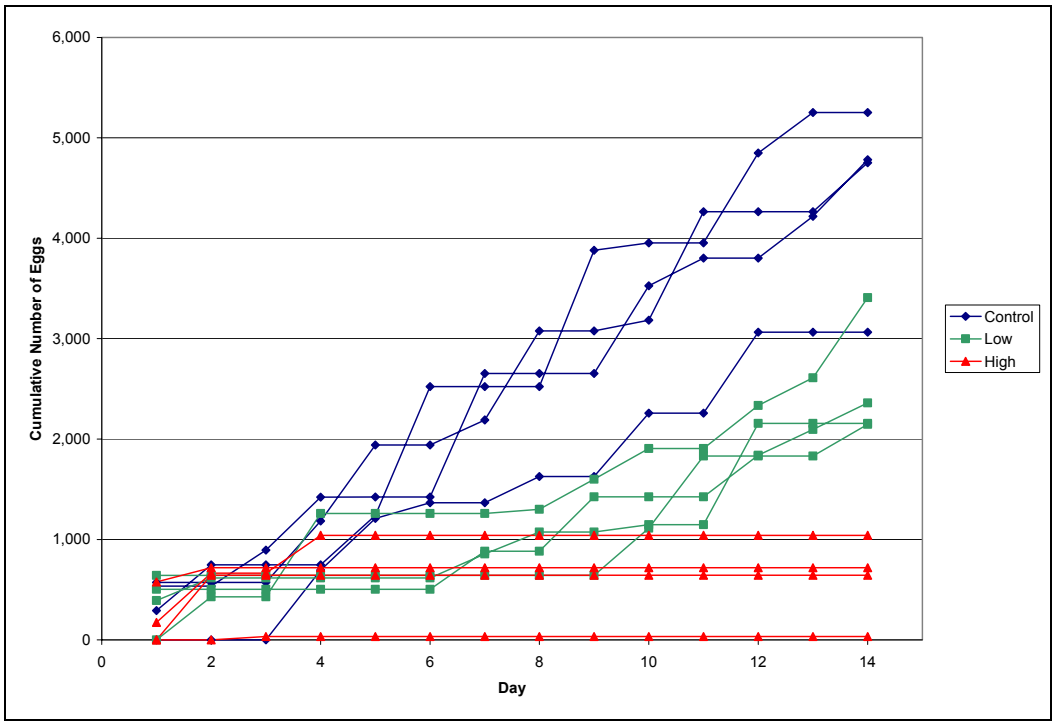

Figure 6.15. Total egg production by replicate per treatment for the EPA 14-Day fadrozole assay.

Fecundity per Female Reproductive Day—During the EPA 14-Day Fadrozole assay, the maximum number of female reproductive days was achieved for all treatments (Table 6.14). The number of eggs produced per female reproductive day in the Control treatment varied from 54.7 eggs to 93.8 eggs and from 38.3 eggs to 60.9 eggs in the Low concentration (Figure6.16). For the High concentration, the number of eggs produced per female reproductive day ranged from 0.6 eggs to 18.6 eggs. Because no fish died during the assay, the statistical results reported here are the same as those reported for total fecundity. Females in the High concentration produced significantly fewer eggs per reproductive day than those in the Control treatment and the Low concentration (Kruskal-Wallis, $H = 9.27$, $p = 0.010$, df = 2). The achieved power for this assay was 82%, and the sample size required to detect a significant difference from the Control treatment at 80% power was 4 (Table 6.14).

Table 6.14. Summary statistics and power estimates for fecundity per female reproductive day for the EPA 14-Day Fadrozole assay.

Level	Mean Number of Reproductive Days [1]	N	Mean	Stdev	CV	Achieved Power [2]	Sample Size Required [3]
control	56	4	79.7	17.2	22%	82%	4
low	56	4	45.0	10.8	24%		
high	56	4	10.9	7.5	69%		

[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.

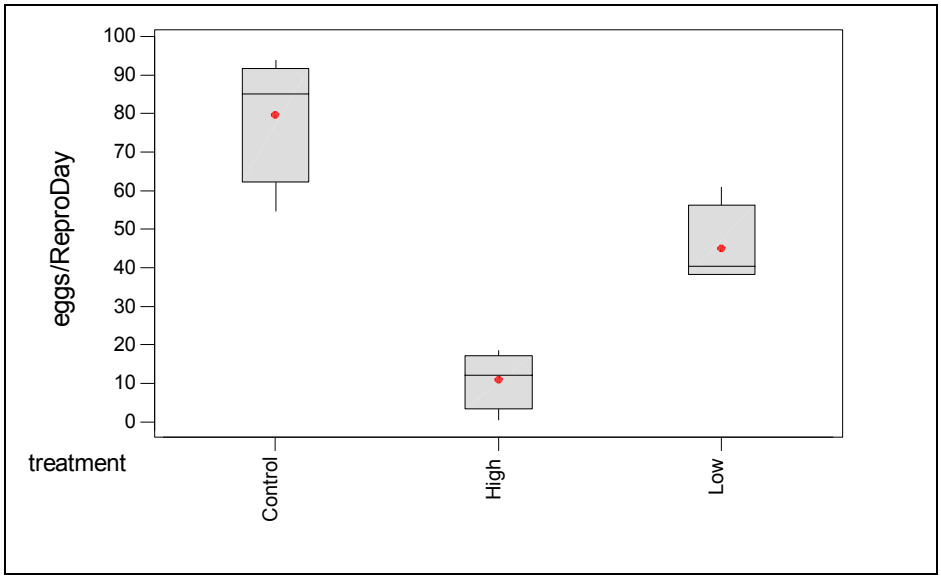

Figure 6.16. Boxplot of the number of eggs produced per female reproductive day by treatment for the EPA 14-Day Fadrozole assay. The box represents the interquartile range, whiskers represent the data range, the horizontal line is the median value, and the circle is the mean value.

Eggs on Tiles/Dishes—The mean number of eggs laid on the tiles among the treatments during the EPA 14-Day Fadrozole assay varied from 551 eggs for the High concentration to 4,015 eggs for the Control treatment (Appendix E, Table 4.3). The number of eggs on dishes ranged from 59 eggs for the High concentration to 448 eggs for the Control treatment. 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 (Appendix E, Figure 4.2). There were no significant differences in this mean proportional difference among treatments (Kruskal-Wallis, $H = 0.81$, $p = 0.668$, df = 2).

6.1.9 Fertilization Success

Total Fertilization—The total (tiles + dishes) fertilization success rates for most treatment replicates during the EPA 14-Day Fadrozole assay were high, ranging from 0.997 (Control-treatment and Low-concentration replicates) to 1.00 (replicates from all treatments) (Figure 6.17). The proportion of eggs fertilized for the High concentration was 1.00 for all replicates. No significant differences in mean fertilization success rates (Table 6.15) among treatments were detected (Kruskal-Wallis, $H = 5.93$, $p = 0.052$, df = 2). The achieved power for this assay was 48%, and the sample size required to detect a significant difference from the Control treatment at 80% power was 7 (Table 6.15).

Table 6.15. Summary statistics and power estimates for the proportion of eggs fertilized for the EPA 14-Day Fadrozole assay.

Level	N	Mean	Stdev	CV	Achieved Power [1]	Sample Size Required [2]
control	4	0.998	0.001	0.1%	48%	7
low	4	0.999	0.002	0.2%		
high	4	1.000	0	0.0%		

[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.

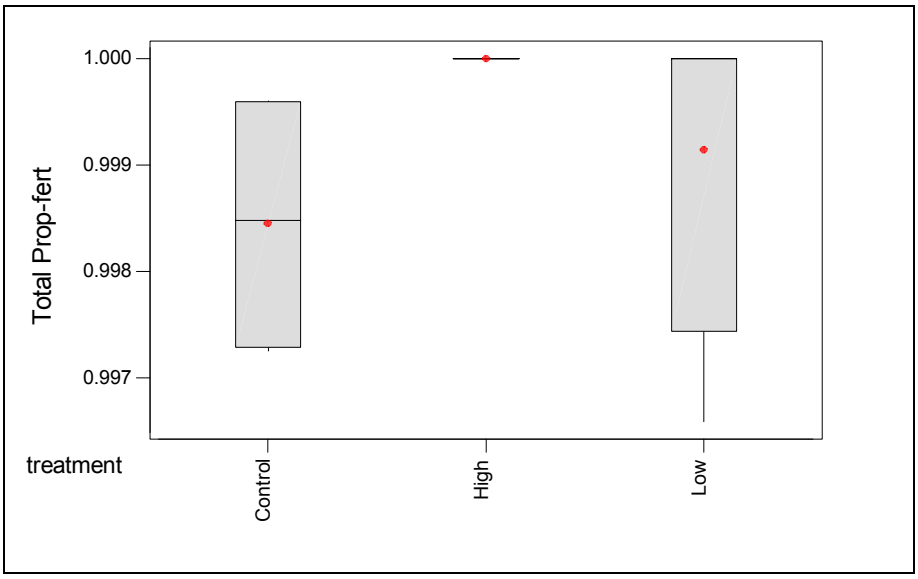

Figure 6.17. Boxplot of the proportion of eggs fertilized by treatment for the EPA 14-Day Fadrozole assay. The box represents the interquartile range, whiskers represent the data range, the horizontal line is the median value, and the circle is the mean value.

Fertilization of Eggs on Tiles and Dishes— The fertilization success rates for all treatment replicates for eggs laid on tiles during the EPA 14-Day Fadrozole assay were high, ranging from 0.996 (Low concentration replicate) to 1.00 (High- and Low-concentration replicates) (Appendix E, Figure 4.3). No significant differences in mean fertilization success rates (Appendix E, Table 4.4) among treatments were detected (Kruskal-Wallis, $H = 5.93$, $p = 0.052$, df = 2). The fertilization success rates for all treatment replicates for eggs laid on dishes during the assay were high, ranging from 0.995 (Control-treatment

replicate) to 1.00 (several replicates, including all treatments) (Appendix E, Figure 4.4). No significant differences in mean fertilization success rates (Appendix E, Table 4.4) among treatments were detected (Kruskal-Wallis, $H = 2.00$, $p = 0.368$, df = 2).

6.1.10 Hatchability and Larval Development

Eggs were collected during the 14-Day pre-exposure period for the evaluation of hatchability. The proportion of fertilized eggs that hatched ranged from 0.96 to 1.00 for tanks that would be used for the Control treatment, from 0.78 to 0.96 for tanks that would constitute the Low concentration, and from 0.98 to 1.00 for tanks that would be assigned to the High concentration during the 14-Day exposure assay. Among the tanks evaluated during the pre-exposure period, but that were not used in the 14-Day assay, the mean proportion of fertilized eggs that hatched was 0.98. There was a significant difference detected among treatments in the proportion of eggs that hatched (Kruskal-Wallis, $H = 8.42$, $p = 0.038$, df = 3). The proportion of eggs that hatched in tanks destined for the Low concentration treatment was significantly less than that for eggs laid in tanks that would be used for the High concentration.

Eggs were collected during the EPA 14-Day Fadrozole 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 0.84 to 1.00 for the Low concentration (Figure 6.18). No eggs were available to evaluate hatchability for the High concentration because the evaluation was started on Day 12 of the assay and no eggs were laid in those tanks after Day 4. There were no significant differences between the Control treatment and the Low concentration in the proportion of eggs that hatched (Kruskal-Wallis, $H = 0.05$, $p = 0.821$, 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 6.16).

Table 6.16. Summary statistics and power estimates for the proportion of fertile eggs that hatched for the EPA 14-Day Fadrozole assay.

Level	N	Mean	Stdev	CV	Achieved Power [1]	Sample Size Required [2]
control	6	0.973	0.031	3%	5%	>1000
low	7	0.959	0.062	6%		
high	0	–	–	–		

[1] Calculated from arcsine square-root transformed data; with sample size = 6.
[2] To detect a significant difference from control treatment based on maximum achieved absolute difference; calculated on arcsine square-root transformed data.

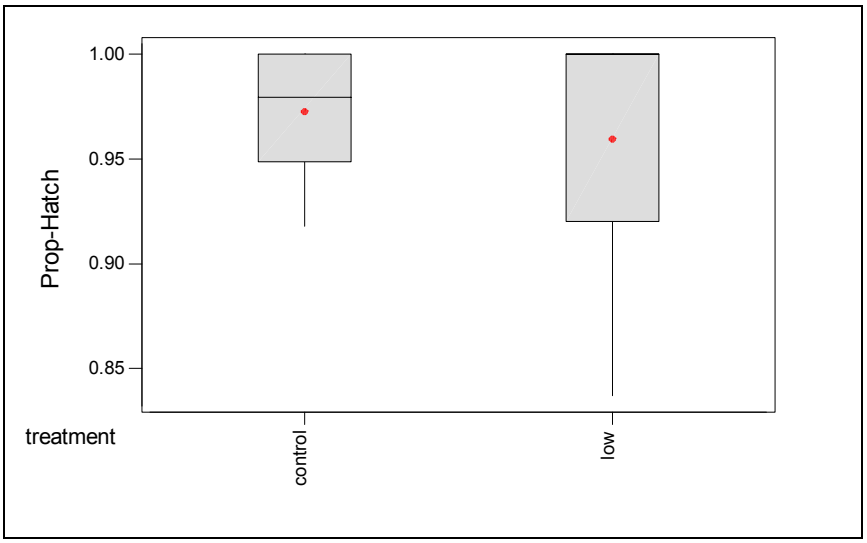

Figure 6.18. Boxplot of the proportion of fertile eggs that hatched by treatment for the EPA 14-Day Fadrozole assay. The box represents the interquartile range, whiskers represent the data range, the horizontal line is the median value, and the circle is the mean value.

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) was 0.95 (sd = 0.05) for all tanks that would be used in the Control treatment. The mean proportion of normal larvae in the remaining treatments was 0.97 (sd = 0.03) in the Low concentration and 0.98 (sd = 0.02) in the High concentration. Among the tanks evaluated during the pre-exposure period, but that were not used in the 21-Day assay, the mean proportion of normal larvae was 0.97 (sd = 0.01). There were no significant differences among treatments in the proportion of eggs that hatched (Kruskal-Wallis, $H = 1.38$, $p = 0.710$, df = 3).

Eggs were collected during the EPA 14-Day Fadrozole assay for the evaluation of larval development. The proportion of larvae that developed normally (i.e., that showed no morphological abnormalities) ranged from 0.74 to 1.00 for the Control treatment and from 0.92 to 1.00 for the Low concentrations (Figure 6.19). There were no larvae available form the High concentration for the evaluation of development. There were no significant differences between the Control treatment and the Low concentration in the proportion of eggs that hatched (Kruskal-Wallis, $H = 0.08$, $p = 0.772$, df = 1). 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 402 (Table 6.17).

Table 6.17. Summary statistics and power estimates for the proportion of normal larvae for the EPA 14-Day Fadrozole assay.

Level	N	Mean	Stdev	CV	Achieved Power [1]	Sample Size Required [2]
control	6	0.916	0.123	13%	6%	402
low	7	0.970	0.026	3%		
high	0	–	–	–		

[1] Calculated from arcsine square-root transformed data; with sample size = 6.
[2] To detect a significant difference from control treatment based on maximum achieved absolute difference; calculated on arcsine square-root transformed data.

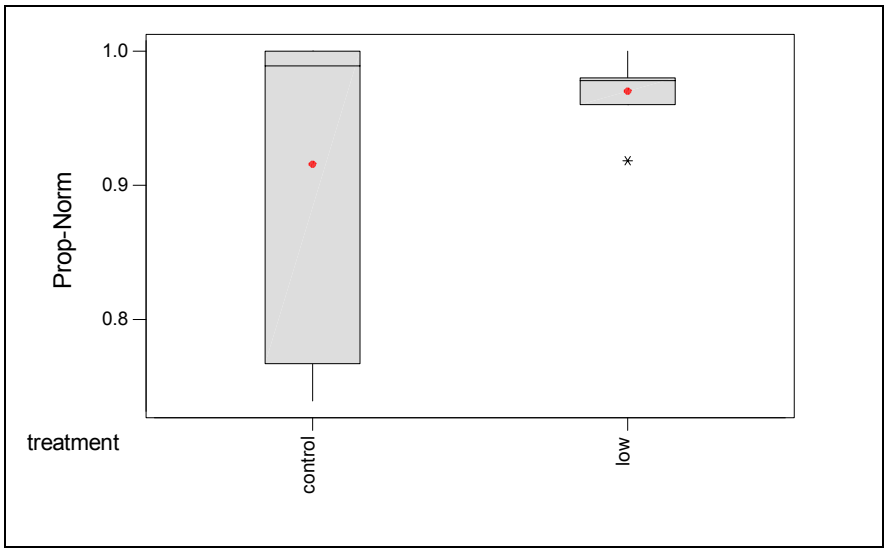

Figure 6.19. Boxplot of the proportion of normal larvae by treatment for the EPA 14-Day Fadrozole assay. The box represents the interquartile range, whiskers represent the data range, the horizontal line is the median value, the circle is the mean value, and the asterisk represents a probable outlier.

6.1.11 Body Weight

The body weight of females used in the EPA 14-Day Fadrozole assay ranged from 1.5 g to 3.6 g. A significant difference in mean body weight among treatments was detected (Kruskal-Wallis, $H = 12.33$, $p = 0.002$, df = 2). The mean body weight of females exposed to the High concentration was significantly greater than that of females in the Control treatment. The body weight of males used in the EPA 14-Day Fadrozole assay ranged from 2.5 g to 7.5 g. There were no significant differences in mean body weight among treatments (Kruskal-Wallis, $H = 2.90$, $p = 0.235$, df = 2).

6.2 EPA 21-Day Assay for Fadrozole

The EPA 21-Day Fadrozole assay was conducted from March 10, 2003, to March 25, 2003 (pre-exposure assay), and from March 25, 2003, to April 15, 2003 (exposure assay).

6.2.1 Survival

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

6.2.2 Vitellogenin

Vitellogenin concentrations in Control treatment females used during the EPA 21-Day fadrozole assay ranged from 2,667,000 ng/mL to 10,890,000 ng/mL (Figure 6.20). Among females exposed to the two fadrozole concentrations, vitellogenin concentrations ranged from 0 ng/mL (not detected) to 2,768,500 ng/mL. Significant differences in the mean vitellogenin concentration per treatment (Table 6.18) were detected (Kruskal-Wallis, $H = 39.41$, $p = <0.001$, df = 2). Vitellogenin concentrations in Control treatment females were significantly greater than those in females exposed to the Low and High fadrozole

concentrations. Additionally, vitellogenin concentrations in females from the Low concentration were greater than those in females from the High concentration. The achieved power for this endpoint was 100%, and the sample size required to detect a significant difference from the Control treatment at 80% power was 3 (Table 6.18).

Table 6.18. Summary statistics and power estimates for female vitellogenin concentrations (ng/mL) for the EPA 21-Day Fadrozole assay.

Level	N	Mean	Stdev	CV	Achieved Power [1]	Sample Size Required [2]
control	16	5,949,000	2,252,237	38%	100%	3
low	16	1,197,869	754,539	63%		
high	16	4,914	10,679	217%		

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

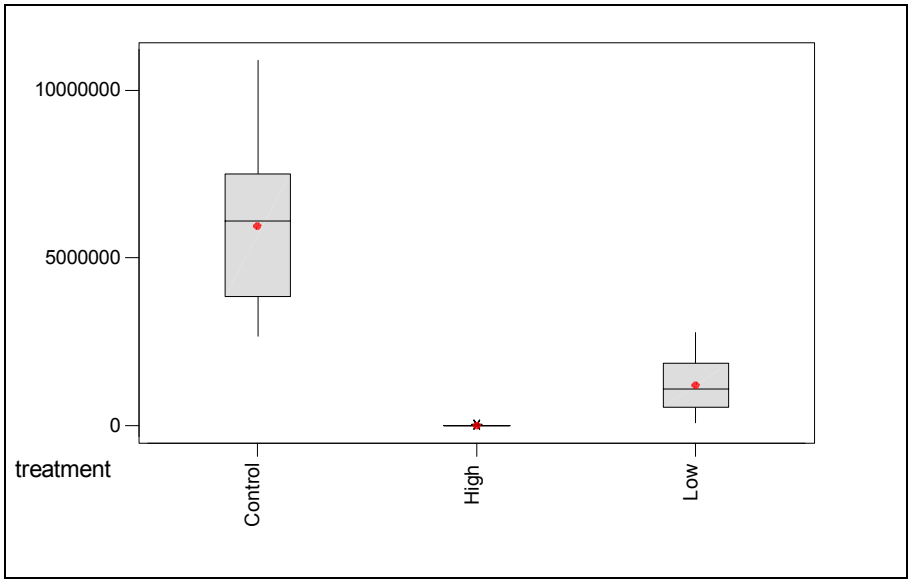

Figure 6.20. Boxplot of female vitellogenin concentration (ng/mL) by treatment for the EPA 21-Day Fadrozole assay. The box represents the interquartile range, whiskers represent the data range, the horizontal line is the median value, and the circle is the mean value.

Vitellogenin concentrations in Control treatment males used during the EPA 21-Day fadrozole assay ranged from 168 ng/mL to 8,288 ng/mL (Figure 6.21). Among males exposed to the two fadrozole concentrations, vitellogenin concentrations ranged from 14 ng/mL to 15,097 ng/mL. No significant differences in the mean vitellogenin concentration per treatment (Table 6.19) were detected (Kruskal-Wallis, $H = 0.85$, $p = 0.655$, 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 67 (Table 6.19).

Table 6.19. Summary statistics and power estimates for male vitellogenin concentrations (ng/mL) for the EPA 21-Day Fadrozole assay.

Level	N	Mean	Stdev	CV	Achieved Power [1]	Sample Size Required [2]
control	8	2,334	2,639	113%	13%	67
low	8	3,576	5,554	155%		
high	8	2,074	2,792	135%		

[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.

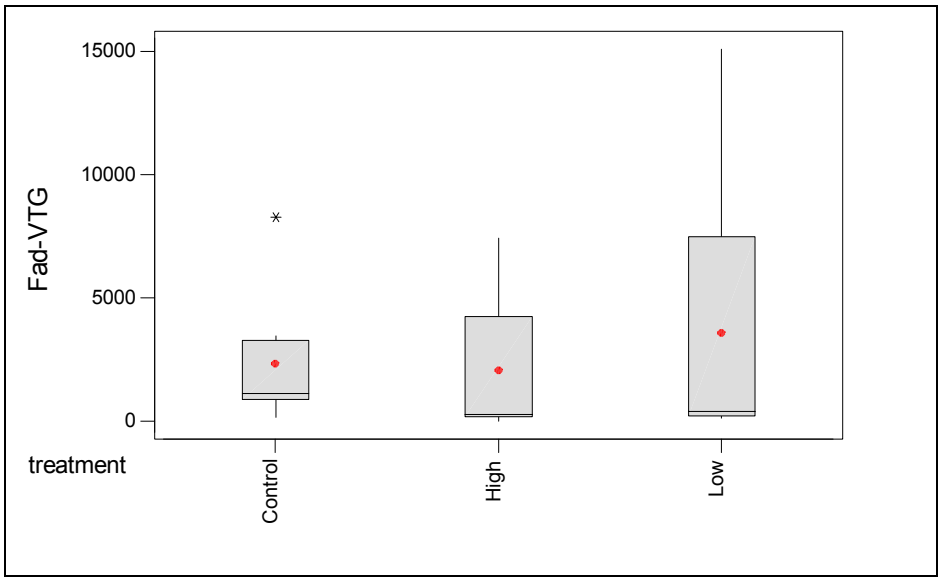

Figure 6.21. Boxplot of male vitellogenin concentration (ng/mL) by treatment for the EPA 21-Day Fadrozole assay. The box represents the interquartile range, whiskers represent the data range, the horizontal line is the median value, the circle is the mean value, and the asterisk represents a probable outlier.

6.2.3 Appearance / Secondary Sex Characteristics

All of the females used during the EPA 21-Day Fadrozole assay exhibited typical female morphology (no fat pad, no tubercles, no vertical banding, ovipositor present).

All of the males used during the EPA 21-Day Fadrozole assay exhibited typical male morphology (fat pads, tubercles, vertical banding, no ovipositor present).

6.2.4 Gonadosomatic Index

The range of GSI values calculated for females in the all treatments varied from three- to seven-fold (Figure 6.22), and the overall variability within the treatment was moderate to high (CVs = 28%–31%; Table 6.20). The highest value (GSI = 22.2) was obtained for a female exposed to the High-fadrozole concentration. One female exposed to the Low-fadrozole concentration had a GSI value of 21.3. A significant difference in the mean GSI value per treatment (Table 6.20) was detected (Kruskal-Wallis, H =

10.50, $p = 0.005$, df = 2). The mean GSI value for females from the High concentration was greater than that for females from the Control treatment. The achieved power for this endpoint was 80%, and the sample size required to detect a significant difference from the Control treatment at 80% power was 16 (Table 6.20).

Table 6.20. Summary statistics and power estimates for female gonadosomatic index data for the EPA 21-Day Fadrozole assay.

Level	N	Mean	Stdev	CV	Achieved Power [1]	Sample Size Required [2]
control	16	10.3	2.9	28%	80%	16
low	16	13.3	4.1	31%		
high	16	15.2	4.8	31%		

[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.

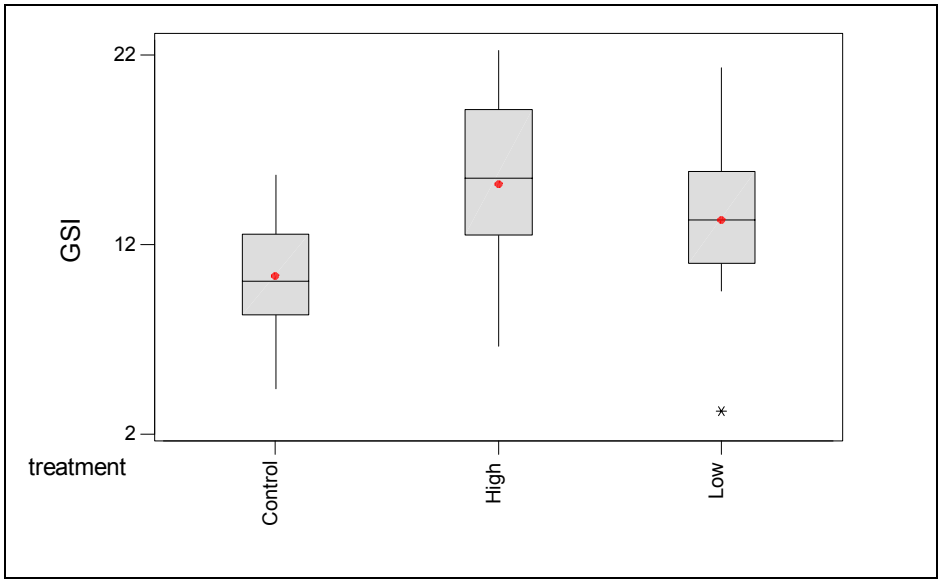

Figure 6.22. Boxplot of female GSI by treatment for the EPA 21-Day Fadrozole assay. The box represents the interquartile range, whiskers represent the data range, the horizontal line is the median value, the circle is the mean value, and the asterisk represents a probable outlier.

The range of most GSI values calculated for males during the EPA 21-Day Fadrozole assay, was small, ranging from 0.7 to 1.8 (Figure 6.23), which approximates the typical range for reproductively-active male fathead minnows. One male in the Low concentration had a GSI value of 2.3, attributable to its relatively high gonad weight of 0.10 g and moderate body size (4.5 g). The three large males did not have abnormally high GSI values (0.8–1.6), which reflects their proportionally high gonad weights (0.07 g–0.13 g). There were significant differences in mean GSI values (Table 6.21) among treatments (Kruskal-Wallis, $H = 10.19$, $p = 0.006$, df = 2). The mean GSI values for fish from the High and Low concentrations were greater than the mean value for fish from the Control treatment. The achieved power

for this endpoint was 87%, and the sample size required to detect a significant difference from the Control treatment at 80% power was 7 (Table 6.21).

Table 6.21. Summary statistics and power estimates for male gonadosomatic index data for the EPA 21-Day Fadrozole assay.

Level	N	Mean	Stdev	CV	Achieved Power [1]	Sample Size Required [2]
control	8	1.00	0.20	20%	87%	7
low	8	1.37	0.45	33%		
high	8	1.52	0.18	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.

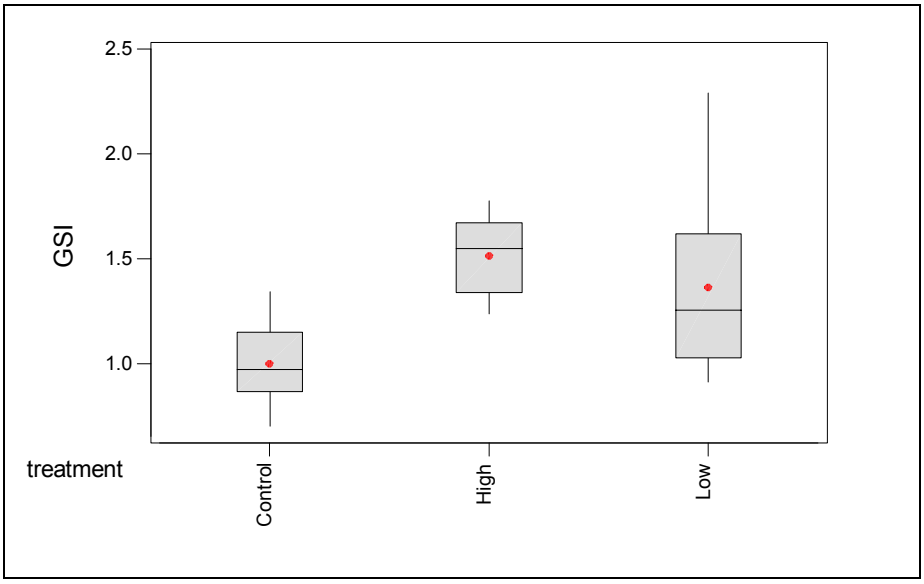

Figure 6.23. Boxplot of male GSI by treatment for the EPA 21-Day Fadrozole assay. The box represents the interquartile range, whiskers represent the data range, the horizontal line is the median value, and the circle is the mean value.

6.2.5 Female Gonad Histology

Histological analyses were conducted on the ovaries of 48 females exposed to fadrozole 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 Fadrozole assay revealed significant differences among treatments (Kruskal-Wallis, $H = 34.09$, $p = <0.001$, df = 2). The mean ovarian stage of females exposed to the High concentration was significantly less than that of females from the Control treatment or the Low concentration.

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 from the Control treatment and Low concentration ranged from Stage 1a to Stage 5 (see Methods for a description of the stages (Figure 6.24). Ova from fish from the High concentration ranged from Stage 1a to Stage 4. Variability within treatments for each stage, particularly for Stage 5, was very high as indicated by CVs that ranged as high as 400% (Table 6.22). Statistical analyses showed that within each of the five developmental stages, except Stage 5, there were significant differences among treatments in the proportion of cells occurring in the stage (Table 6.22). The proportion of ova in Stage 1a was significantly greater in the Control-treatment females than in High-concentration females. The proportion of ova in Stage 1b was significantly greater in the Control-treatment females than in High- or Low-concentration females. The proportion of ova in Stage 2 was significantly greater in the Control-treatment and High-concentration females than in Low-concentration females. The proportion of ova in Stage 3 was significantly greater in the High-concentration females than in Control-treatment or Low-concentration females. The proportion of ova in Stage 4 was significantly less in the High-concentration females than in Control-treatment or Low-concentration females.

Table 6.22. Descriptive statistics of the proportion of ovarian cells in each developmental stage for females from the EPA 21-Day Fadrozole 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	0.064	0.020	31%	0.049	0.026	53%	0.037	0.020	54%	10.43	0.005*
1b	0.246	0.059	24%	0.180	0.067	37%	0.170	0.066	39%	9.75	0.008*
2	0.230	0.030	13%	0.180	0.034	19%	0.211	0.053	25%	13.55	0.001*
3	0.243	0.055	23%	0.305	0.105	34%	0.425	0.148	35%	14.94	0.001*
4	0.180	0.118	66%	0.240	0.109	46%	0.015	0.018	115%	28.14	<0.001**
5	0.006	0.017	288%	0.000	0.001	400%	0	0	--	2.18	0.337

* $p < 0.01$

** $p < 0.001$

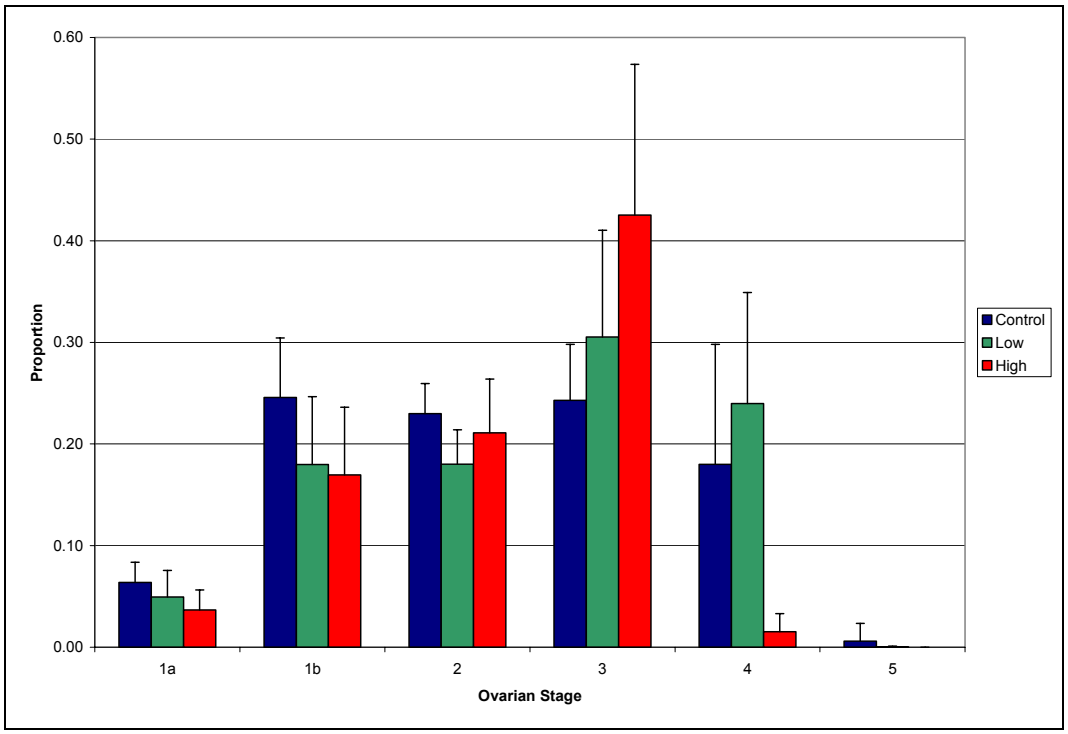

Figure 6.24. Frequency histogram showing the quantitative developmental staging of ovaries for each treatment of the EPA 21-Day Fadrozole assay. For each treatment, the columns represent the grand mean proportion of cells in each stage and the bars represent the standard deviation.

Atretic Follicles—The mean proportion of atretic follicles per 300 follicles (counted per fish) ranged from 0.008 follicles for females in the Control treatment to 0.126 follicles for females in the High concentration (Figure 6.25). One fish from the High concentration 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, $H = 24.42$, $p < 0.001$, df = 2). The proportion of atretic follicles per 300 follicles in fish from the High concentration was greater than those of fish from the remaining two treatments. Additionally, the proportion of atretic follicles per 300 follicles in fish from the Low concentration was greater than that in fish from the Control treatment.

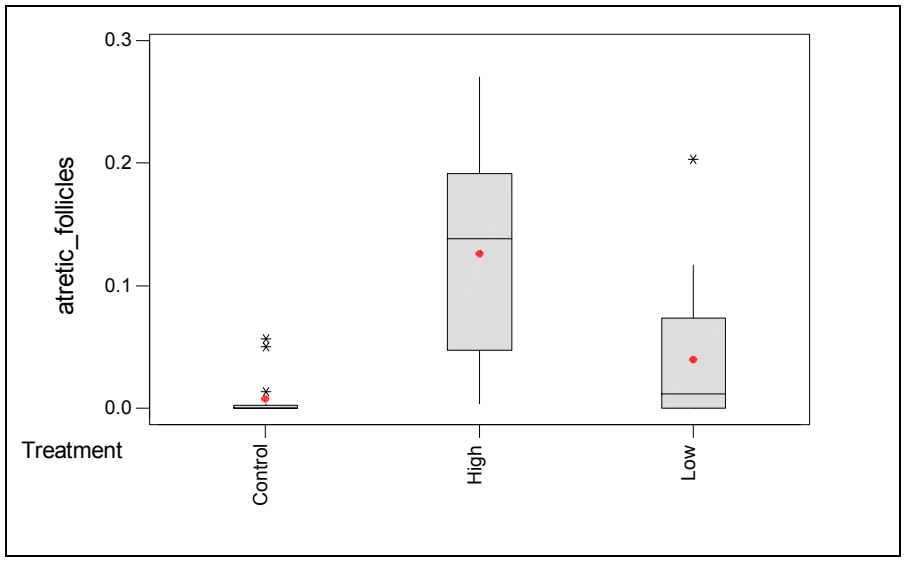

Figure 6.25. Boxplot of the proportion of atretic follicles per 300 follicles by treatment for the EPA 21-Day Fadrozole assay. The box represents the interquartile range, whiskers represent the data range, the horizontal line is the median value, the circle is the mean value, and asterisks represent probable outliers.

Corpora Lutea—The mean proportion of corpora lutea per 300 follicles (counted per fish) ranged from 0.006 for females in the Low concentration to 0.024 for females in the Control treatment (Figure 6.26). Variability within each treatment was very high, ranging from 132% for the Control treatment to 388% for the High concentration. Despite the high variability, there was a significant difference in the mean proportion of corpora lutea among treatments (Kruskal-Wallis, $H = 7.65$, $p = 0.022$, df = 2). The proportion of corpora lutea in fish from the Control treatment was significantly greater than that in fish from the High concentration.

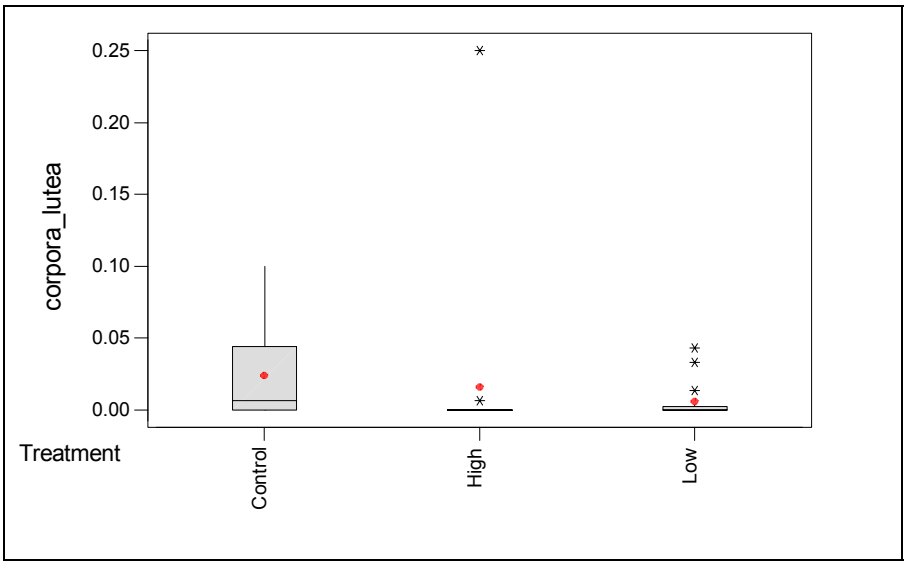

Figure 6.26. Boxplot of the proportion of corpora lutea per 300 follicles by treatment for the EPA 21-Day Fadrozole assay. The box represents the interquartile range, whiskers represent the data range, the horizontal line is the median value, the circle is the mean value, and asterisks represent probable outliers.

Observations—One fish from the Low concentration were observed to have most of the atretic follicles at the margins of the ovaries. One fish from the High concentration were observed to have inflammatory cells in the ovary. Five other fish from the High concentration had atretic follicles that were Stage 3 (one fish) or Stage 4 (four fish) cells.

6.2.6 Male Gonad Histology

General Testes Staging—Testes from 24 males exposed to fadrozole during the EPA 21-Day Fadrozole 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 (88 fields) or Stage 5 (8 fields) development. Ninety-four of the 96 microscopic fields examined in the 8 Low-concentration treatment males showed Stage 4 (85 fields) or Stage 5 (9 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 (69 fields) or Stage 5 (27 fields) development. Statistical analysis of the mean staging from 12 microscopic fields per fish revealed no significant differences among treatments (Kruskal-Wallis, $H = 3.92$, $p = 0.141$, 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 6.27). Variability within treatments for each stage was very high as indicated by CVs that ranged as high as 198% (Table 6.23). Statistical analyses showed that there were significant differences among treatments in the proportion of cells in any of the developmental stages (Table 6.23). The mean proportion of cells in developmental Stage 3a was greater in fish from the Low concentration than in fish from the Control treatment. The observed proportions of cells in developmental Stage 3b ranked higher in fish from the Low concentration and the Control treatment than in fish from the High concentration. The mean proportion of cells in developmental Stage

5 was greater in fish from the Low concentration than in fish from the High concentration. Therefore, there did not appear to be an effect on testicular developmental stage associated with fadrozole dose.

Table 6.23. Descriptive statistics of the proportion of testes cells in each developmental stage for males from the EPA 21-Day Fadrozole 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	0.004	0.006	147%	0.002	0.003	151%	0.001	0.002	198%	1.30	0.523
2b	0.019	0.016	84%	0.014	0.010	68%	0.010	0.008	80%	1.26	0.532
3a	0.092	0.054	59%	0.204	0.079	39%	0.141	0.091	64%	6.52	0.038*
3b	0.292	0.110	38%	0.274	0.082	30%	0.176	0.079	45%	6.18	0.046*
4	0.235	0.093	40%	0.223	0.048	22%	0.162	0.076	47%	3.51	0.173
5	0.358	0.139	39%	0.283	0.135	48%	0.510	0.199	39%	6.50	0.039*

* $p < 0.05$

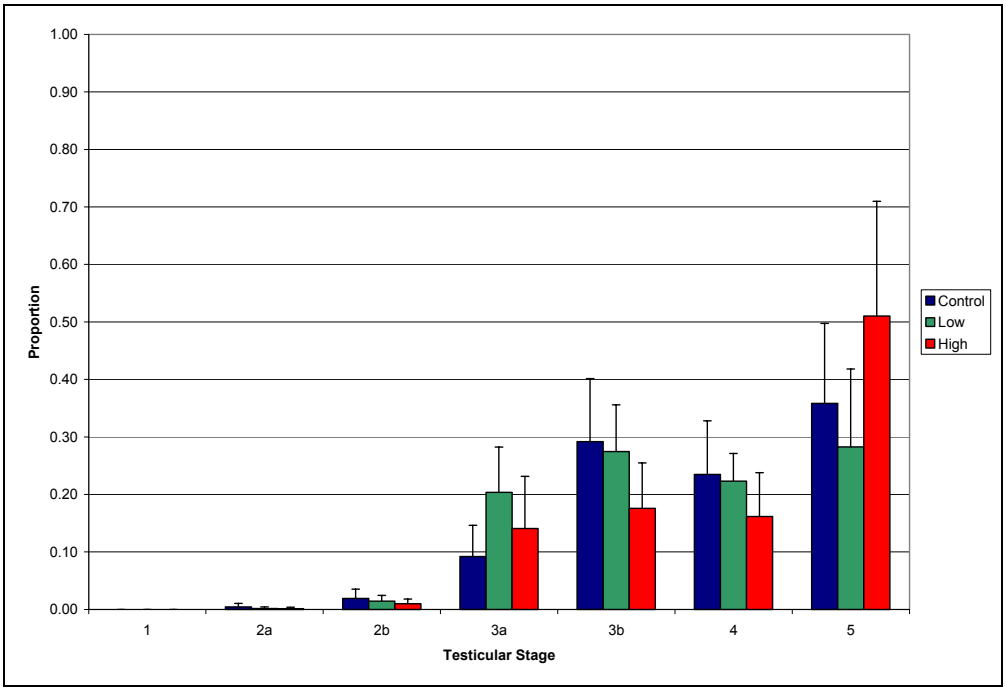

Figure 6.27. Frequency histogram showing the quantitative developmental staging of testes for each treatment of the EPA 21-Day Fadrozole assay. For each treatment, the columns represent the grand mean proportion of cells in each stage and the bars represent the standard deviation.

Tubule Diameter—The average diameter of the seminiferous tubules of males from the Control treatment ranged from 104.2 µm to 136.1 µm (Figure 6.28). Tubule diameters of males from the two test concentrations ranged from 101.1 µm to 202.2 µm. A significant difference in the mean tubule diameter per treatment (Table 6.24) was detected (Kruskal-Wallis, $H = 12.89$, $p = 0.002$, df = 2). The mean tubule

diameter in males form the High concentration was greater than those in males from the Low concentration and the Control treatment. The achieved power for this endpoint was 97%, and the sample size required to detect a significant difference from the Control treatment at 80% power was 5 (Table 6.24).

Table 6.24. Summary statistics and power estimates for male seminiferous tubule diameter data for the EPA 21-Day Fadrozole assay.

Level	N	Mean	Stdev	CV	Achieved Power [1]	Sample Size Required [2]
control	8	115.0	10.8	9%	97%	5
low	8	123.5	12.7	10%		
high	8	151.8	25.0	16%		

[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.

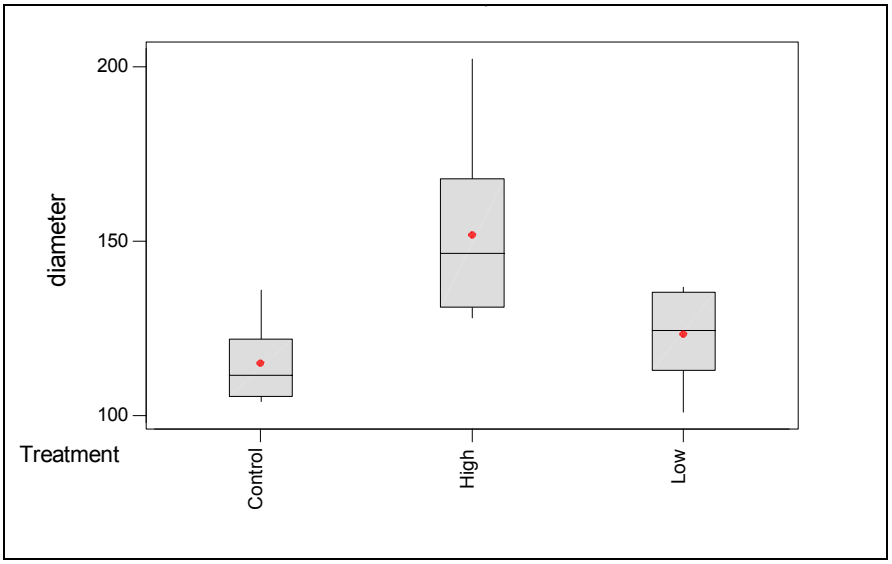

Figure 6.28. Boxplot of male seminiferous tubule diameter (μm) by treatment for the EPA 21-Day Fadrozole assay. The box represents the interquartile range, whiskers represent the data range, the horizontal line is the median value, and the circle is the mean value.

Observations—No Sertoli cell proliferation was observed. Four males from the High concentration were found to have focal proliferation of Leydig cells. One male from the High concentration had focal proliferation of secondary spermatogonia. Four males from the High concentration had multifocal proliferation of secondary spermatogonia. In two of these fish, the proliferation extended into the lumen and included Stages 3b, 4, and 5 cells. In one fish, the proliferation extended into mature tubules. No testicular atrophy was recorded and no ovatestes were observed for any treatment.

6.2.7 Plasma Steroid Concentrations

Estradiol—Estradiol concentrations in Control-treatment females used during the EPA 21-Day fadrozole assay ranged from 372 pg/mL to 5,847 pg/mL (Figure 6.29). Among females exposed to the Low-fadrozole concentrations, estradiol concentrations ranged from 401 pg/mL to 2,587 pg/mL. No females from the High concentration were available for estradiol determination. A significant difference in the mean estradiol concentration per treatment (Table 6.25) was detected (Kruskal-Wallis, $H = 5.16$, $p = 0.023$, df = 2). The mean estradiol concentration in females from the Low concentration was less than that in females from the Control treatment. 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 17 (Table 6.25).

Table 6.25. Summary statistics and power estimates for female estradiol concentrations (pg/mL) for the EPA 21-Day Fadrozole assay.

Level	N	Mean	Stdev	CV	Achieved Power [1]	Sample Size Required [2]
control	16	2,861	1,492	52%	40%	17
low	7	1,343	756	56%		
high	0	–	–	–		

[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.

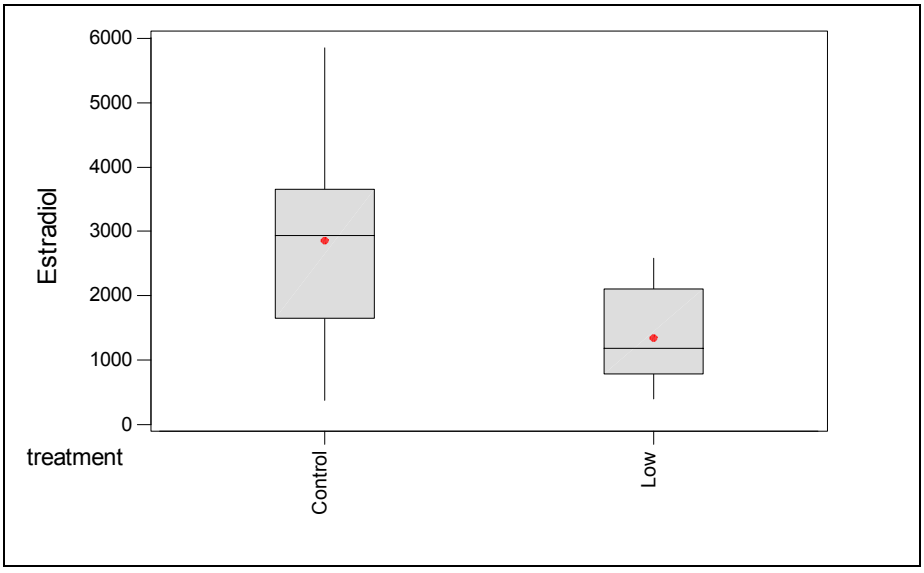

Figure 6.29. Boxplot of female estradiol concentration (pg/mL) by treatment for the EPA 21-Day Fadrozole assay. The box represents the interquartile range, whiskers represent the data range, the horizontal line is the median value, and the circle is the mean value.

Estradiol concentrations in Control treatment males used during the EPA 21-Day Fadrozole assay ranged from 0 pg/mL (not detected) to 193 pg/mL (Figure 6.29). Estradiol was not detected in males exposed to

the Low-fadrozole concentrations (4 individuals). No males from the High concentration were available for estradiol determination (Table 6.26).

Table 6.26. Summary statistics and power estimates for male estradiol concentrations (pg/mL) for the EPA 21-Day Fadrozole assay.

Level	N	Mean	Stdev	CV	Achieved Power [1]	Sample Size Required [1]
control	8	70	96	138%	–	–
low	4	0	0	–		
high	0	–	–	–		

[1] Not calculated.

Testosterone—Testosterone concentrations in Control-treatment females used during the EPA 21-Day Fadrozole assay ranged from 199 pg/mL to 3,828 pg/mL (Figure 6.30). Among females exposed to the two fadrozole concentrations, testosterone concentrations ranged from 561 pg/mL to 7,275 pg/mL. Significant differences in the mean testosterone concentration per treatment (Table 6.27) were detected (Kruskal-Wallis, $H = 13.68$, $p = 0.001$, df = 2). The mean testosterone concentration in females from the Control treatment was less than those in females from the Low and High concentrations. The achieved power for this endpoint was 93%, and the sample size required to detect a significant difference from the Control treatment at 80% power was 10 (Table 6.27).

Table 6.27. Summary statistics and power estimates for female testosterone concentrations (pg/mL) for the EPA 21-Day Fadrozole assay.

Level	N	Mean	Stdev	CV	Achieved Power [1]	Sample Size Required [2]
control	16	1,096	938	86%	93%	10
low	13	1,977	834	42%		
high	16	2,928	1,951	67%		

[1] Calculated from natural log transformed data; with sample size = 13.
[2] To detect a significant difference from control treatment based on maximum achieved absolute difference; calculated on natural log transformed data.

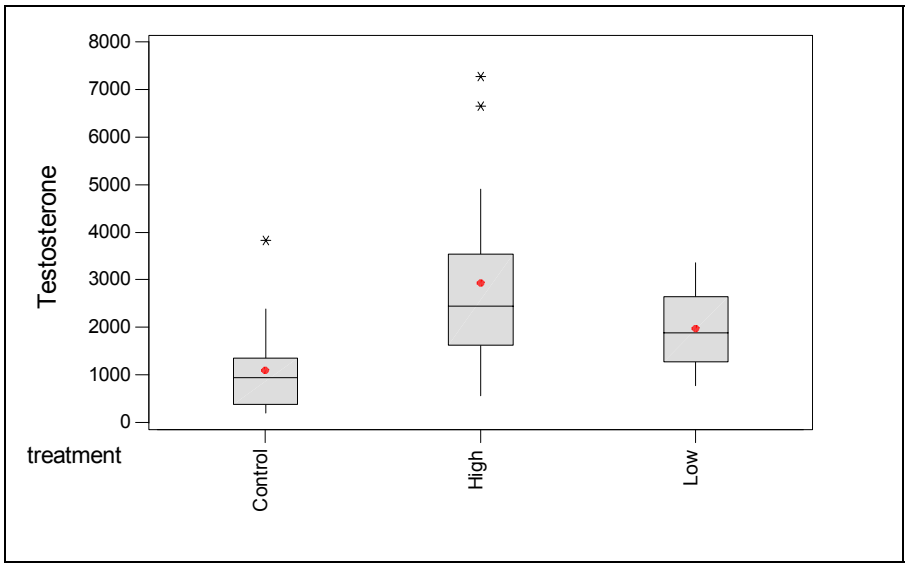

Figure 6.30. Boxplot of female testosterone concentration (pg/mL) by treatment for the EPA 21-Day Fadrozole assay. The box represents the interquartile range, whiskers represent the data range, the horizontal line is the median value, the circle is the mean value, and the asterisks represent probable outliers.

Testosterone concentrations in Control treatment males used during the EPA 21-Day Fadrozole assay ranged from 953 pg/mL to 3,926 pg/mL (Figure 6.31). Among males exposed to the two fadrozole concentrations, testosterone concentrations ranged from 3,282 pg/mL to 15,262 pg/mL. Significant differences in the mean testosterone concentration per treatment (Table 6.28) were detected (Kruskal-Wallis, $H = 14.92$, $p = 0.001$, df = 2). The mean testosterone concentration in males from the Control treatment was less than those in males from the Low and High concentrations. The achieved power for this endpoint was 100%, and the sample size required to detect a significant difference from the Control treatment at 80% power was 4 (Table 6.28).

Table 6.28. Summary statistics and power estimates for male testosterone concentrations (pg/mL) for the EPA 21-Day Fadrozole assay.

Level	N	Mean	Stdev	CV	Achieved Power [1]	Sample Size Required [2]
control	8	2,208	1,116	51%	100%	4
low	8	6,593	1,600	24%		
high	8	9,030	4,252	47%		

[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.

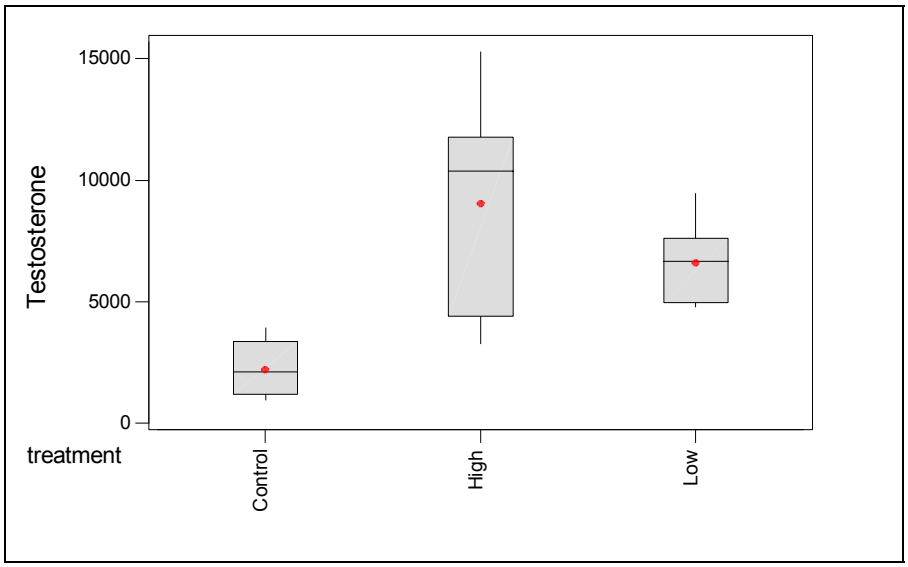

Figure 6.31. Boxplot of male testosterone concentration (pg/mL) by treatment for the EPA 21-Day Fadrozole assay. The box represents the interquartile range, whiskers represent the data range, the horizontal line is the median value, and the circle is the mean value.

11-ketotestosterone—11-ketotesosterone concentrations in Control-treatment females used during the EPA 21-Day Fadrozole assay ranged from 0 pg/mL (not detected) to 594 pg/mL (Figure 6.32). However, 11-ketotestosterone was only detected in 2 of the 16 females from the Control treatment that were analyzed. Among females exposed to the two fadrozole concentrations, testosterone concentrations ranged from 0 pg/mL (not detected) to 1,966 pg/mL. A significant difference in the mean 11-ketotestosterone concentration per treatment (Table 6.29) was detected (Kruskal-Wallis, $H = 9.05$, $p = 0.011$, df = 2). The mean 11-ketotestosterone concentration in females from the Control treatment was less than those in females from the High concentration. However, the achieved power for this endpoint was only 48%, because of the high variability in the Control response. The sample size required to detect a significant difference from the Control treatment at 80% power was 22 (Table 6.29).

Table 6.29. Summary statistics and power estimates for female 11-ketotestosterone concentrations (pg/mL) for the EPA 21-Day Fadrozole assay.

Level	N	Mean	Stdev	CV	Achieved Power [1]	Sample Size Required [2]
control	16	71	193	274%	48%	22
low	11	369	377	102%		
high	16	515	593	115%		

[1] Calculated from natural log transformed data; with sample size = 13.
[2] To detect a significant difference from control treatment based on maximum achieved absolute difference; calculated on natural log transformed data.

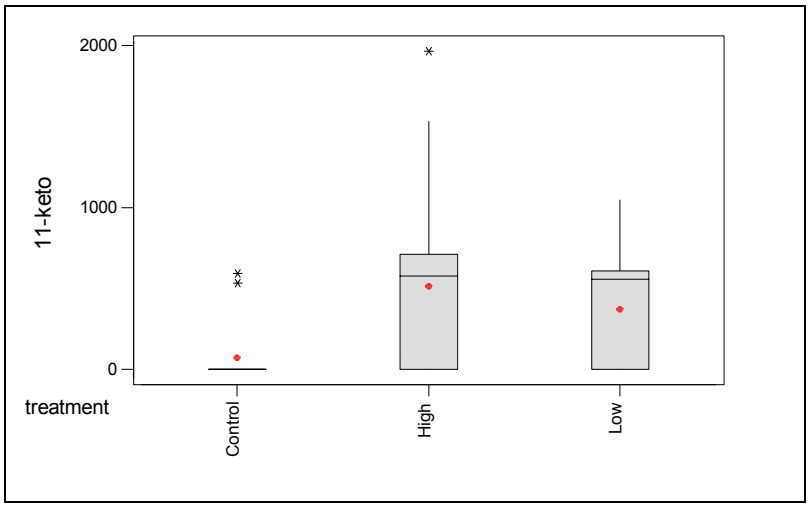

Figure 6.32. Boxplot of female 11-ketotestosterone concentration (pg/mL) by treatment for the EPA 21-Day Fadrozole assay. The box represents the interquartile range, whiskers represent the data range, the horizontal line is the median value, the circle is the mean value, and the asterisks represent probable outliers.

11-ketotesosterone concentrations in Control treatment males used during the EPA 21-Day Fadrozole assay ranged from 4,697 pg/mL to 43,298 pg/mL (Figure 6.33). Among males exposed to the two fadrozole concentrations, 11-ketotesosterone concentrations ranged from 14,292 pg/mL to 192,900 pg/mL. Significant differences in the mean 11-ketotesosterone concentration per treatment (Table 6.30) were detected (Kruskal-Wallis, $H = 14.89$, $p = 0.001$, df = 2). The mean 11-ketotestosterone concentration in males from the Control treatment was less than those in males from the Low and High concentrations. The achieved power for this endpoint was 100%, and the sample size required to detect a significant difference from the Control treatment at 80% power was 4 (Table 6.30).

Table 6.30. Summary statistics and power estimates for male 11-ketotesosterone concentrations (pg/mL) for the EPA 21-Day Fadrozole assay.

Level	N	Mean	Stdev	CV	Achieved Power [1]	Sample Size Required [2]
control	8	19,770	15,109	76%	100%	4
low	8	130,695	47,538	36%		
high	8	77,822	54,034	69%		

[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.

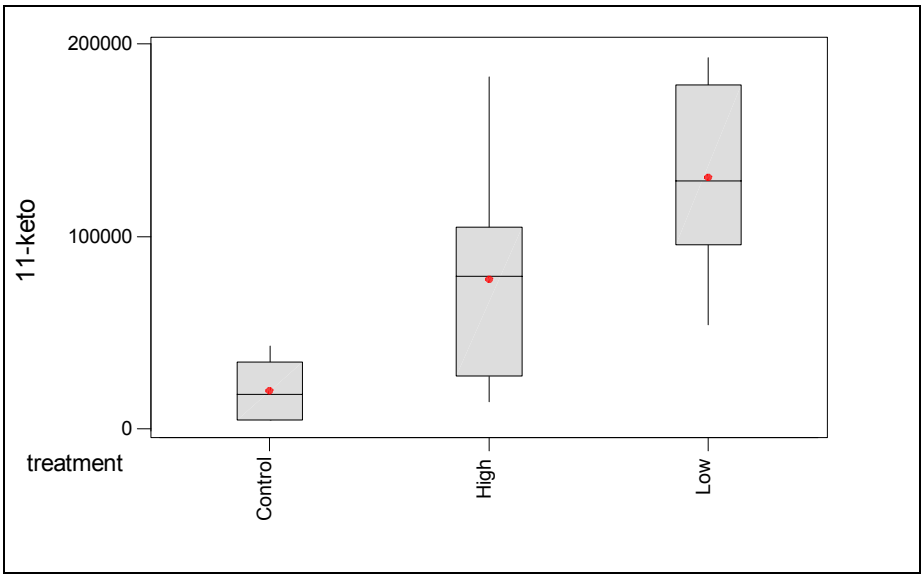

Figure 6.33. Boxplot of male 11-ketotesosterone concentration (pg/mL) by treatment for the EPA 21-Day Fadrozole assay. The box represents the interquartile range, whiskers represent the data range, the horizontal line is the median value, and the circle is the mean value.

6.2.8 Fecundity

Total Fecundity—A 15-day pre-exposure evaluation of total egg production was performed. Total 15-day counts among the three treatments in the exposure assay (individual tank values summed for each treatment) ranged from about 17,000 eggs to 22,000 eggs (Figure 6.34). A significant difference in the mean 15-day egg production among the groups of replicates evaluated during the pre-exposure assay was detected (Kruskal-Wallis, $H = 8.74$, $p = 0.033$, df = 3). However, the only significant difference was that the numbers of eggs laid in the tanks that were not subsequently used during the 21-Day fadrozole assay were lower than the number for the tanks that would eventually constitute the High concentration during the assay. There were no differences in the numbers of eggs laid among the tanks that would eventually be used for the three treatments during the 21-Day assay.

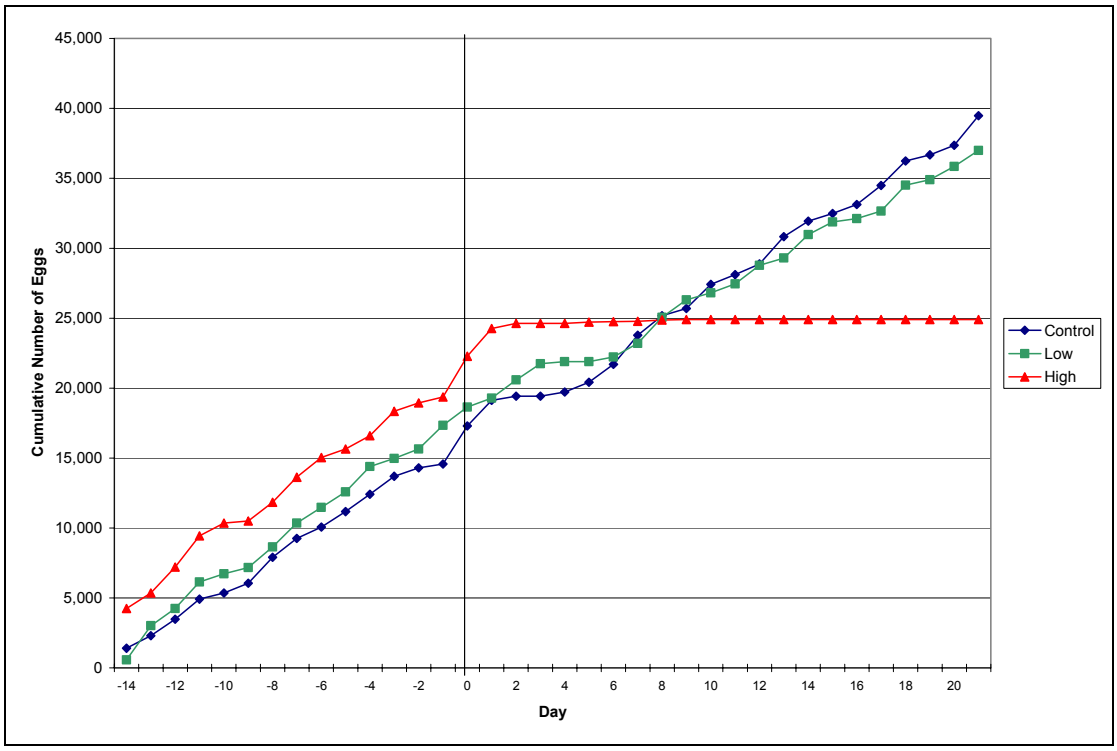

Figure 6.34. Total egg production per treatment for the 15-Day pre-exposure assay.

During the EPA 21-Day Fadrozole assay, total counts in the Control treatment were reasonably consistent among replicates, varying from 5,193 eggs to 6,131 eggs (Figure 6.35). Variability in total egg production among Low-concentration replicates was somewhat greater, ranging from 3,224 eggs to 6,356 eggs. Total counts among the High-concentration replicates varied from 413 eggs to 922 eggs. Statistical analysis of square-root transformed egg counts showed significant among-treatment differences (Kruskal-Wallis, $H = 8.00$, $p = 0.018$, df = 2) in mean total numbers of eggs produced (Table 6.31). The mean total number of eggs produced by females in the High concentration was significantly less than the mean total numbers of eggs produced by females in the Control treatment and the Low concentration. The achieved power for this assay was 100%, and the sample size required to detect a significant difference from the Control treatment at 80% power was 2 (Table 6.31).

Table 6.31. Summary statistics and power estimates for total fecundity data for the EPA 21-Day Fadrozole assay.

Level	N	Mean	Stdev	CV	Achieved Power [1]	Sample Size Required [2]
control	4	5548	421	8%	100%	2
low	4	4587	1305	28%		
high	4	654	210	32%		

[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.

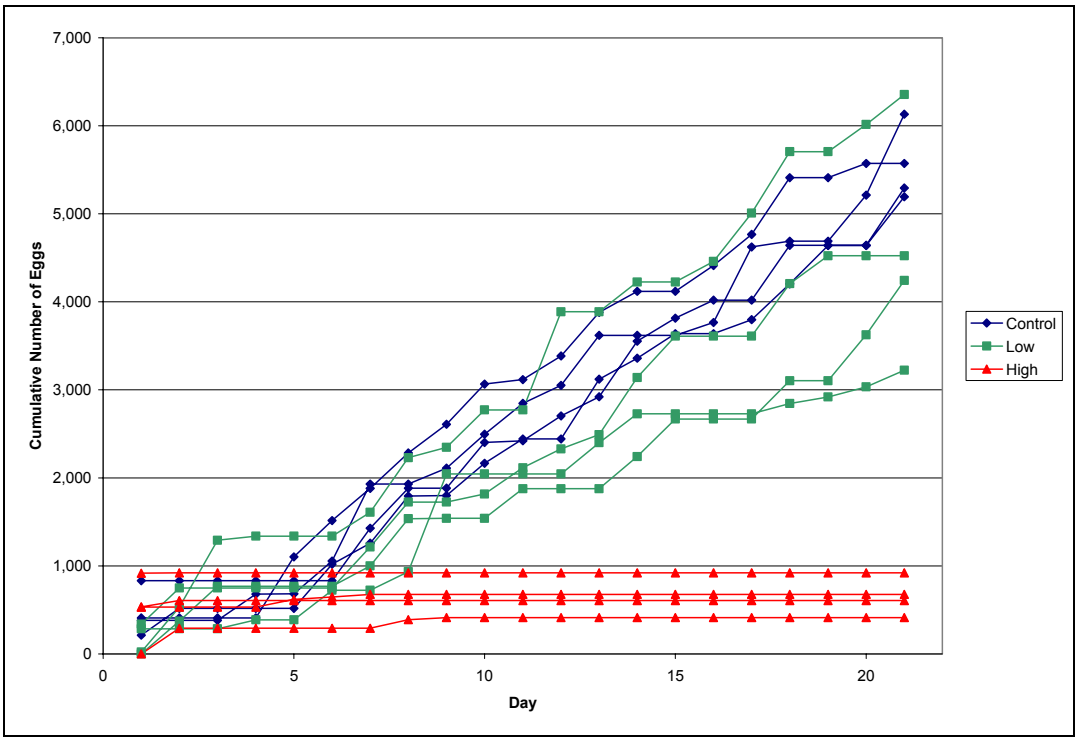

Figure 6.35. Total egg production by replicate per treatment for the EPA 21-Day fadrozole assay.

Fecundity per Female Reproductive Day—During the 15-Day pre-exposure evaluation, the mean number of eggs produced per female reproductive day ranged from 61.1 eggs/day for the tanks that would be used for the Control treatment to 104.4 eggs/day for the tanks that would be used for the High concentration during the 21-day exposure assay. The mean number of eggs produced per female reproductive day for the tanks that would not be used during the 21-day exposure assay was 60.6 eggs/day. There were no significant differences among treatments in the mean numbers of eggs produced per reproductive day during the pre-exposure period (Kruskal-Wallis, $H = 7.65$, $p = 0.054$, df = 2). The largest difference in eggs per female reproductive day was between the tanks that would constitute the High concentration and those that were not subsequently used during the exposure assay.

During the EPA 21-Day Fadrozole assay, the maximum number of female reproductive days was achieved for all treatments (Table 6.32). The number of eggs produced per female reproductive day varied from 61.8 eggs to 73.0 eggs in the Control treatment and from 38.4 to 75.7 in the Low concentration (Figure 6.36). For the High concentration, the number of eggs produced per female reproductive day ranged from 4.9 eggs to 11.0 eggs. Because no fish died during the assay, the statistical results reported here are the same as those reported for total fecundity. The number of eggs produced per day by females in the High concentration was significantly less than the numbers produced by females in the Control treatment and the Low concentration (Kruskal-Wallis, $H = 8.00$, $p = 0.018$, df = 2). The achieved power for this assay was 100%, and the sample size required to detect a significant difference from the Control treatment at 80% power was 2 (Table 6.32).

Table 6.32. Summary statistics and power estimates for fecundity per female reproductive day for the EPA 21-Day Fadrozole assay.

Level	Mean Number of Reproductive Days [1]	N	Mean	Stdev	CV	Achieved Power [2]	Sample Size Required [3]
control	84	4	66.0	5.0	8%	100%	2
low	84	4	54.6	15.5	28%		
high	84	4	7.8	2.5	32%		

[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.

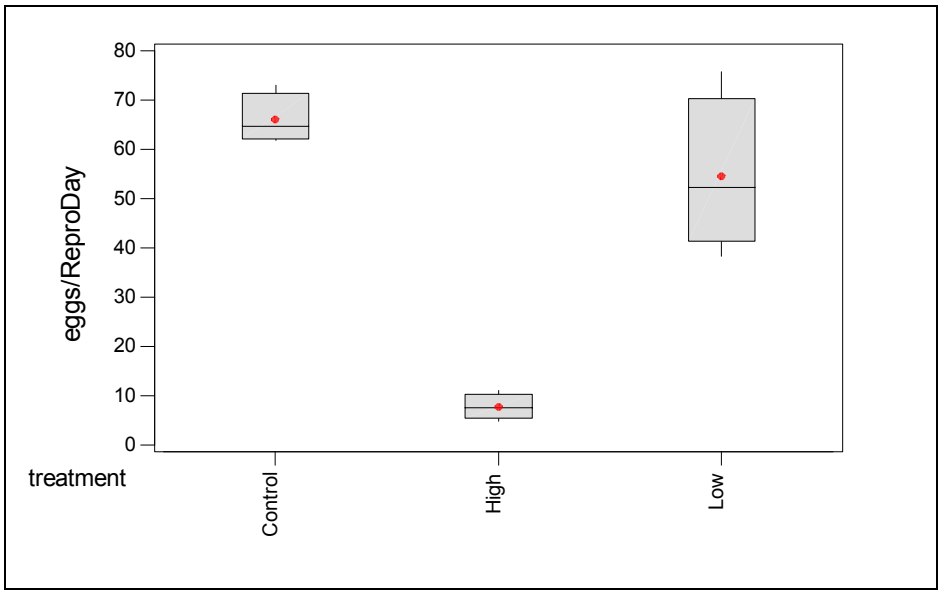

Figure 6.36. Boxplot of the number of eggs produced per female reproductive day by treatment for the EPA 21-Day Fadrozole assay. The box represents the interquartile range, whiskers represent the data range, the horizontal line is the median value, and the circle is the mean value.

Eggs on Tiles/Dishes— The mean number of eggs laid on the tiles during the 15-day pre-exposure assay varied from 2,859 eggs for the tanks that would be used for the Control treatment to 3,791 eggs for the tanks that would be used for the High concentration. The mean number of eggs on dishes ranged from 1,464 eggs for the Control treatment to 1,781 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 15-day pre-exposure assay (Kruskal-Wallis, $H = 7.26$, $p = 0.064$, df = 3).

The mean number of eggs laid on the tiles among the treatments during the EPA 21-Day Fadrozole assay varied from 534 eggs for the High concentration to 4,938 eggs for the Control treatment (Appendix E;

Table 4.7). The number of eggs on dishes ranged from 120 eggs for the High concentration to 610 eggs for the Control treatment. 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 (Appendix E; Figure 4.6). A significant difference in this mean proportional difference among treatments was detected (Kruskal-Wallis, $H = 6.96$, $p = 0.031$, df = 2). Proportionally more eggs occurred on tiles in the Control treatment than in the High-concentration treatment.

6.2.9 Fertilization Success

Total Fertilization—Eggs were collected during the 15-day pre-exposure period for the evaluation of fertilization success rate. The mean proportion of eggs fertilized in the Control treatment was 0.997 [standard deviation (sd) = 0.006], 1.000 (sd = 0.0003) in the Low concentration, and 1.000 (sd = 0.0001) in the High concentration. The mean proportion of eggs fertilized in the replicates that were not used in the 21-day exposure assay was 1.000 (sd = 0.0005). There were no significant differences among treatments in the proportion of eggs that were fertilized (Kruskal-Wallis, $H = 1.17$, $p = 0.760$, df = 3).

The total (tiles + dishes) fertilization success rates for all treatment replicates during the EPA 21-Day Fadrozole assay were high, ranging from 0.995 (High-concentration replicate) to 1.00 (High-concentration and Control-treatment replicates) (Figure 6.37). No significant differences in mean fertilization success rates (Table 6.33) among treatments were detected (Kruskal-Wallis, $H = 1.56$, $p = 0.457$, df = 2). The achieved power for this assay was 6%, and the sample size required to detect a significant difference from the Control treatment at 80% power was 201 (Table 6.33).

Table 6.33. Summary statistics and power estimates for the proportion of eggs fertilized for the EPA 21-Day Fadrozole assay.

Level	N	Mean	Stdev	CV	Achieved Power [1]	Sample Size Required [2]
control	4	0.999	0.001	0.1%	6%	201
low	4	0.999	0.001	0.1%		
high	4	0.999	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.

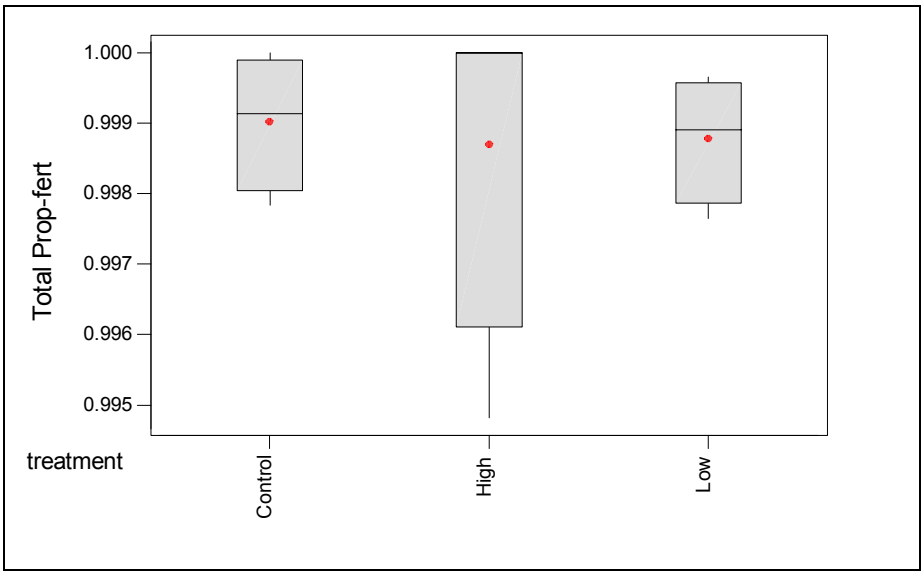

Figure 6.37. Boxplot of the proportion of eggs fertilized by treatment for the EPA 21-Day Fadrozole assay. The box represents the interquartile range, whiskers represent the data range, the horizontal line is the median value, and the circle is the mean value.

Fertilization of Eggs on Tiles and Dishes—During the 15-Day pre-validation assay, there were no significant differences in the fertilization success rates among treatments for eggs laid on tiles (Kruskal-Wallis, $H = 1.17$, $p = 0.760$, df = 3) or on dishes as all eggs that occurred on dishes were fertilized. The fertilization success rates for all treatment replicates for eggs laid on tiles during the EPA 21-Day Fadrozole assay were high, ranging from 0.992 (High-concentration replicate) to 1.00 (High-concentration and Control-treatment replicates) (Appendix E, Figure 4.7). No significant differences in mean fertilization success rates (Appendix E, Table 4.8) among treatments were detected (Kruskal-Wallis, $H = 1.56$, $p = 0.457$, df = 2). The fertilization success rates for all treatment replicates for eggs laid on dishes during the assay were high, ranging from 0.998 (Control treatment replicate) to 1.00 (all High and Low-concentration replicates) (Appendix E, Figure 4.8). No significant differences in mean fertilization success rates (Appendix E, Table 4.8) among treatments were detected (Kruskal-Wallis, $H = 2.00$, $p = 0.368$, df = 2).

6.2.10 Hatchability and Larval Development

Eggs were collected during the 15-day pre-exposure period for the evaluation of hatchability. The mean proportion of fertilized eggs that hatched in the Control treatment was 0.93 [standard deviation (sd) = 0.14], 0.99 (sd = 0.02) in the Low concentration, and 0.97 (sd = 0.06) in the High concentration. There were no significant differences among treatments in the proportion of eggs that hatched (Kruskal-Wallis, $H = 0.50$, $p = 0.779$, df = 2).

Eggs were collected during the EPA 21-Day Fadrozole assay for the evaluation of hatchability. The proportion of fertilized eggs that hatched ranged from 0.88 to 1.00 in the Control treatment and from 0.83 to 1.00 for the Low concentration (Figure 6.38). No eggs were available to evaluate hatchability for the High concentration because the evaluation was started on Day 12 of the assay and no eggs were laid in those tanks after Day 9. There were no significant differences between the Low concentration and the Control treatment in the proportion of eggs that hatched (Kruskal-Wallis, $H = 0.14$, $p = 0.708$, df = 1).

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 767 (Table 6.34).

Table 6.34. Summary statistics and power estimates for the proportion of fertile eggs that hatched for the EPA 21-Day Fadrozole assay.

Level	N	Mean	Stdev	CV	Achieved Power [1]	Sample Size Required [2]
control	11	0.962	0.045	5%	6%	767
low	11	0.942	0.071	8%		
high	0	–	–	–		

[1] Calculated from arcsine square-root transformed data; with sample size = 11.
[2] To detect a significant difference from control treatment based on maximum achieved absolute difference; calculated on arcsine square-root transformed data.

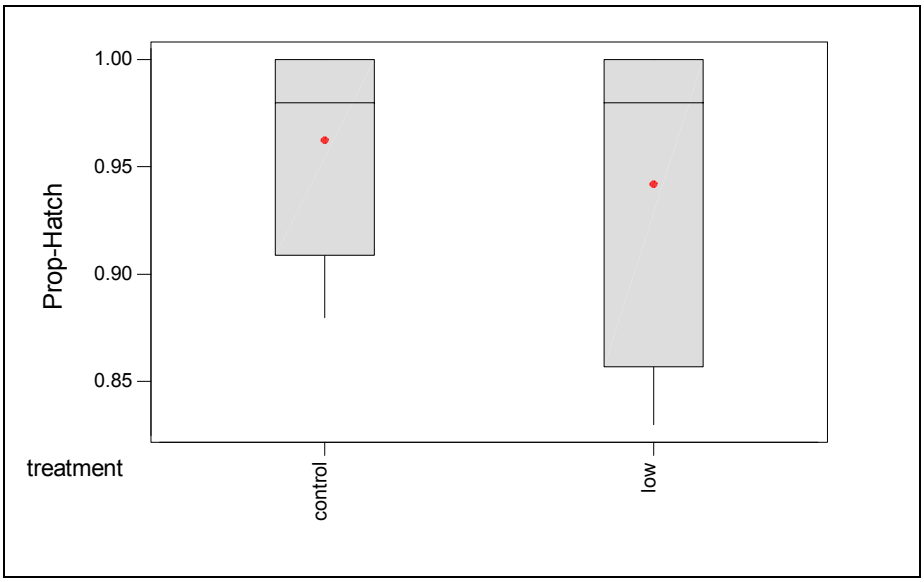

Figure 6.38. Boxplot of the proportion of fertile eggs that hatched by treatment for the EPA 21-Day Fadrozole assay. The box represents the interquartile range, whiskers represent the data range, the horizontal line is the median value, and the circle is the mean value.

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 0.97 (sd = 0.02). The mean proportion of normal larvae in the remaining treatments was 0.99 (sd = 0.01) in the Low concentration and 0.96 (sd = 0.03) in the High concentration. There were no significant differences among treatments in the proportion of eggs that hatched (Kruskal-Wallis, $H = 3.77$, $p = 0.152$, df = 2).

Eggs were collected during the EPA 21-Day Fadrozole 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 Low concentration (Figure 6.39). Again, no larvae were available in the High concentration to evaluate development. There

were no significant differences between the Low concentration and the Control treatment in the proportion of eggs that hatched (Kruskal-Wallis, $H = 0.76$, $p = 0.382$, df = 1). 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 102 (Table 6.35).

Table 6.35. Summary statistics and power estimates for the proportion of normal larvae for the EPA 21-Day Fadrozole assay.

Level	N	Mean	Stdev	CV	Achieved Power [1]	Sample Size Required [2]
control	11	0.955	0.059	6%	13%	102
low	11	0.917	0.107	12%		
high	0	–	–	–		

[1] Calculated from arcsine square-root transformed data; with sample size = 11.
[2] To detect a significant difference from control treatment based on maximum achieved absolute difference; calculated on arcsine square-root transformed data.

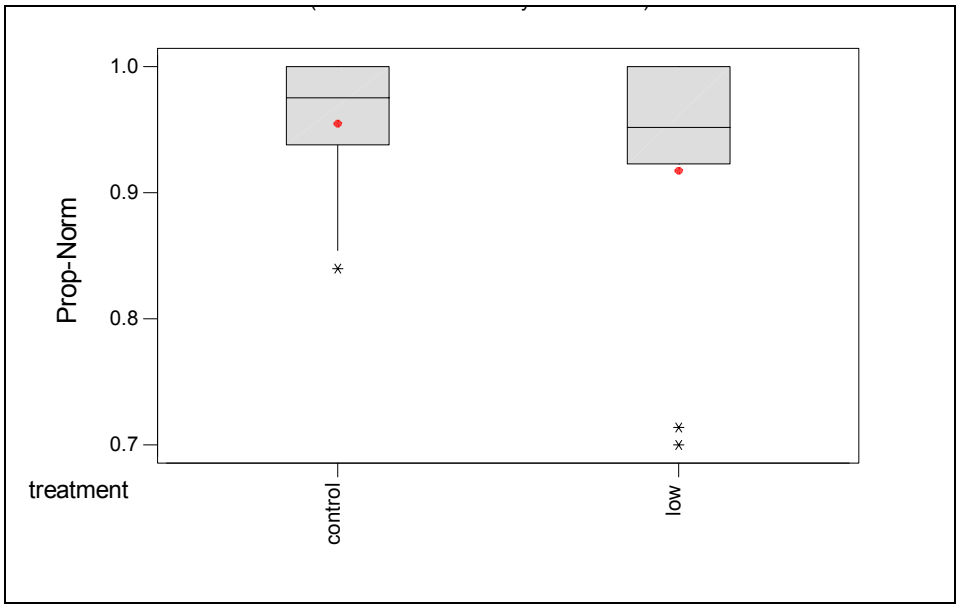

Figure 6.39. Boxplot of the proportion of normal larvae by treatment for the EPA 21-Day Fadrozole assay. The box represents the interquartile range, whiskers represent the data range, the horizontal line is the median value, the circle is the mean value, and asterisks represent probable outliers.

6.2.11 Body Weight

The body weight of females used in the EPA 21-Day Fadrozole assay ranged from 1.5 g to 4.4 g. There were significant differences in mean body weight among all treatments (Kruskal-Wallis, $H = 24.62$, $p = <0.001$, df = 2). The mean body weight of females in the High concentration was greater than that of females in the other two treatments and mean body weight of females in the Low concentration was greater than that of females in the Control treatment. The body weight of males used in the EPA 21-Day Fadrozole assay ranged from 3.8 g to 9.2 g. Three males were unusually large (Figure 6.40). Two that

were exposed to the High concentration weighed 9.2 g and 6.6 g. One male from the Control treatment weighed 8.3 g. However, there were no significant differences in mean body weight among treatments (Kruskal-Wallis, $H = 1.94$, $p = 0.380$, df = 2).

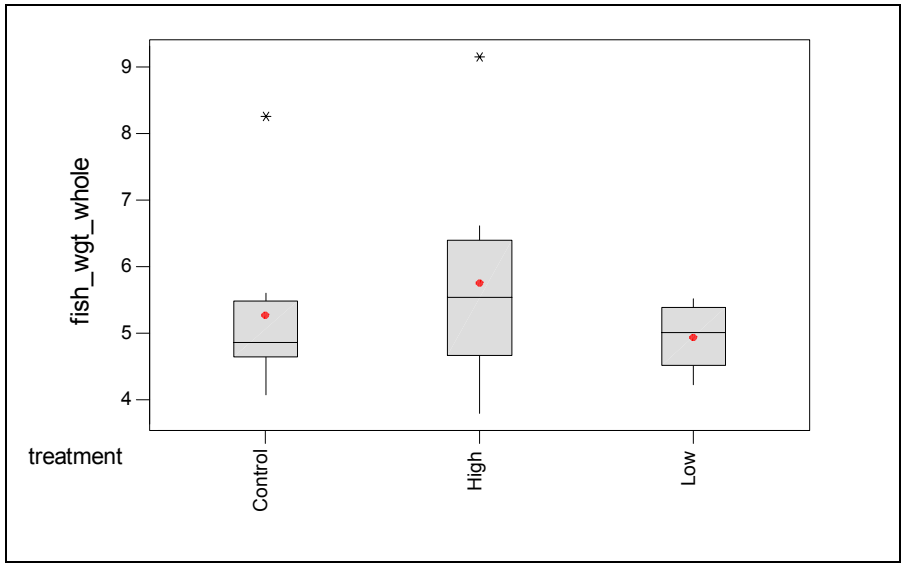

Figure 6.40. Boxplot of the body weights of males by treatment for the EPA 21-Day Fadrozole assay. The box represents the interquartile range, whiskers represent the data range, the horizontal line is the median value, the circle is the mean value, and asterisks represent probable outliers.

6.3 Non-spawning Adult 14-day Assay for Fadrozole

The Non-spawning Adult 14-Day Fadrozole assay was conducted from April 1, 2003 to April 15, 2003 (exposure assay).

6.3.1 Survival

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

6.3.2 Vitellogenin

Vitellogenin concentrations in Control treatment females used during the Non-spawning Adult 14-Day fadrozole assay ranged from 2,245,000 ng/mL to 11,020,000 ng/mL (Figure 6.41). Among females exposed to the three fadrozole concentrations, vitellogenin concentrations ranged from 0 ng/mL (not detected) to 1,612,000 ng/mL. Significant differences in the mean vitellogenin concentration among treatments (Table 6.36) were detected (Kruskal-Wallis, $H = 32.71$, $p = <0.001$, df = 2). Mean vitellogenin concentrations among Control-treatment females were significantly greater than the mean concentrations for females exposed to the High, and Medium fadrozole concentrations. Additionally, the mean concentration of vitellogenin from Low concentration females was significantly greater than those from females in the Medium and High fadrozole concentrations. The achieved power for this endpoint was 100%, and the sample size required to detect a significant difference from the Control treatment at 80% power was 3 (Table 6.36).

Table 6.36. Summary statistics and power estimates for female vitellogenin concentrations (ng/mL) for the Non-spawning Adult 14-Day Fadrozole assay.

Level	N	Mean	Stdev	CV	Achieved Power [1]	Sample Size Required [2]
control	10	4,934,800	2,619,357	53%	100%	3
low	10	793,075	447,921	56%		
medium	10	18,431	30,153	164%		
high	9	8,962	17,857	199%		

[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.

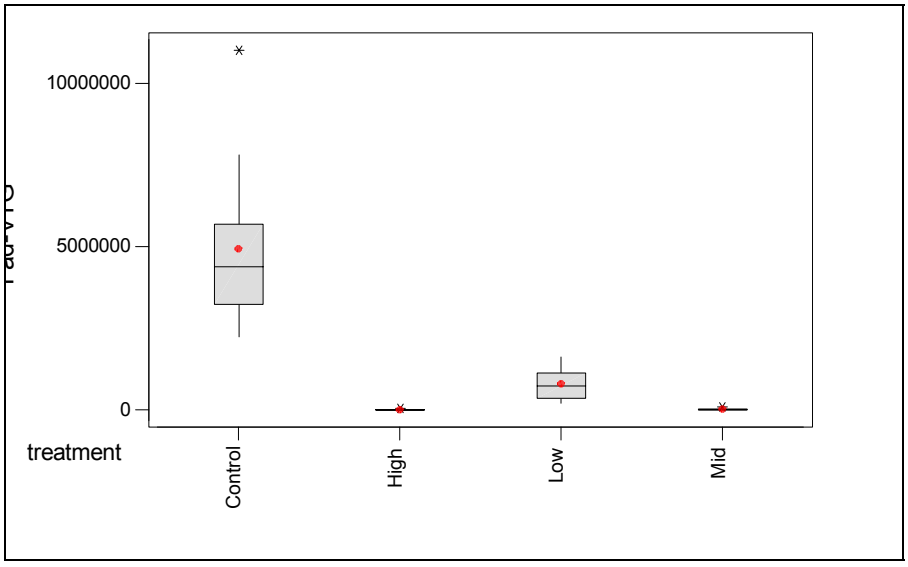

Figure 6.41. Boxplot of female vitellogenin concentration (ng/mL) by treatment for the Non-spawning Adult 14-Day Fadrozole assay. The box represents the interquartile range, whiskers represent the data range, the horizontal line is the median value, the circle is the mean value, and asterisks represent probable outliers.

Vitellogenin concentrations in Control treatment males used during the Non-spawning Adult 14-Day fadrozole assay ranged from 0 ng/mL (not detected) to 3,734 ng/mL (Figure 6.42). Among most males exposed to the three fadrozole concentrations, vitellogenin concentrations ranged from 0 ng/mL (not detected) to 829 ng/mL. One male exposed to the High fadrozole concentration had a vitellogenin concentration of 6,377 ng/mL and a second had a concentration of 5,498 ng/mL. No significant differences in the mean vitellogenin concentration per treatment (Table 6.37) were detected (Kruskal-Wallis, $H = 1.67$, $p = 0.644$, 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 174 (Table 6.37).

Table 6.37. Summary statistics and power estimates for male vitellogenin concentrations (ng/mL) for the Non-spawning Adult 14-Day Fadrozole assay.

Level	N	Mean	Stdev	CV	Achieved Power [1]	Sample Size Required [2]
control	10	675	1,261	187%	9%	174
low	10	74	82	111%		
	10	192	236	123%		
high	10	1,395	2,422	174%		

[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.

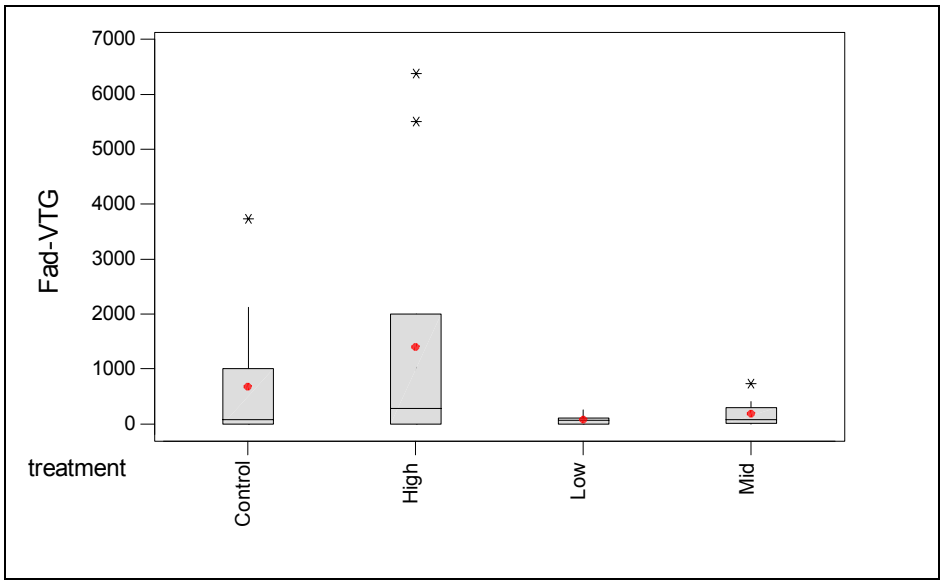

Figure 6.42. Boxplot of male vitellogenin concentration (ng/mL) by treatment for the Non-spawning Adult 14-Day Fadrozole assay. The box represents the interquartile range, whiskers represent the data range, the horizontal line is the median value, the circle is the mean value, and asterisks represent probable outliers.

6.3.3 Appearance / Secondary Sex Characteristics

Most of the females used during the Non-spawning Adult 14-Day Fadrozole assay showed normal female morphology. One female from the High concentration had the fat pad typical of males.

Morphological development among males used during the Non-spawning Adult 14-Day Fadrozole assay varied among treatments (Figure 6.43). Three males, one exposed to the Medium concentration and two exposed to the High concentration, lacked tubercles. Two males exposed to the Medium concentration also lacked fat pads. Sixteen of the 40 males used during the assay lacked vertical banding. There was no consistent dose-related pattern to these variations in morphology.

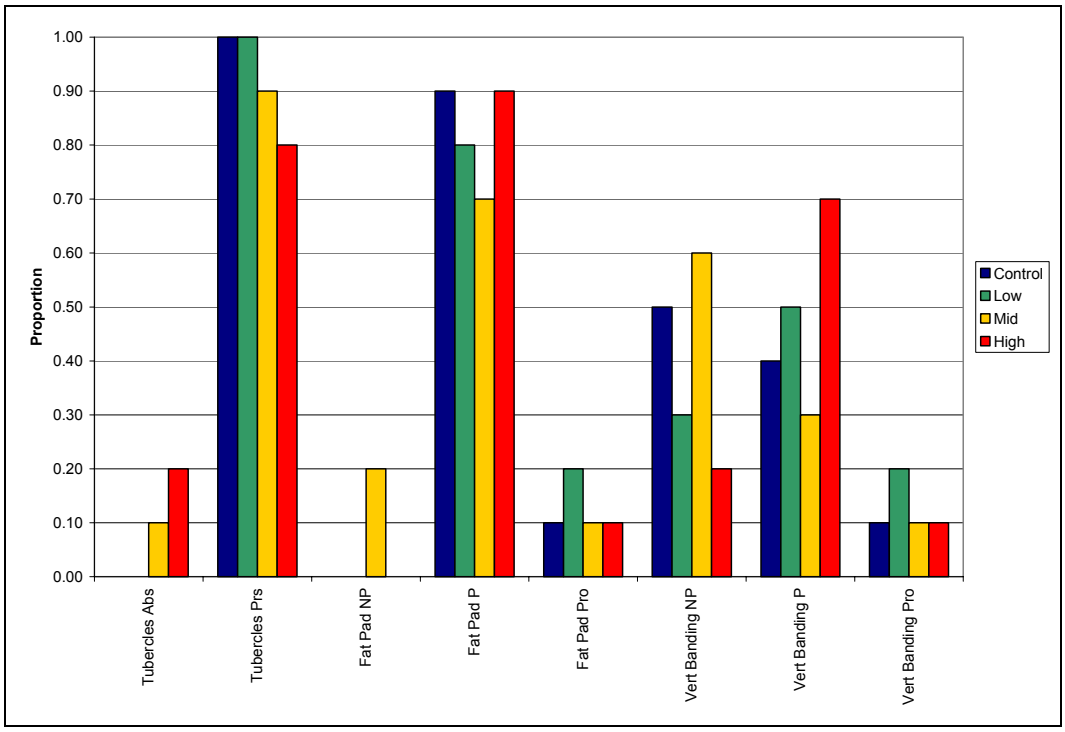

Figure 6.43. Secondary sex characteristics of males used during the Non-spawning Adult 14-Day Fadrozole assay.

6.3.4 Gonadosomatic Index

The range of GSI values calculated for females in the all treatments varied from about two- to eight-fold (Figure 6.44), and the overall within-treatment variability was moderate (CVs = 2%–51%; Table 6.38). The highest female GSI value was 21.7 (one fish in the Low concentration), but several fish had GSI values ~19–20. A significant difference in mean GSI values (Table 6.38) among treatments was detected (Kruskal-Wallis, $H = 9.21$, $p = 0.027$, df = 3). The mean GSI value for females exposed to the High fadrozole concentration was significantly less than the mean GSI for females from the Control treatment. The achieved power for this endpoint was 59%, and the sample size required to detect a significant difference from the Control treatment at 80% power was 15 (Table 6.38).

Table 6.38. Summary statistics and power estimates for female gonadosomatic index data for the Non-spawning Adult 14-Day Fadrozole assay.

Level	N	Mean	Stdev	CV	Achieved Power [1]	Sample Size Required [2]
control	10	15.3	3.1	20%	59%	15
low	10	13.5	3.9	29%		
medium	10	10.5	3.9	38%		
high	10	10.2	5.2	51%		

[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.

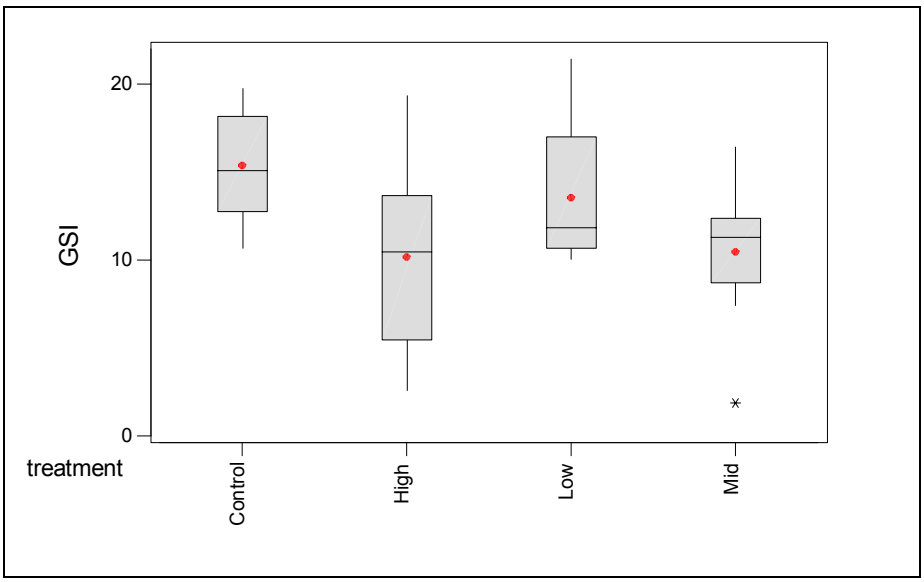

Figure 6.44. Boxplot of female GSI by treatment for the Non-spawning Adult 14-Day Fadrozole assay. The box represents the interquartile range, whiskers represent the data range, the horizontal line is the median value, the circle is the mean value, asterisks represent probable outliers.

The range of most GSI values calculated for males during the Non-spawning Adult 14-Day Fadrozole assay, was large, ranging from 0.5 to 2.1 (Figure 6.45), which approximates the typical range for reproductively-active male fathead minnows. Overall within-treatment variability was moderate to high (CVs = 30%–63%; Table 6.39). The highest and lowest male GSI values were 2.4 and 2.6 (for one fish from the High concentration and one from the Control treatment) and 0.1 and 0.4 (two fish from the Control treatment), respectively. However, there were no significant differences in mean GSI values (Table OECD14FAD GSI-2) among treatments (Kruskal-Wallis, $H = 1.29$, $p = 0.731$, df = 3). The achieved power for this endpoint was 15%, and the sample size required to detect a significant difference from the Control treatment at 80% power was 68 (Table 6.39).

Table 6.39. Summary statistics and power estimates for male gonadosomatic index data for the Non-spawning Adult 14-Day Fadrozole assay.

Level	N	Mean	Stdev	CV	Achieved Power [1]	Sample Size Required [2]
control	10	1.19	0.75	63%	15%	68
low	10	1.27	0.57	45%		
medium	10	1.24	0.37	30%		
high	10	1.45	0.52	36%		

[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.

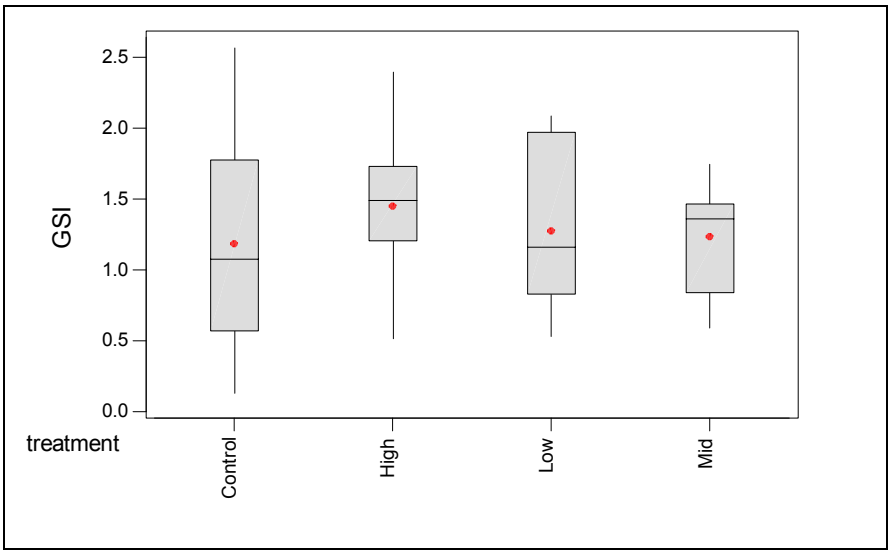

Figure 6.45. Boxplot of male GSI by treatment for the Non-spawning Adult 14-Day Fadrozole assay. The box represents the interquartile range, whiskers represent the data range, the horizontal line is the median value, and the circle is the mean value.

6.3.5 Female Gonad Histology

Histological analyses were conducted on the ovaries of 40 females exposed to fadrozole 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 Fadrozole assay revealed no significant differences among treatments (Kruskal-Wallis, $H = 3.93$, $p = 0.269$, 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 Medium and High concentrations ranged from Stage 1a to Stage 5 (see Methods for a description of the stages), whereas ova from females from the Control treatment and Low concentration showed Stage 1a to Stage 4 development (Figure 6.46). Variability within treatments for each stage was very high as indicated by CVs that ranged as high as 316% (Table 6.40). Statistical analyses showed that there was a significant difference among treatments in the proportion of cells in developmental Stages 1a, Stage 3, and Stage 4, but the were no significant differences among treatments in the proportion of cells in the developmental Stages 1b, 2, and 5 (Table 6.40). The proportion of cells in developmental Stage 1a in the Low and Medium concentrations were significantly greater than those in the High concentration. The proportion of cells in developmental Stage 3 in the High concentration was significantly greater than that in the Control treatment. The proportion of cells in developmental Stage 4 in the Medium concentration was significantly less than that in the Control treatment.

Table 6.40. Descriptive statistics of the proportion of ovarian cells in each developmental stage for females from the Non-spawning Adult 14-Day Fadrozole 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	0.050	0.022	45%	0.064	0.030	47%	0.075	0.028	37%	0.037	0.013	34%	13.34	0.004**
1b	0.284	0.071	25%	0.277	0.117	42%	0.277	0.075	27%	0.197	0.085	43%	7.41	0.060
2	0.159	0.057	36%	0.141	0.066	47%	0.203	0.070	34%	0.175	0.054	31%	4.55	0.208
3	0.143	0.054	38%	0.195	0.065	33%	0.226	0.088	39%	0.266	0.103	39%	9.88	0.020*
4	0.281	0.123	44%	0.247	0.156	63%	0.134	0.095	71%	0.157	0.109	69%	8.23	0.041*
5	0.000	0.000	–	0.000	0.000	–	0.000	0.001	316%	0.001	0.002	316%	2.05	0.561

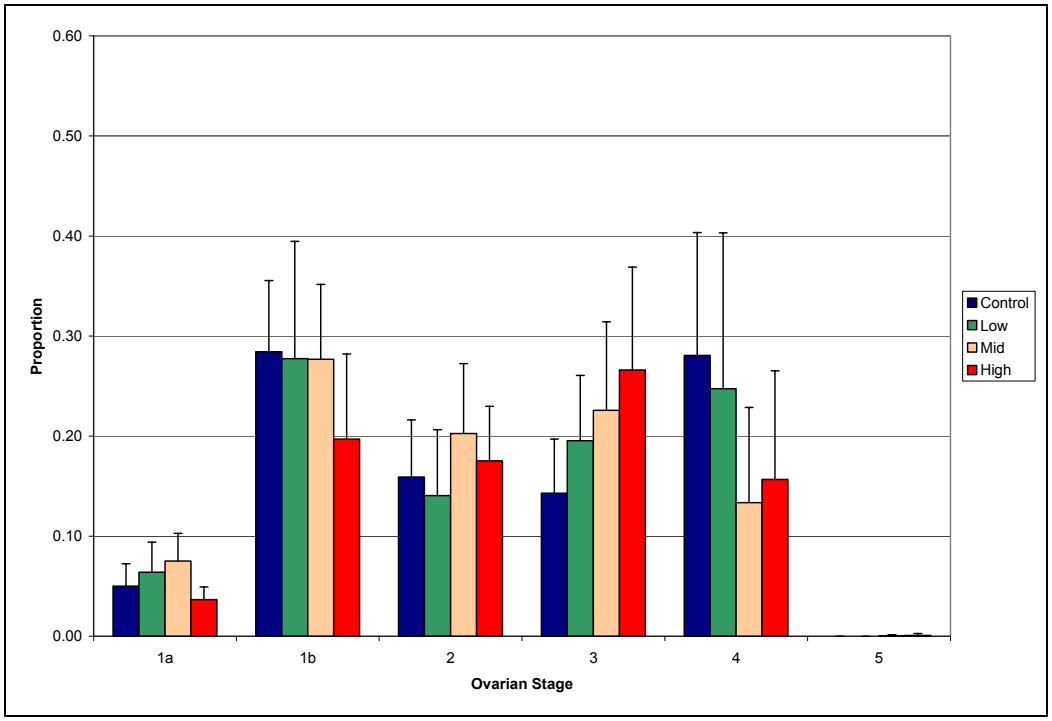

Figure 6.46. Frequency histogram showing the quantitative developmental staging of ovaries for each treatment of the Non-spawning Adult 14-Day Fadrozole assay. For each treatment, the columns represent the grand mean proportion of cells in each stage and the bars represent the standard deviation.

Atretic Follicles—The mean proportion of atretic follicles per 300 follicles (counted per fish) ranged from 0.060 follicles for females in the Medium concentration to 0.166 follicles for females in the High concentration treatment (Figure 6.47). There were significant differences in the mean proportion of atretic follicles among treatments (Kruskal-Wallis, $H = 11.34$, $p = 0.010$, df = 3). The proportion of atretic follicles in females from the High concentration was greater than those in females from the other three treatments.

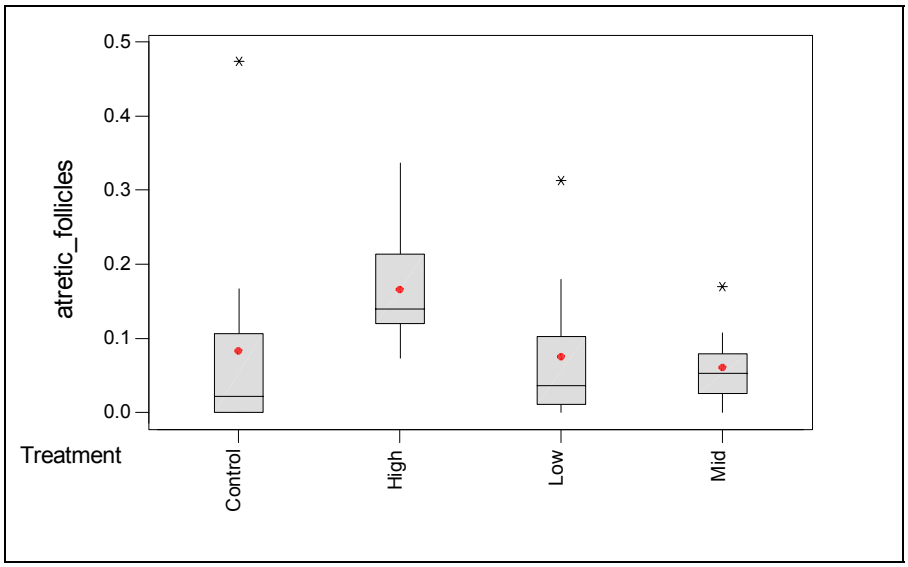

Figure 6.47. Boxplot of atretic follicles per 300 follicles by treatment for the Non-spawning Adult 14-Day Fadrozole assay. The box represents the interquartile range, whiskers represent the data range, the horizontal line is the median value, the circle is the mean value, and asterisks represent probable outliers.

Corpora Lutea—The mean proportion of corpora lutea per 300 follicles (counted per fish) ranged from 0.0003 for females in the Low concentration and Control treatment to 0.025 for females in the Medium concentration (Figure 6.48). There were no significant differences in the mean proportion of corpora lutea among treatments (Kruskal-Wallis, $H = 5.90$, $p = 0.116$, df = 3).

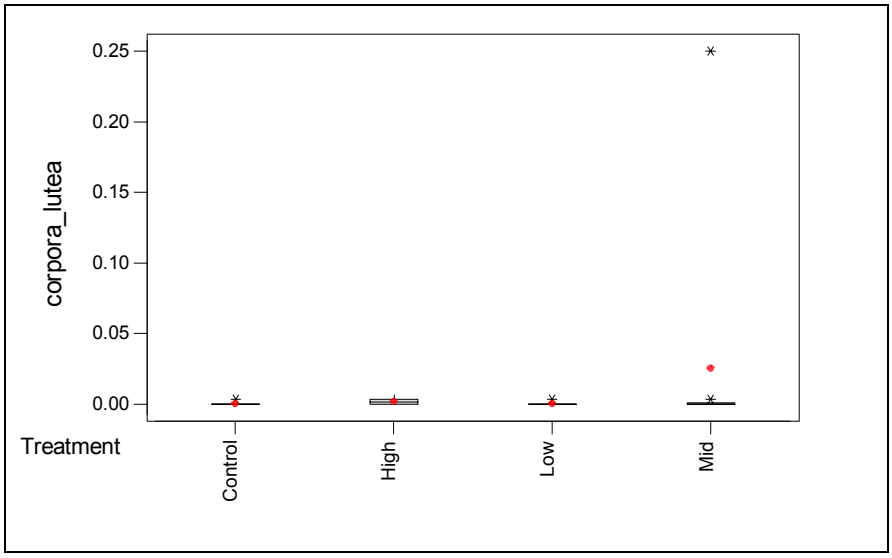

Figure 6.48. Boxplot of corpora lutea per 300 follicles by treatment for the Non-spawning Adult 14-Day Fadrozole assay. The box represents the interquartile range, whiskers represent the data range, the horizontal line is the median value, the circle is the mean value, and asterisks represent probable outliers.

Observations—One female from the Control treatment was observed to have many Stage 4 cells didn't develop and became atretic. Six females from the High concentration had atretic follicles that were Stage 4 cells. One of these females also had atretic follicles that were Stage 3 cells.

6.3.6 Male Gonad Histology

General Testes Staging—Testes from 40 males exposed to fadrozole during the Non-spawning Adult 14-Day Fadrozole assay were examined to determine the general developmental condition. However, the cassette containing a gonad sample for one male from the Control treatment contained too little tissue to permit a definitive evaluation and this male was excluded from the analyses. 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 8 of the 108 microscopic fields examined in the 9 Control treatment males showed Stage 4 (36 fields) or Stage 5 (64 fields) development. Most of the 120 microscopic fields examined in the 10 Low-concentration treatment males showed Stage 4 (58 fields) or Stage 5 (56 fields) development. Six microscopic fields showed Stage 3 development. In the 10 Medium concentration males available for examination, all of the 120 microscopic fields showed Stage 4 (64 fields) or Stage 5 (56 fields) development. In the 10 High concentration males available for examination, 115 of the 120 microscopic fields showed Stage 4 (62 fields) or Stage 5 (53 fields) development. Statistical analysis of the mean staging from 12 microscopic fields per fish revealed that no significant differences among treatments (Kruskal-Wallis, $H = 0.74$, $p = 0.863$, 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 6.49). Variability within treatments for each stage was very high as indicated by CVs that ranged as high as 175% (Table 6.41). There were no significant differences among treatments in the proportion of cells in any of the developmental stages (Table 6.41). Therefore, there was no effect on testicular developmental stage associated with fadrozole dose.

Table 6.41. Descriptive statistics of the proportion of testes cells in each developmental stage for males from the Non-spawning Adult 14-Day Fadrozole assay and results of the Kruskal-Wallis Test (df = 2) comparing treatments.

	Control (n = 9)			Low (n = 10)			Medium (n = 10)			High (n = 10)			Kruskal-Wallis	
Stage													*H*	*p*
	Mean	Stdev	CV	Mean	Stdev	CV	Mean	Stdev	CV	Mean	Stdev	CV		
1	0	0	–	0	0	–	0	0	–	0	0	–	–	–
2a	0.004	0.005	119%	0.003	0.005	152%	0.004	0.005	115%	0.001	0.002	175%	2.54	0.467
2b	0.021	0.032	149%	0.054	0.083	154%	0.027	0.029	108%	0.040	0.055	136%	1.01	0.799
3a	0.080	0.108	135%	0.092	0.085	92%	0.049	0.048	98%	0.108	0.110	102%	1.74	0.628
3b	0.134	0.101	75%	0.120	0.078	65%	0.097	0.090	93%	0.111	0.092	83%	0.86	0.835
4	0.116	0.067	58%	0.072	0.059	81%	0.115	0.077	67%	0.116	0.051	44%	3.93	0.269
5	0.644	0.269	42%	0.659	0.238	36%	0.708	0.191	27%	0.624	0.228	37%	0.40	0.940

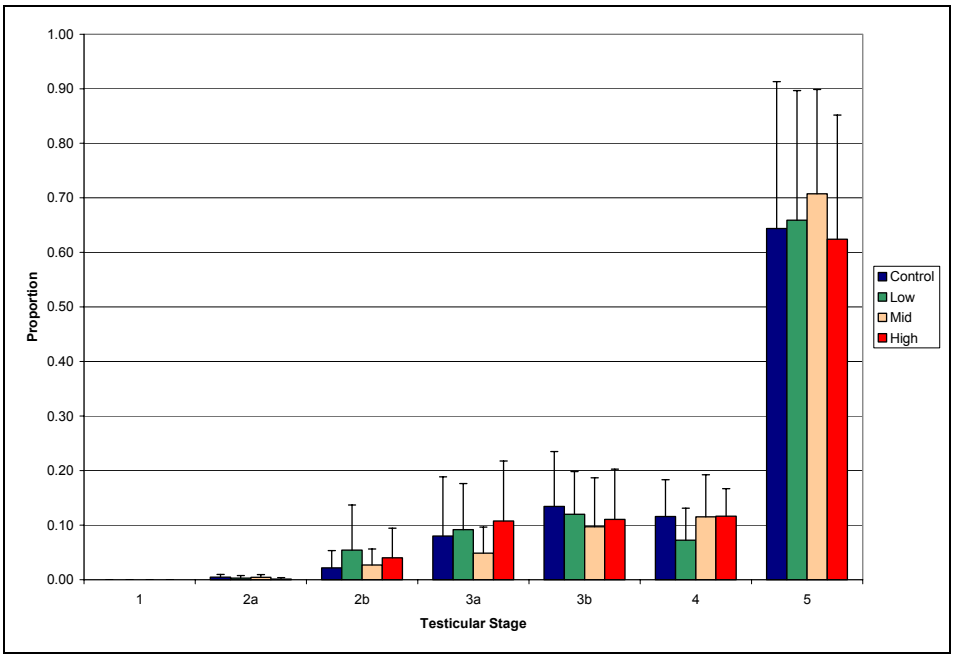

Figure 6.49. Frequency histogram showing the quantitative developmental staging of testes for each treatment of the Non-spawning Adult 14-Day Fadrozole assay. For each treatment, the columns represent the grand mean proportion of cells in each stage and the bars represent the standard deviation.

Tubule Diameter—The diameter of the seminiferous tubules of males from the Control treatment ranged from 121.7 µm to 267.5 µm (Figure 6.50). Tubule diameters of males from the three test concentrations ranged from 82.2 µm to 294.7 µm. No significant differences in the mean tubule diameter per treatment (Table 6.42) were detected (Kruskal-Wallis, $H = 4.03$, $p = 0.258$, df = 3). 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 36 (Table 6.42).

Table 6.42. Summary statistics and power estimates for male seminiferous tubule diameter data for the Non-spawning Adult 14-Day Fadrozole assay.

Level	N	Mean	Stdev	CV	Achieved Power [1]	Sample Size Required [2]
control	9	193.8	55.4	29%	23%	36
low	10	189.2	58.4	31%		
medium	10	149.0	42.1	28%		
high	10	168.2	63.7	38%		

[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.

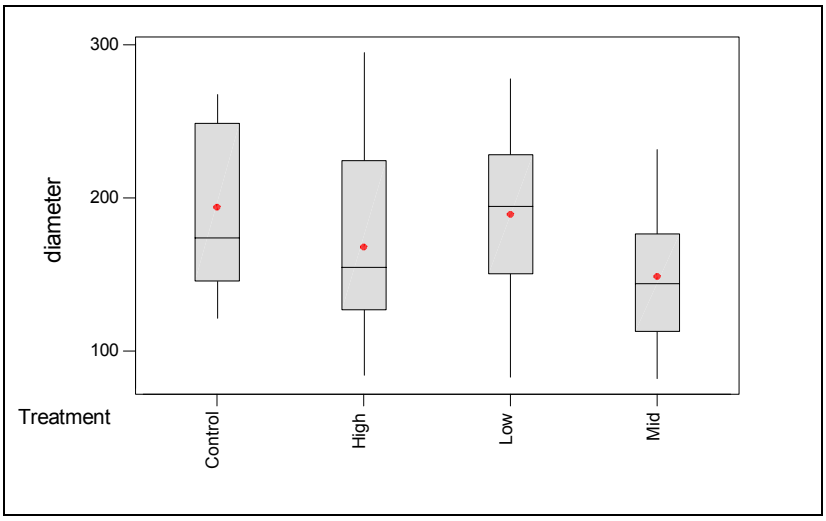

Figure 6.50. Boxplot of seminiferous tubule diameter (μm) by treatment for the Non-spawning Adult 14-Day Fadrozole assay. The box represents the interquartile range, whiskers represent the data range, the horizontal line is the median value, and the circle is the mean value.

Observations—One male from the High concentration showed Sertoli cell proliferation. One male exposed to the Low concentration and one male from the Medium concentration showed multifocal Leydig cell proliferation. Three males exposed to the High concentration were found to have focal or multifocal Leydig cell proliferation. No testicular atrophy and no ovatestes were observed in males from any treatment. Several males were observed to have abnormal testicular development (Table 6.43).

Table 6.43. Histological observations for males exposed to concentrations of fadrozole during the Non-spawning Adult 14-D assay.

Fish ID	Concentration	Leydig Cell Proliferation	Observations
218733	Control	None	Multifocal basophilic cystic structures with concentric laminations inside tubules and ducts, up to 45 μm diameter
218736	Control	None	Transition from stage 3b to stage 4 results in foci of nuclear fragments and spermatids and mixed stage nuclear fragments in tubule lumina. Large numbers of Stage 2 b cells in tubules
218737	Control	None	Multifocal proliferation of secondary spermatogonia
218738	Control	None	Stage 4 ova in section – probably an artifact
218750	Low	None	Tubules in stage 5 appeared to have smaller diameter than normal Multifocal partial divisions of Stage 3b and Stage 4 cells in tubule lumina Abnormal developmental sequence—a high concentration Stage 2b cells and very few Stage 4 cells

Fish ID	Concentration	Leydig Cell Proliferation	Observations
			Tubule lumina occluded in some cases
218756	Low	Multifocal	Multifocal proliferation of secondary spermatogonia
218762	Medium	None	Tubules in stage 5 appear to have smaller diameter than normal Proliferation of state 2b and 3a cells along tubules filled with mature sperm
218763	Medium	None	Large numbers of stage 2b cells in tubules
218764	Medium	None	Tubules in stage 5 appeared to have smaller diameter than normal
218765	Medium	None	Cystic structures (concretions) in some tubule lumina – irregular to 20 μm diameter
218767	Medium	None	Stage 3 ova in section – probably an artifact
218782	High	multifocal	Most tubules normal, but some with multifocal areas of Sertoli cell proliferation and Leydig cell proliferation Also, possible secondary spermatocyte proliferation.
218783	High	None	Multifocal proliferation of secondary spermatogonia A few syncytia of Stage 5 cells A few clumps of Stage 3b and Stage 4 cells in tubule lumina
218784	High	None	Stage 3 ova in section – probably an artifact
218787	High	None	Necrotic cells in tubule lumina associated with dense clusters of sperm Multifocal proliferation of secondary spermatogonia
218788	High	None	Multifocal proliferation of secondary spermatogonia, filled with Stage 5 cells Occasional incomplete division of Stage 3 cells , which appear necrotic Abnormal appearing arrangement of components of spermatogenesis Areas where tubule lumina occluded with apparent Stage 2b cells and some necrotic cells in tubule lumina
218789	High	None	Clumps of Stage 5 cells in tubule lumina

6.3.7 Plasma Steroid Concentrations

Estradiol—Estradiol concentrations in Control-treatment females used during the Non-spawning Adult 14-Day Fadrozole assay ranged from 0 pg/mL (not detected) to 3,502 pg/mL (Figure 6.51). Among females exposed to the three fadrozole concentrations, estradiol concentrations ranged from 0 pg/mL (not detected) to 1,956 pg/mL. A significant difference in the mean estradiol concentration per treatment (Table 6.44) was detected (Kruskal-Wallis, $H = 18.33$, $p < 0.001$, df = 2). The mean estradiol concentration in females from the High concentration was less than that in females from the Control treatment. The achieved power for this endpoint was 54%, and the sample size required to detect a significant difference from the Control treatment at 80% power was 15 (Table 6.44).

Table 6.44. Summary statistics and power estimates for female estradiol concentrations (pg/mL) for the Non-spawning Adult 14-Day Fadrozole assay.

Level	N	Mean	Stdev	CV	Achieved Power [1]	Sample Size Required [2]
control	10	1,519	1,132	75%	53%	15
low	10	727	524	72%		
medium	9	320	457	143%		
high	10	142	129	90%		

[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.

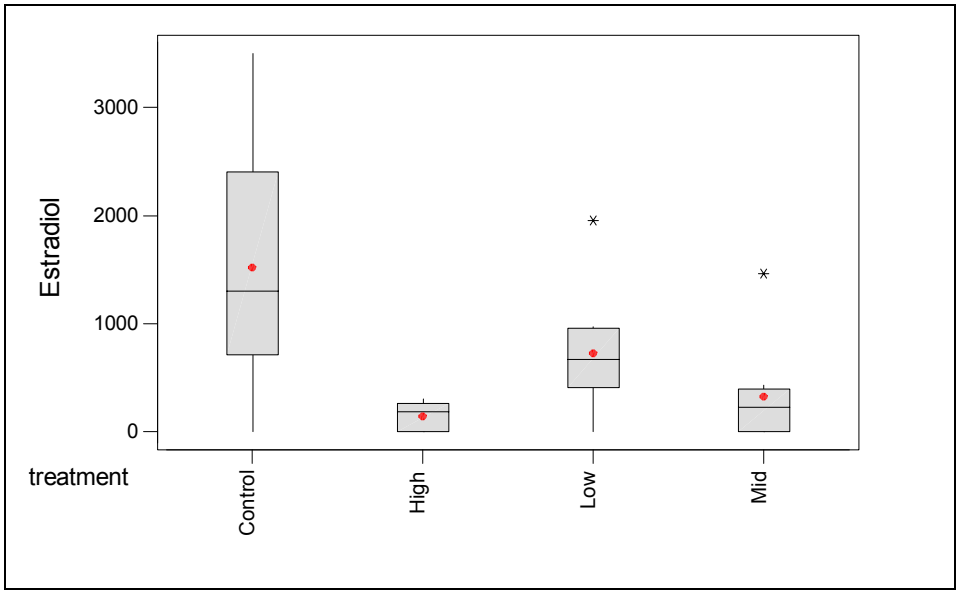

Figure 6.51. Boxplot of female estradiol concentration (pg/mL) by treatment for the Non-spawning Adult 14-Day Fadrozole assay. The box represents the interquartile range, whiskers represent the data range, the horizontal line is the median value, the circle is the mean value, and the asterisks represent probable outliers.

Estradiol was detected only in one male exposed to the Medium concentration during the Non-spawning Adult 14-Day Fadrozole assay (1,814 pg/mL). Estradiol was not detected in males from the Control treatment (9 individuals) or in those exposed to the Low and High concentrations (10 individuals per treatment) (Table 6.45).

Table 6.45. Summary statistics and power estimates for male estradiol concentrations (pg/mL) for the Non-spawning Adult 14-Day Fadrozole assay.

Level	N	Mean	Stdev	CV	Achieved Power [1]	Sample Size Required [1]
control	9	0	0	–	–	–
low	10	0	0	–		
medium	10	181	574	316%		
high	10	0	0	–		

[1] Not calculated.

Testosterone—Testosterone concentrations in Control-treatment females used during the Non-spawning Adult 14-Day Fadrozole assay ranged from 146 pg/mL to 2,053 pg/mL (Figure 6.52). Among females exposed to the three fadrozole concentrations, testosterone concentrations ranged from 394 pg/mL to 7,314 pg/mL. No significant differences in the mean testosterone concentration per treatment (Table 6.46) were detected (Kruskal-Wallis, $H = 4.61$, $p = 0.203$, df = 2. The achieved power for this endpoint was 29%, and the sample size required to detect a significant difference from the Control treatment at 80% power was 26 (Table 6.46).

Table 6.46. Summary statistics and power estimates for female testosterone concentrations (pg/mL) for the Non-spawning Adult 14-Day Fadrozole assay.

Level	N	Mean	Stdev	CV	Achieved Power [1]	Sample Size Required [2]
control	9	1,095	712	65%	29%	26
low	10	1,730	822	48%		
medium	8	986	407	41%		
high	9	2,067	2,084	101%		

[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.

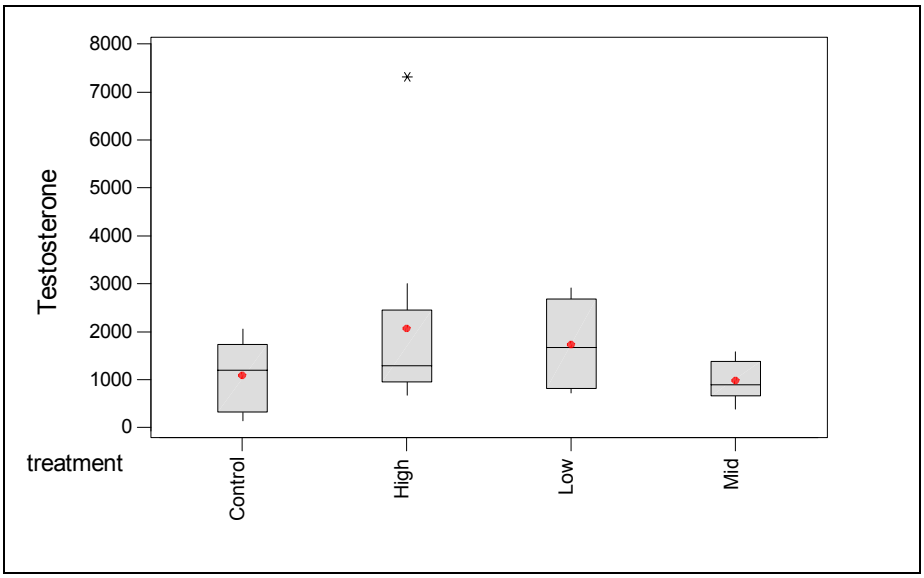

Figure 6.52. Boxplot of female testosterone concentration (pg/mL) by treatment for the Non-spawning Adult 14-Day Fadrozole assay. The box represents the interquartile range, whiskers represent the data range, the horizontal line is the median value, the circle is the mean value, and the asterisk represents a probable outlier.

Testosterone concentrations in Control treatment males used during the Non-spawning Adult 14-Day Fadrozole assay ranged from 1,005 pg/mL to 2,603 pg/mL (Figure 6.53). Among males exposed to the three fadrozole concentrations, testosterone concentrations ranged from 0 pg/mL (not detected) to 13,606 pg/mL. No significant differences in the mean testosterone concentration per treatment (Table 6.47) were detected (Kruskal-Wallis, $H = 3.28$, $p = 0.350$, df = 2). The achieved power for this endpoint was 21%, and the sample size required to detect a significant difference from the Control treatment at 80% power was 41 (Table 6.47).

Table 6.47. Summary statistics and power estimates for male testosterone concentrations (pg/mL) for the Non-spawning Adult 14-Day Fadrozole assay.

Level	N	Mean	Stdev	CV	Achieved Power [1]	Sample Size Required [2]
control	9	1,889	493	26%	21%	41
low	10	1,658	805	49%		
medium	10	1,413	813	58%		
high	10	3,256	3,860	119%		

[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.

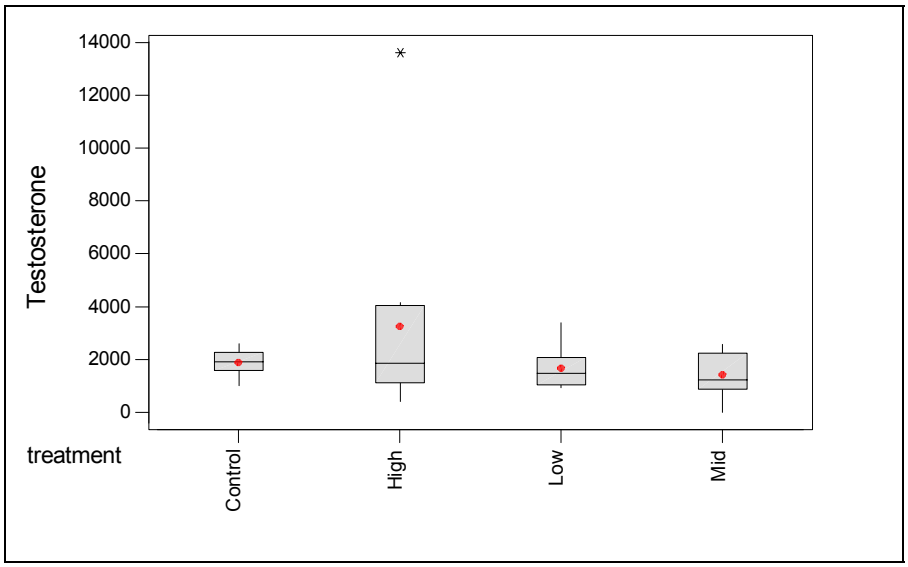

Figure 6.53. Boxplot of male testosterone concentration (pg/mL) by treatment for the Non-spawning Adult 14-Day Fadrozole assay. The box represents the interquartile range, whiskers represent the data range, the horizontal line is the median value, the circle is the mean value, and the asterisk represents a probable outlier.

11-ketotestosterone—11-ketotesosterone was not detected in Control-treatment females, nor in females exposed to the Low fadrozole concentration, during the Non-spawning Adult 14-Day Fadrozole assay (Figure 6.54). 11-ketotesosterone was detected in one of seven females exposed to the Medium concentration (519 pg/mL). Among the 10 females from the High fadrozole concentrations, 11-ketotestosterone was detected in 6, and the concentrations for those ranged from 329 pg/mL to 897 pg/mL. Significant differences in the mean 11-ketotestosterone concentration per treatment (Table 6.48) were detected (Kruskal-Wallis, $H = 11.23$, $p = 0.011$, df = 2). 11-ketotestosterone was primarily detected in females from the High concentration, with one female from the Medium concentration also having a detectable level of the compound. The achieved power for this endpoint was 59%, and the sample size required to detect a significant difference from the Control treatment at 80% power was 8 (Table 6.48).

Table 6.48. Summary statistics and power estimates for female 11-ketotestosterone concentrations (pg/mL) for the Non-spawning Adult 14-Day Fadrozole assay.

Level	N	Mean	Stdev	CV	Achieved Power [1]	Sample Size Required [2]
control	7	0	0		59%	8
low	5	0	0			
medium	7	74	196	264%		
high	10	397	378	95%		

[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.

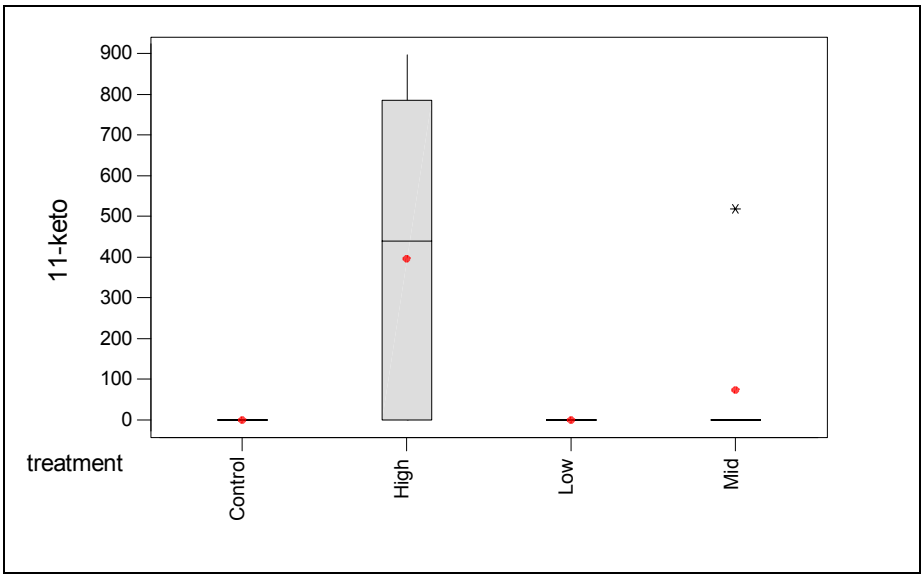

Figure 6.54. Boxplot of female 11-ketotestosterone concentration (pg/mL) by treatment for the Non-spawning Adult 14-Day Fadrozole assay. The box represents the interquartile range, whiskers represent the data range, the horizontal line is the median value, the circle is the mean value, and the asterisk represents a probable outlier.

11-ketotesosterone concentrations in Control treatment males used during the Non-spawning Adult 14-Day Fadrozole assay ranged from 0 pg/mL (not detected) to 19,106 pg/mL (Figure 6.55). Among males exposed to the three fadrozole concentrations, 11-ketotesosterone concentrations ranged from 0 pg/mL (not detected) to 57,374 pg/mL. No significant differences in the mean 11-ketotesosterone concentration per treatment (Table 6.49) were detected (Kruskal-Wallis, $H = 1.32$, $p = 0.724$, 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 170 (Table 6.49).

Table 6.49. Summary statistics and power estimates for male 11-ketotesosterone concentrations (pg/mL) for the Non-spawning Adult 14-Day Fadrozole assay.

Level	N	Mean	Stdev	CV	Achieved Power [1]	Sample Size Required [2]
control	10	7,585	6,499	86%	9%	170
low	10	7,422	8,541	115%		
medium	10	6,101	7,505	123%		
high	10	12,535	17,396	139%		

[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.

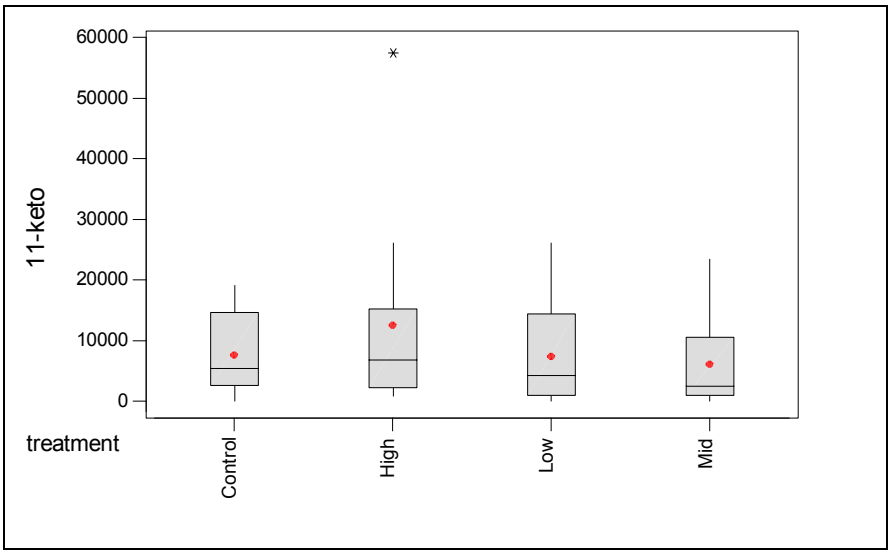

Figure 6.55. Boxplot of male 11-ketotesosterone concentration (pg/mL) by treatment for the Non-spawning Adult 14-Day Fadrozole assay. The box represents the interquartile range, whiskers represent the data range, the horizontal line is the median value, the circle is the mean value, and the asterisk represents a probable outlier.

6.3.8 Body Weight and Length

The body weight of females used in the Non-spawning Adult 14-Day Fadrozole assay ranged from 1.7 g to 4.8 g (Figure 6.56). There were no significant differences in mean body weight (natural log transformed) among treatments (Kruskal-Wallis, $H = 1.05$, $p = 0.790$, df = 3). 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 186 (Table 6.50).

The body (fork) length of females used in the Non-spawning Adult 14-Day Fadrozole assay ranged from 46 mm to 62 mm (Figure 6.57). There were no significant differences in mean body weight (natural log transformed) among treatments (Kruskal-Wallis, $H = 1.96$, $p = 0.581$, 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 53 (Table 6.51).

Table 6.50. Summary statistics and power estimates for female body weight (g) data for the Non-spawning Adult 14-Day Fadrozole assay.

Level	N	Mean	Stdev	CV	Achieved Power [1]	Sample Size Required [2]
control	10	2.9	0.8	27%	8%	186
low	10	2.7	0.6	23%		
medium	10	2.9	0.7	24%		
high	10	2.9	0.5	18%		

[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.

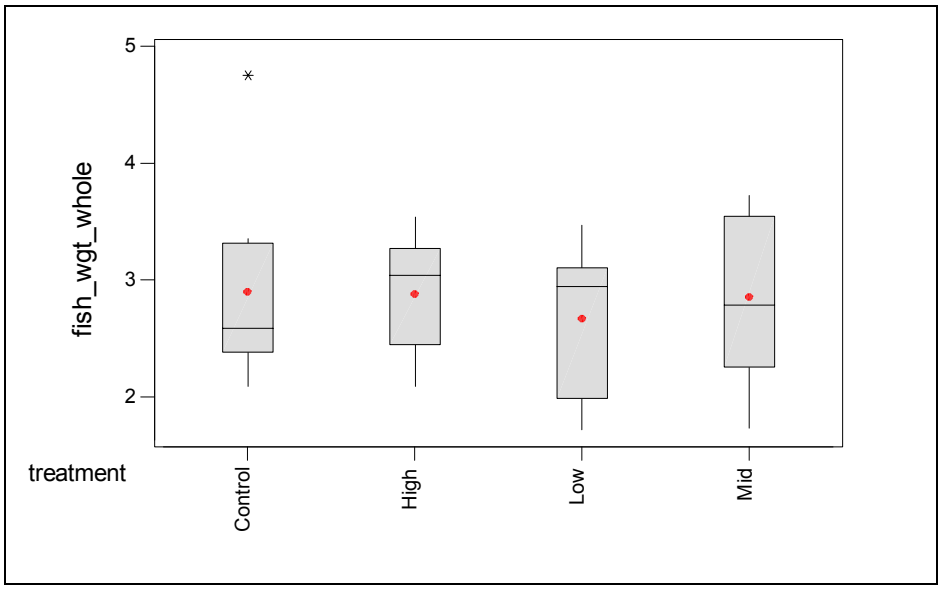

Figure 6.56. Boxplot of female body weight (g) by treatment for the Non-spawning Adult 14-Day Fadrozole assay. The box represents the interquartile range, whiskers represent the data range, the horizontal line is the median value, the circle is the mean value, and the asterisk represents a probable outlier.

Table 6.51. Summary statistics and power estimates for female body length (mm) data for the Non-spawning Adult 14-Day Fadrozole assay.

Level	N	Mean	Stdev	CV	Achieved Power [1]	Sample Size Required [2]
control	10	54.2	3.4	6%	18%	53
low	10	54.3	5.4	10%		
medium	10	55.4	4.5	8%		
high	10	56.9	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.

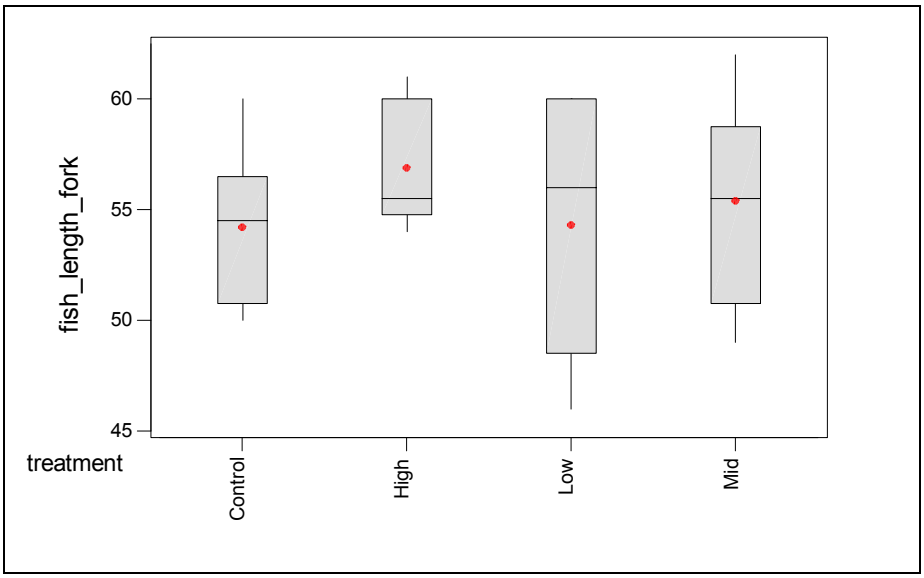

Figure 6.57. Boxplot of female body length (mm) by treatment for the Non-spawning Adult 14-Day Fadrozole assay. The box represents the interquartile range, whiskers represent the data range, the horizontal line is the median value, and the circle is the mean value.

The body weight of males used in the Non-spawning Adult 14-Day Fadrozole assay ranged from 3.9 g to 8.1 g (Figure 6.58). There were no significant differences in mean body weight among treatments (Kruskal-Wallis, $H = 3.33$, $p = 0.343$, df = 3). The achieved power for this endpoint was 21%, and the sample size required to detect a significant difference from the Control treatment at 80% power was 46 (Table 6.52).

The body (fork) length of males used in the Non-spawning Adult 14-Day Fadrozole assay ranged from 57 mm to 80 mm (Figure 6.59). There were no significant differences in mean body weight (natural log transformed) among treatments (Kruskal-Wallis, $H = 5.89$, $p = 0.117$, 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 32 (Table 6.53).

Table 6.52. Summary statistics and power estimates for male body weight (g) data for the Non-spawning Adult 14-Day Fadrozole assay.

Level	N	Mean	Stdev	CV	Achieved Power [1]	Sample Size Required [2]
control	10	5.8	1.3	23%	21%	46
low	10	5.5	1.0	19%		
medium	10	5.1	0.7	14%		
high	10	5.6	0.8	13%		

[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.

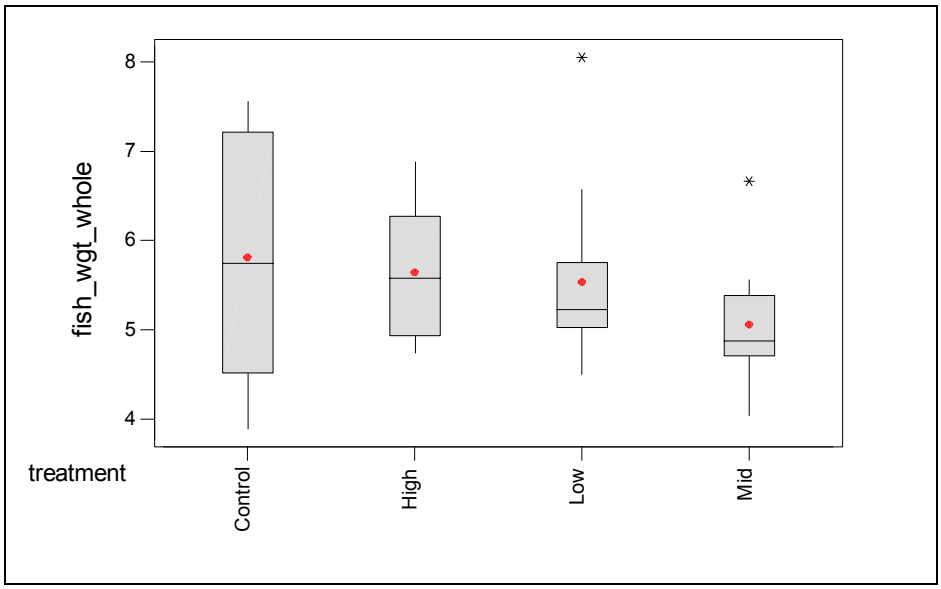

Figure 6.58. Boxplot of male body weight (g) by treatment for the Non-spawning Adult 14-Day Fadrozole assay. The box represents the interquartile range, whiskers represent the data range, the horizontal line is the median value, the circle is the mean value, and asterisks represent probable outliers.

Table 6.53. Summary statistics and power estimates for male body length (mm) data for the Non-spawning Adult 14-Day Fadrozole assay.

Level	N	Mean	Stdev	CV	Achieved Power [1]	Sample Size Required [2]
control	10	70.8	6.5	9%	30%	32
low	10	70.2	4.5	6%		
medium	10	66.4	3.9	6%		
high	10	70.3	4.7	7%		

[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.

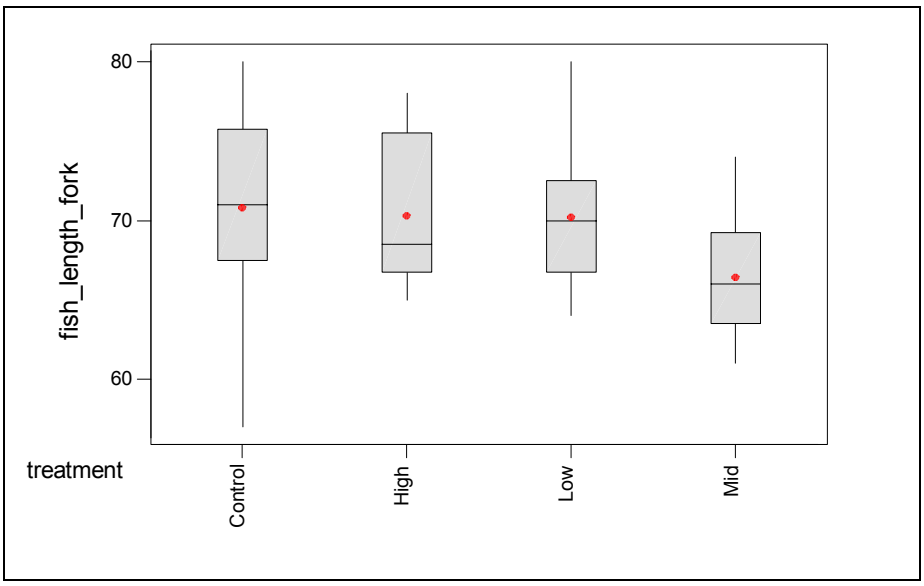

Figure 6.59. Boxplot of male body length (mm) by treatment for the Non-spawning Adult 14-Day Fadrozole assay. The box represents the interquartile range, whiskers represent the data range, the horizontal line is the median value, and the circle is the mean value.