3.0 RESULTS: METHOXYCHLOR

3.1 EPA 14-Day Assay for Methoxychlor

The EPA 14-Day Methoxychlor assay was conducted from September 29, 2002, to October 14, 2002 (pre-exposure assay), and from October 14, 2002, to October 28, 2002 (exposure assay).

3.1.1 Survival

All males in the Control treatment and all males and females in the Low-concentration treatment survived the EPA 14-Day Methoxychlor assay. However, two females in the Control treatment died (88% survival). Both males in two High-concentration tanks died before the end of the 14-day exposure and those tanks were terminated (male survival = 50%). In the two High-concentration tanks that completed the assay, six of the eight females survived (75%).

3.1.2 Vitellogenin

Vitellogenin concentrations in Control-treatment females used during the EPA 14-Day Methoxychlor assay ranged from 229,750 ng/mL to 5,470,500 ng/mL (Figure 3.1). Among females exposed to the two methoxychlor concentrations, vitellogenin concentrations ranged from 2,595 ng/mL to 2,177,500 ng/mL. Significant differences in the mean vitellogenin concentration per treatment (Table 3.1) were detected (Kruskal-Wallis, $H = 10.91$, $p = 0.004$, df = 2). Vitellogenin concentrations in Control-treatment females were significantly greater than those in females exposed to the Low concentration. The achieved power for this endpoint was 47%, and the sample size required to detect a significant difference from the Control treatment at 80% power was 10 (Table 3.1).

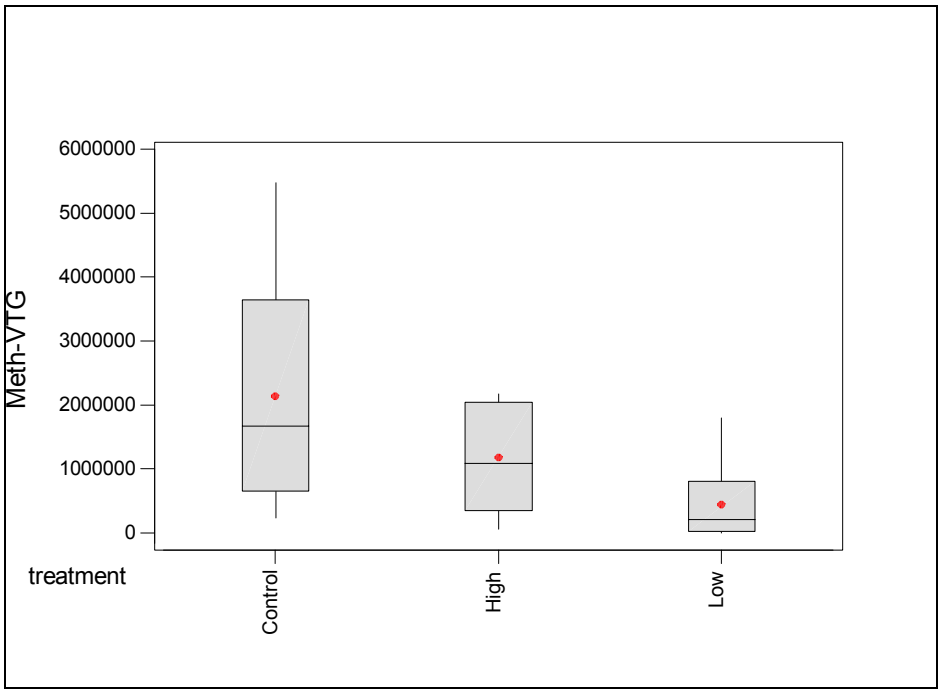

Figure 3. 1. Boxplot of female vitellogenin concentration (ng/mL) by treatment for the EPA 14-Day Methoxychlor assay. The box represents the interquartile range, whiskers represent the data range, the horizontal line is the median value, and the circle is the mean value.

Table 3.1. Summary statistics and power estimates for female vitellogenin concentrations (ng/mL) for the EPA 14-Day Methoxychlor assay.

Level	N	Mean	Stdev	CV	Achieved Power [1]	Sample Size Required [2]
Control	14	2,136,139	1,685,109	79%	47%	10
Low	13	449,451	564,871	126%		
High	5	1,174,907	877,963	75%		

[1] Calculated from natural log transformed data; sample size = 5.

[2] Size required to detect a significant difference from Control treatment based on maximum achieved absolute difference; calculated on natural log transformed data.

Vitellogenin concentrations in Control-treatment males used during the EPA 14-Day Methoxychlor assay ranged from 0 ng/mL (not detected) to 711 ng/mL (Figure 3.2). Among males exposed to the two methoxychlor concentrations, vitellogenin concentrations ranged from 0 ng/mL to 1,130,000 ng/mL. No significant differences in the mean vitellogenin concentration per treatment (Table 3.2) were detected (Kruskal-Wallis, $H = 0.31$, $p = 0.856$, df = 2). The achieved power for this endpoint was 10%, and the sample size required to detect a significant difference from the Control treatment at 80% power was 51 (Table 3.2).

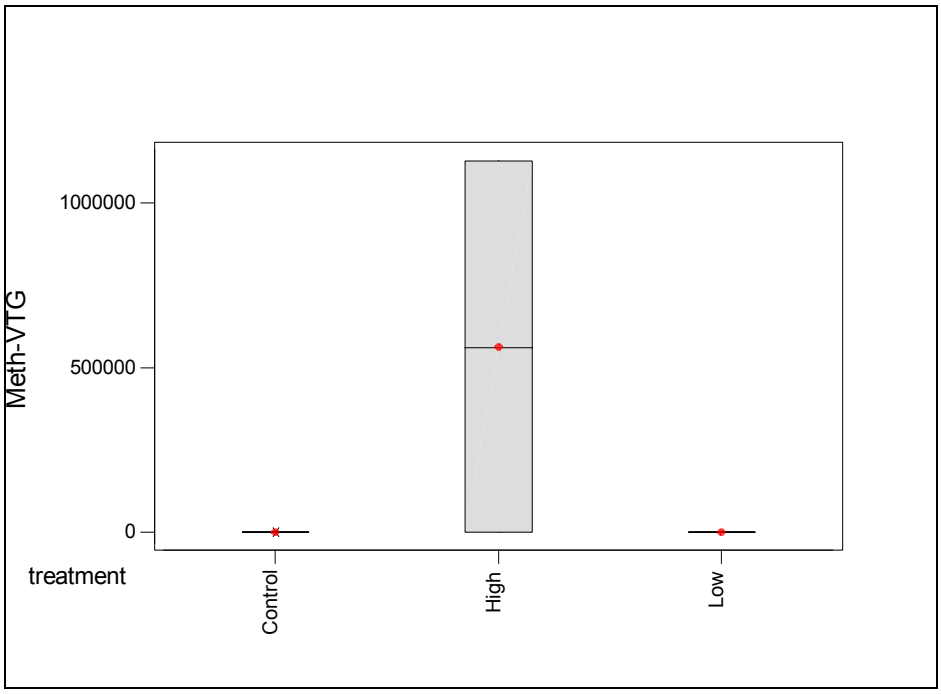

Figure **3.2.** Boxplot of male vitellogenin concentration (ng/mL) by treatment for the EPA 14-Day Methoxychlor assay. The box represents the interquartile range, whiskers represent the data range, the horizontal line is the median value, and the circle is the mean value.

Table 3.2. Summary statistics and power estimates for male vitellogenin concentrations (ng/mL) for the EPA 14-Day Methoxychlor assay.

Level	N	Mean	Stdev	CV	Achieved Power [1]	Sample Size Required [2]
Control	7	181	250	138%	10%	51
Low	7	362	509	141%		
High	4	562,500	649,532	115%		

[1] Calculated from natural log transformed data; sample size = 4.

[2] Size required to detect a significant difference from Control treatment based on maximum achieved absolute difference; calculated on natural log transformed data.

3.1.3 Appearance / Secondary Sex Characteristics

All of the females used during the EPA 14-Day Methoxychlor assay exhibited typical female morphology (no fat pad, no tubercles, ovipositor present) except that three females from the Control treatment lacked ovipositors.

Most males used during the EPA 14-Day Methoxychlor assay had typical male morphological features. One male from the Control treatment lacked a dorsal fat pad. Six males, at least one from each treatment, lacked vertical banding.

3.1.4 Gonadosomatic Index

The range of GSI values calculated for females in all treatments varied from three- to five-fold (Figure 3.3), and the overall variability within the treatment was moderate (CVs = 32%–46%) (Table 3.3). The highest female GSI values were about 24 (one fish each in the Control treatment and the High concentration). There were no significant differences in mean GSI values among treatments (Kruskal-Wallis, $H = 4.77$, $p = 0.092$, df = 2) (Table 3.3). The achieved power for this endpoint was 19%, and the sample size required to detect a significant difference from the Control treatment at 80% power was 31 (Table 3.3).

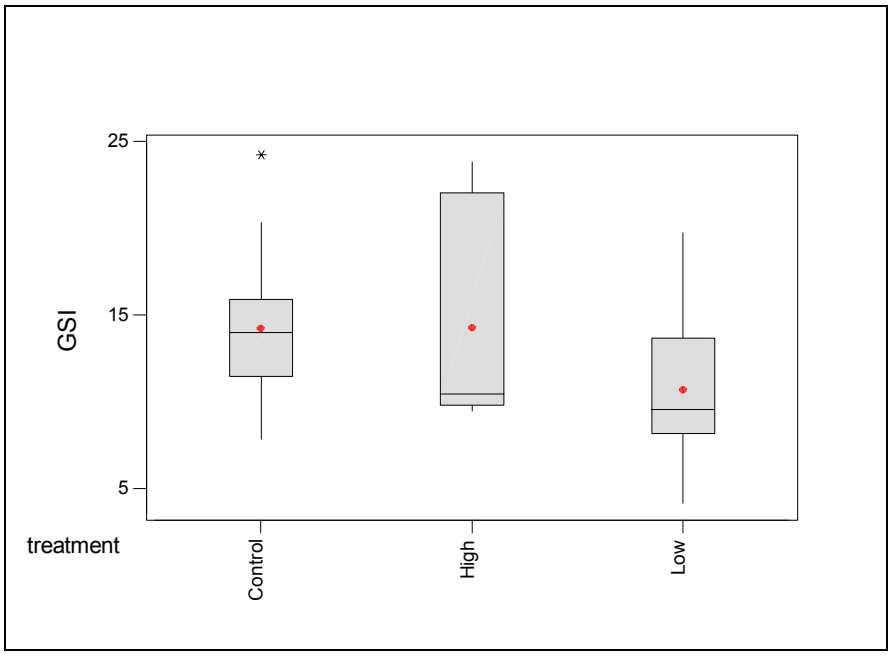

Figure 3.3. Boxplot of female GSI by treatment for the EPA 14-Day Methoxychlor assay. The box represents the interquartile range, whiskers represent the data range, the horizontal line is the median value, the circle is the mean value, and asterisks represent probable outliers.

Table 3.3. Summary statistics and power estimates for female gonadosomatic index data for the EPA 14-Day Methoxychlor assay

Level	N	Mean	SD	CV	Achieved Power [1]	Sample Size Required [2]
Control	14	14.2	4.5	32%	19%	31
Low	16	10.7	4.0	37%		
High	6	14.3	6.5	46%		

[1] Calculated from arcsine square-root transformed data; sample size = 6.

[2] Required size to detect a significant difference from Control treatment based on maximum achieved absolute difference; calculated on arcsine square-root transformed data.

The range of most GSI values calculated for males during the EPA 14-Day Methoxychlor assay was small, ranging from 0.7 to 1.7 (Figure 3.4), which approximates the typical range for reproductively-active male fathead minnows. The highest and lowest male GSI values were 1.6 to 1.7 (two fish in the Control treatment, one in the Low concentration) and 0.5 (one fish in the Low concentration), respectively. There were no significant differences in mean GSI values among treatments (Kruskal-Wallis, $H = 2.35$, $p = 0.308$, df = 2) (Table 3.4). The achieved power for this endpoint was 10%, and the sample size required to detect a significant difference from the Control treatment at 80% power was 49 (Table 3.4).

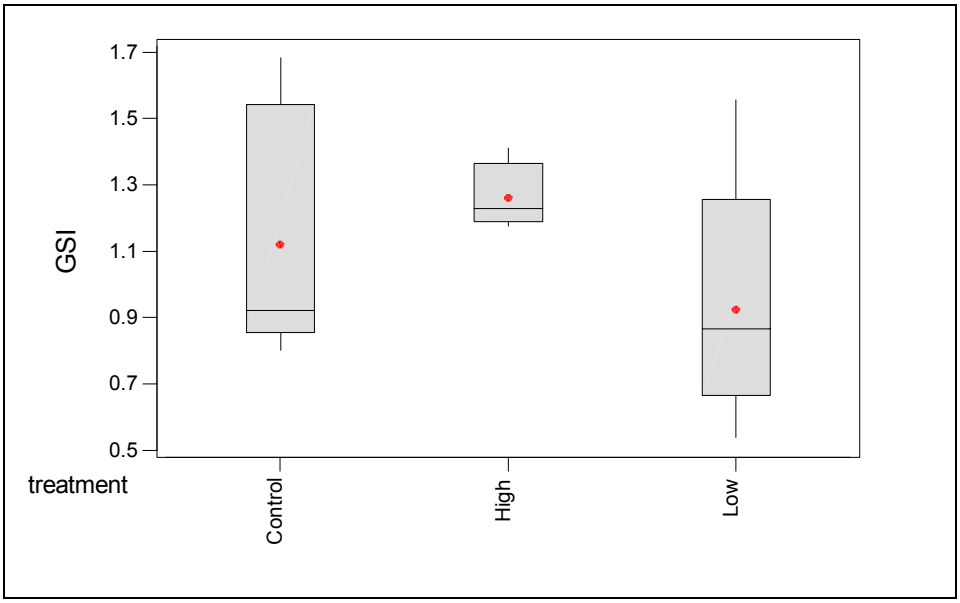

Figure 3.4. Boxplot of male GSI by treatment for the EPA 14-Day Methoxychlor assay. The box represents the interquartile range, whiskers represent the data range, the horizontal line is the median value, and the circle is the mean value.

Table 3.4. Summary statistics and power estimates for male gonadosomatic index data for the EPA 14-Day Methoxychlor assay

Level	N	Mean	SD	CV	Achieved Power [1]	Sample Size Required [2]
Control	8	1.12	0.36	32%	10%	49
Low	7	0.92	0.36	39%		
High	4	1.26	0.10	8%		

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

3.1.5 Female Gonad Histology

Histological analyses were conducted on the ovaries of 35 females exposed to methoxychlor during the EPA 14-day assay. Of these 35 fish, 12 were observed to have moderate-to-diffuse macrophage infiltration into the ovaries. Tissues from three fish with this condition were evaluated with a Gram stain and an acid-fast stain that demonstrated acid-fast-staining structures consistent with mycobacteria. All other fish with a similar macrophage infiltration in the ovaries were also presumed to have mycobacteriosis. At the time of the histological examination, it could not be determined whether the condition was exacerbated by the chemical exposure. Because Control-treatment fish were infected, it was clear that the infection was distributed throughout the population. To determine whether the infection affected the results, the analyses were conducted once with all fish included and then repeated with the infected fish excluded. The results of the second set of analyses (with infected fish removed) were reported only if they resulted in a change in the pattern of statistical significance obtained for the analyses that included all fish.

General Ovary Staging—Statistical analysis of the mean ovarian staging from 12 microscopic fields per fish revealed no significant differences among treatments (Kruskal-Wallis, $H = 4.99$, $p = 0.082$, df = 2). When infected fish were excluded from the analysis, there was a statistical difference among treatments in the mean ovarian staging (Kruskal-Wallis, $H = 10.36$, $p = 0.006$, df = 2). The mean ovarian stage of females from the High concentration was greater than that for females from the Low concentration, but not for females from the Control treatment. Therefore, there was no significant pattern associated with the methoxychlor dose.

Quantitative Ovarian Staging—One hundred cells in each of three sections per female were examined to quantitatively determine the developmental stage of the ovaries. Ova from fish in the Control and High-concentration treatments ranged from Stage 1a to Stage 5 (see Section 2: Methods for a description of the stages), whereas ova from females from the Low-concentration treatment showed Stage 1a to Stage 4 development (Figure 3.5). Variability within treatments for each stage was very high, as indicated by CVs that ranged as high as 245% (Table 3.5). Although statistical analyses showed that there was a significant difference among treatments in the proportion of cells in developmental Stage 5, there were no significant differences among treatments in the proportion of cells in the developmental Stages 1a, 1b, 2, 3, and 4 (Table 3.5). The proportion of cells in developmental Stage 5 in the Low concentration was significantly lower than that in the Control treatment. Therefore, there was no consistent pattern of significant difference associated with the methoxychlor dose. There were no changes in this pattern of statistical significance when the infected fish were excluded from the analysis.

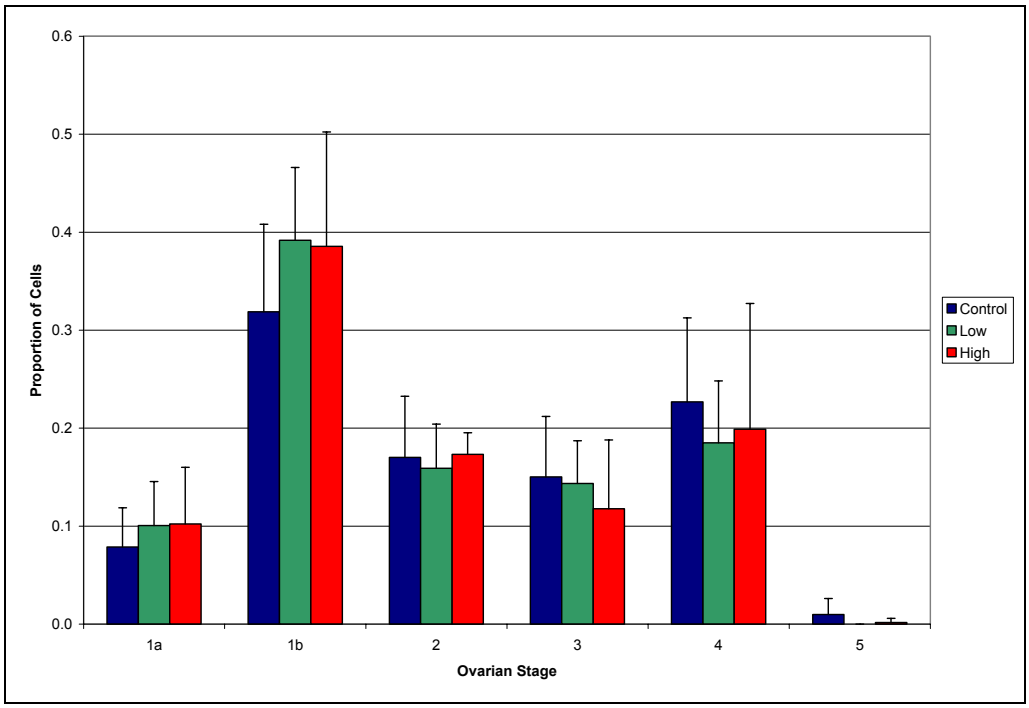

Figure 3.5. Frequency histogram showing the quantitative developmental staging of ovaries for each treatment of the EPA 14-Day Methoxychlor assay. For each treatment, the columns represent the grand mean proportion of cells in each stage and the bars represent the standard deviation.

Table 3.5. Descriptive statistics of the proportion of ovarian cells in each developmental stage for females from the EPA 14-Day Methoxychlor assay and results of the Kruskal-Wallis Test (df = 2) comparing treatments

	Control (n = 16)			Low (n = 16)			High (n = 4)			Kruskal-Wallis	
Stage	**Mean**	**SD**	**CV**	**Mean**	**SD**	**CV**	**Value**	**SD**	**CV**	***H***	***p***
1a	0.079	0.040	51%	0.101	0.045	45%	0.102	0.058	57%	1.33	0.514
1b	0.319	0.089	28%	0.392	0.074	19%	0.386	0.117	30%	4.44	0.108
2	0.170	0.062	37%	0.159	0.045	28%	0.173	0.022	13%	0.88	0.644
3	0.150	0.062	41%	0.144	0.044	30%	0.118	0.070	60%	0.53	0.765
4	0.227	0.086	38%	0.185	0.063	34%	0.199	0.128	65%	1.64	0.440
5	0.010	0.016	167%	0	0	–	0.002	0.004	245%	8.27	0.016*

* $p < 0.05$

Atretic Follicles—The mean proportion of atretic follicles per 300 follicles (counted per fish) ranged from 0.02 for females in the High concentration to 0.04 for females in the Control treatment (Figure 3.6). There were no significant differences in the proportions of atretic follicles among treatments (Kruskal-Wallis, $H = 2.17$, $p = 0.337$, df = 2). There was no change in this pattern of statistical significance when the infected fish were excluded from the analysis.

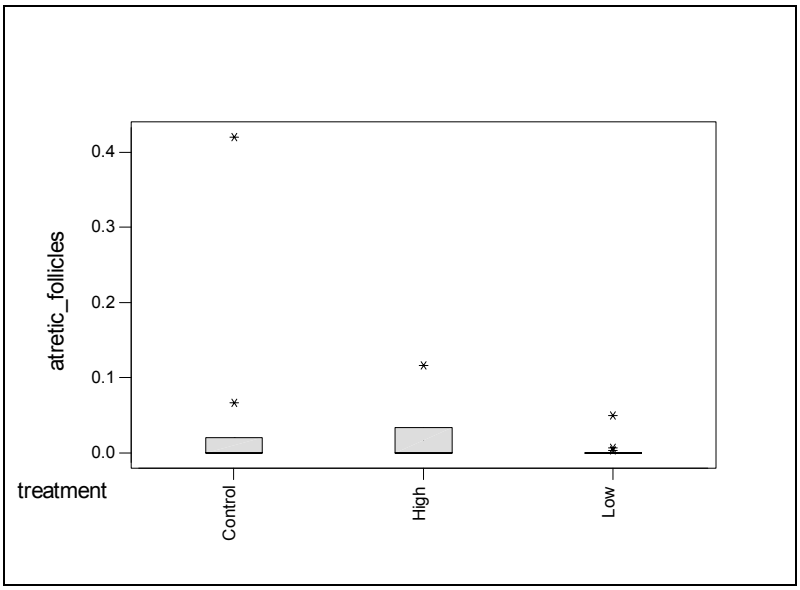

Figure 3.6. Boxplot of the proportion of atretic follicles per 300 follicles by treatment for the EPA 14-Day Methoxychlor assay. The box represents the interquartile range, whiskers represent the data range, the horizontal line is the median value, and asterisks represent probable outliers.

Corpora Lutea—The mean proportion of corpora lutea per 300 follicles (counted per fish) ranged from none for females in the High concentration to 0.06 for females in the Low concentration (Figure 3.7). There was a significant difference in the proportions of corpora lutea among treatments (Kruskal-Wallis, $H = 6.78$, $p = 0.034$, df = 2). The value for the High concentration was significantly lower than those of the other two treatments. There was no change in this pattern of statistical significance when the infected fish were excluded from the analysis.

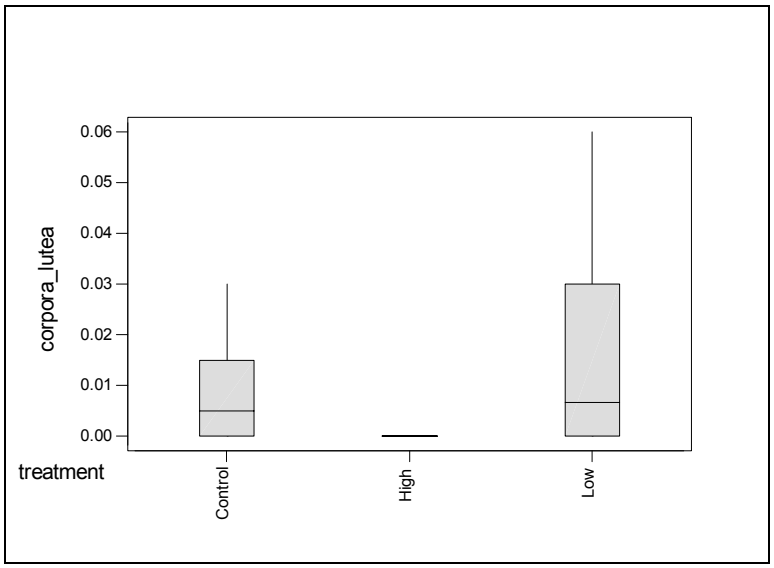

Figure 3.7. Boxplot of the proportion of corpora lutea per 300 follicles by treatment for the EPA 14-Day Methoxychlor assay. The box represents the interquartile range, whiskers represent the data range, and the horizontal line is the median value.

3.1.6 Male Gonad Histology

Testes Staging by Microscopic Field—Testes from males exposed to methoxychlor during the EPA 14-Day Methoxychlor assay were examined to determine their general developmental condition. Males in all treatments had well-developed testes with most showing Stage 4 and Stage 5 development (see Section 2: Methods for description of developmental stages). All of the 96 microscopic fields examined in the 8 Control-treatment males showed Stage 4 (72 fields) or Stage 5 (24 fields) development. All of the 84 fields examined in the 7 Low-concentration treatment males showed Stage 4 development. In the 4 High-concentration males available for examination, all 48 microscopic fields examined showed Stage 4 (42 fields) or Stage 5 (6 fields) development. Statistical analysis of the mean staging from 12 sections per fish revealed no significant differences among treatments (Kruskal-Wallis, $H = 1.99$, $p = 0.371$, 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 of the Control-treatment testes ranged from Stage 1 to Stage 5, whereas testes in males from the Low- and High-concentration treatments showed Stage 2a to Stage 5 development (Figure 3.8). Variability within treatments for each stage was very high, as indicated by CVs that ranged as high as 209% (Table 3.6). Statistical analyses showed that there were no significant differences among treatments in the proportion of cells in any developmental Stage (Table 3.6).

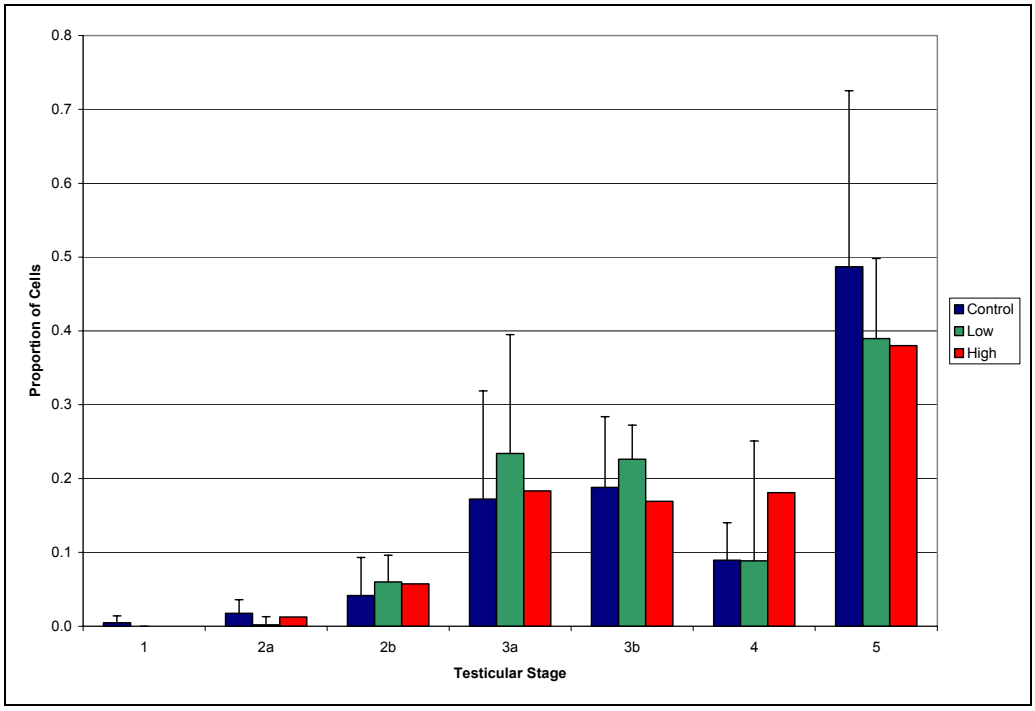

Figure 3.8. Frequency histogram showing the quantitative developmental staging of testes for each treatment of the EPA 14-Day Methoxychlor assay. For each treatment, the columns represent the grand mean proportion of cells in each stage and the bars represent the standard deviation.

Table 3.6. Descriptive statistics of the proportion of testes cells in each developmental stage for males from the EPA 14-Day Methoxychlor assay and results of the Kruskal-Wallis Test (df = 2) comparing treatments

	Control (n = 8)			Low (n = 7)			High (n = 4)			Kruskal-Wallis	
Stage	**Mean**	**SD**	**CV**	**Mean**	**SD**	**CV**	**Value**	**SD**	**CV**	***H***	***p***
1	0.005	0.010	209%	0	–	–	0	–	–	2.90	0.234
2a	0.018	0.019	106%	0.002	0.013	199%	0.013	0.011	88%	4.24	0.120
2b	0.042	0.051	123%	0.060	0.058	154%	0.058	0.036	63%	1.15	0.562
3a	0.172	0.147	85%	0.234	0.183	36%	0.183	0.161	88%	1.03	0.597
3b	0.188	0.096	51%	0.226	0.169	32%	0.169	0.046	27%	2.30	0.317
4	0.090	0.050	56%	0.089	0.181	28%	0.181	0.162	90%	0.30	0.859
5	0.487	0.239	49%	0.390	0.380	41%	0.380	0.109	29%	0.72	0.696

Tubule Diameter—The diameter of the seminiferous tubules of males from the Control treatment ranged from 86.4 µm to 181.7 µm (Figure 3.9). Tubule diameters of males from the two test concentrations ranged from 78.3 µm to 155.6 µm. No significant differences in the mean tubule diameter per treatment were detected (Kruskal-Wallis, $H = 3.24$, $p = 0.198$, df = 2) (Table 3.7). 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 30 (Table 3.7).

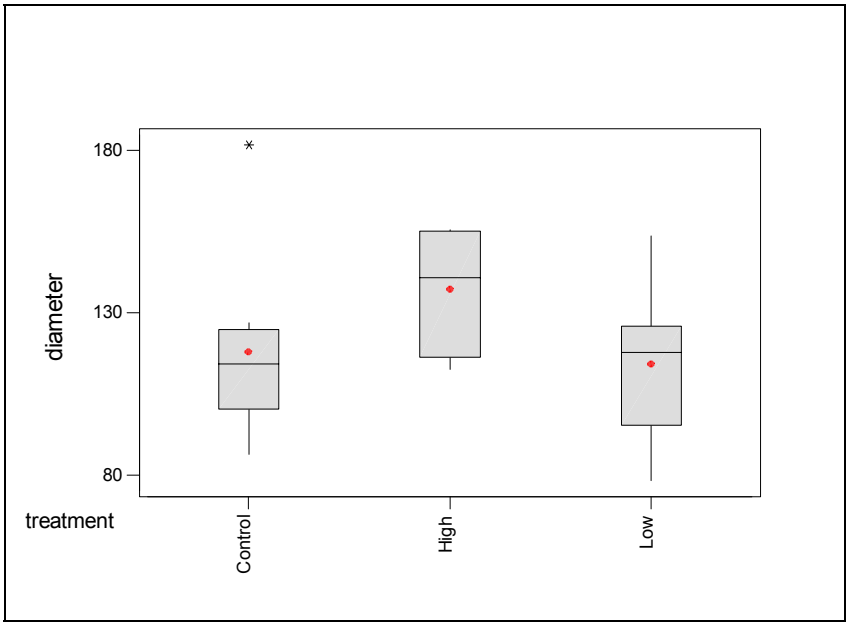

Figure 3.9. Boxplot of seminiferous tubule diameter (µm) by treatment for the EPA 14-Day Methoxychlor assay. The box represents the interquartile range, whiskers represent the data range, the horizontal line is the median value, the circle is the mean value, and asterisks represent probable outliers.

Table 3.7. Summary statistics and power estimates for male seminiferous tubule diameter data for the EPA 14-Day Methoxychlor assay

Level	N	Mean	SD	CV	Achieved Power [1]	Sample Size Required [2]
Control	8	118.0	28.7	24%	13%	30
Low	7	114.2	24.3	21%		
High	4	137.4	21.0	15%		

[1] Calculated from natural log transformed data; sample size = 4.

[2] Required size to detect a significant difference from Control treatment based on maximum achieved absolute difference; calculated on natural log transformed data.

Observations—No Sertoli cell or Leydig cell proliferation was observed. No testicular atrophy was recorded, and no ovatestes were observed for any treatment.

3.1.7 Plasma Steroid Concentrations

Estradiol—Estradiol concentrations in Control-treatment females used during the EPA 14-Day Methoxychlor assay ranged from 0 pg/mL (not detected) to 4,643 pg/mL (Figure 3.10). Among females exposed to the two methoxychlor concentrations, estradiol concentrations ranged from 0 pg/mL (not detected) to 1,349 pg/mL. No significant differences in the mean estradiol concentration per treatment (Table 3.8) were detected (Kruskal-Wallis, $H = 5.94$, $p = 0.051$, df = 2). However, the calculated probability value was only slightly above the critical limit of 0.050. The greatest difference in mean estradiol concentration was between females from the Low concentration (353 pg/mL) and those from the Control treatment (1,670 pg/mL). The achieved power for this endpoint was 20%, and the sample size required to detect a significant difference from the Control treatment at 80% power was 28 (Table 3.8).

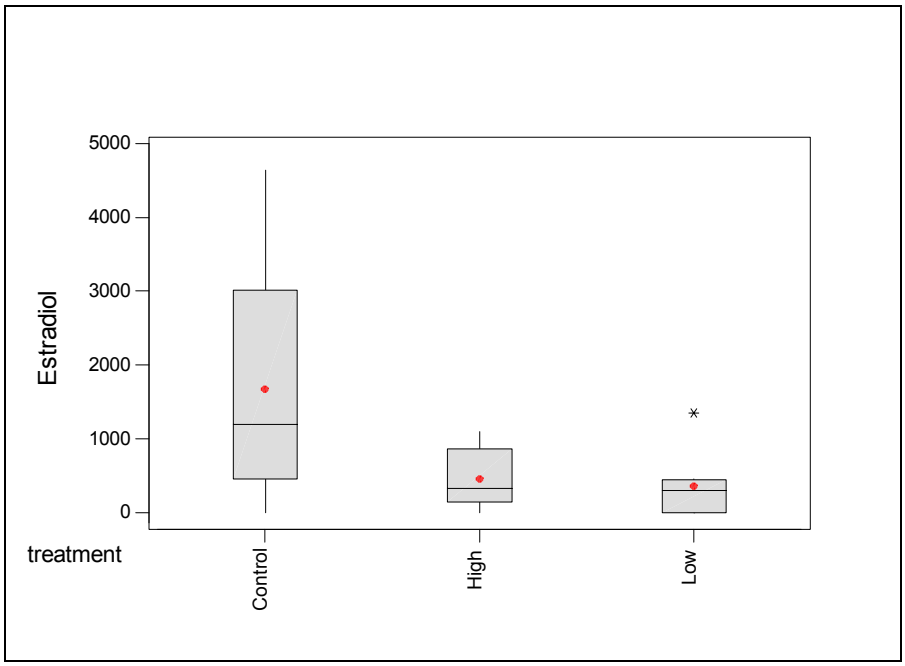

Figure 3.10. Boxplot of female estradiol concentration (pg/mL) by treatment for the EPA 14-Day Methoxychlor assay. The box represents the interquartile range, whiskers represent the data range, the horizontal line is the median value, the circle is the mean value, and the asterisk represents a probable outlier.

Table 3.8. Summary statistics and power estimates for female estradiol concentrations (pg/mL) for the EPA 14-Day Methoxychlor assay.

Level	N	Mean	Stdev	CV	Achieved Power [1]	Sample Size Required [2]
Control	9	1,670	1,568	94%	20%	28
Low	9	353	418	118%		
High	6	455	411	90%		

[1] Calculated from natural log transformed data; sample size = 6.

[2] Size required to detect a significant difference from Control treatment based on maximum achieved absolute difference; calculated on natural log transformed data.

Estradiol concentrations in Control-treatment males used during the EPA 14-Day Methoxychlor assay ranged from 191 pg/mL to 518 pg/mL (Figure 3.11). Among males exposed to the two methoxychlor concentrations, estradiol concentrations ranged from 0 pg/mL (not detected) to 177 pg/mL. A significant difference in the mean estradiol concentration per treatment (Table 3.9) was detected (Kruskal-Wallis, $H = 9.18$, $p = 0.010$, df = 2). The mean estradiol concentration in males from the Low concentration was less than that in males from the Control treatment. An estradiol concentration was obtainable from only one male exposed to the High concentration of methoxychlor. This value (155 pg/mL) was much less than the mean Control-treatment value (299 pg/mL). The achieved power for this endpoint was 11%, and the sample size required to detect a significant difference from the Control treatment at 80% power was 12 (Table 3.9).

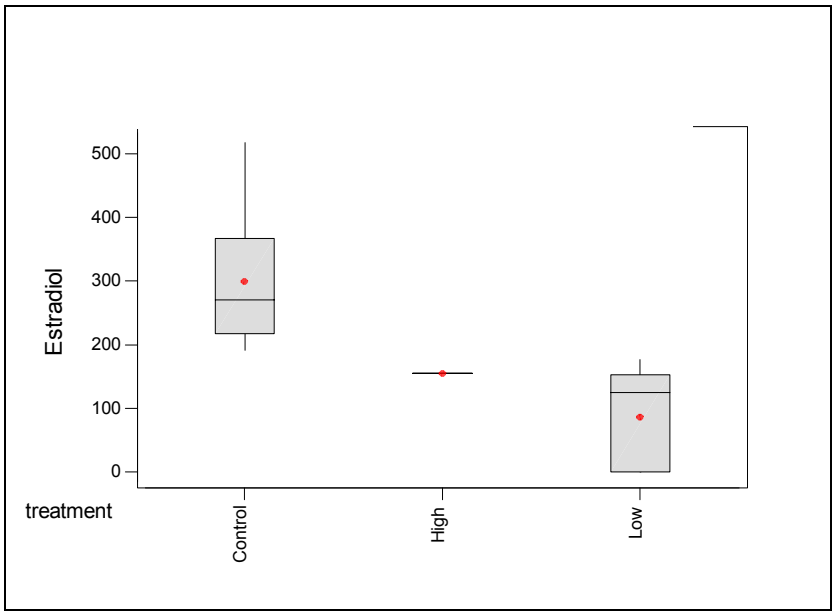

Figure 3. 11. Boxplot of male estradiol concentration (pg/mL) by treatment for the EPA 14-Day Methoxychlor assay. The box represents the interquartile range, whiskers represent the data range, the horizontal line is the median value, and the circle is the mean value.

Table 3.9. Summary statistics and power estimates for male estradiol concentrations (pg/mL) for the EPA 14-Day Methoxychlor assay.

Level	N	Mean	Stdev	CV	Achieved Power [1]	Sample Size Required [2]
Control	7	299	111	37%	11%	12
Low	5	86	81	94%		
High	1	155				

[1] Calculated from natural log transformed data; sample size = 2 (smallest allowable).
[2] Size required to detect a significant difference from Control treatment based on maximum achieved absolute difference; calculated on natural log transformed data.

Testosterone—Testosterone concentrations in Control-treatment females used during the EPA 14-Day Methoxychlor assay ranged from 0 pg/mL (not detected) to 8,269 pg/mL (Figure 3.12). Among females exposed to the two methoxychlor concentrations, testosterone concentrations ranged from 0 pg/mL (not detected) to 7,931 pg/mL. No significant differences in the mean testosterone concentration per treatment (Table 3.10) were detected (Kruskal-Wallis, $H = 0.11$, $p = 0.947$, 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 602 (Table 3.10).

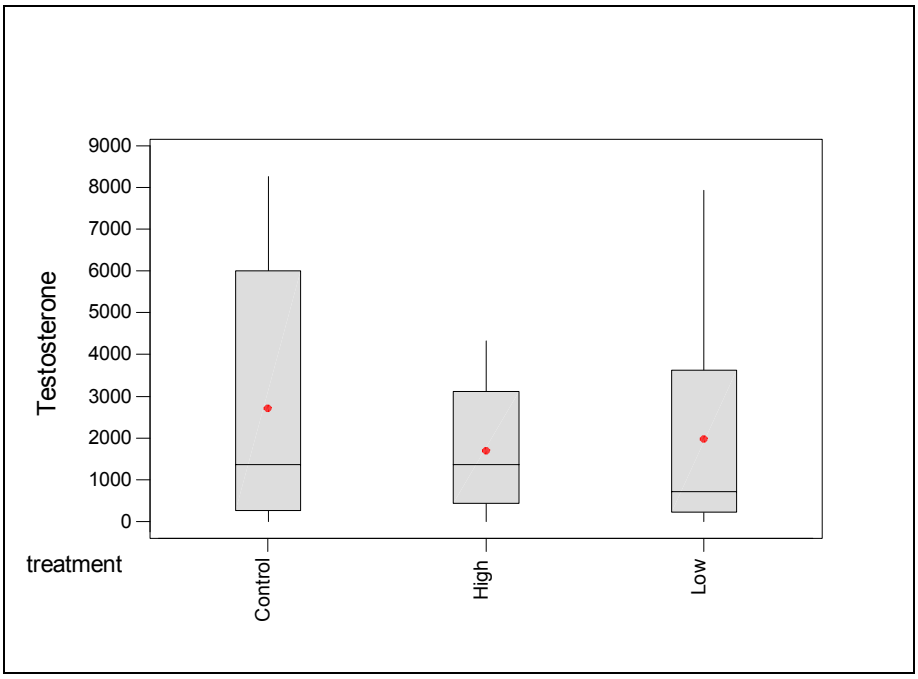

Figure 3.12. Boxplot of female testosterone concentration (pg/mL) by treatment for the EPA 14-Day Methoxychlor assay. The box represents the interquartile range, whiskers represent the data range, the horizontal line is the median value, and the circle is the mean value.

Table 3.10. Summary statistics and power estimates for female testosterone concentrations (pg/mL) for the EPA 14-Day Methoxychlor assay.

Level	N	Mean	Stdev	CV	Achieved Power [1]	Sample Size Required [2]
Control	9	2,719	3,284	121%	5%	602
Low	6	1,972	3,016	153%		
High	5	1,689	1,632	97%		

[1] Calculated from natural log transformed data; sample size = 5.
[2] Size required to detect a significant difference from Control treatment based on maximum achieved absolute difference; calculated on natural log transformed data.

Testosterone concentrations in Control-treatment males used during the EPA 14-Day Methoxychlor assay ranged from 940 pg/mL to 10,682 pg/mL (Figure 3.13). Among males exposed to the two methoxychlor concentrations, testosterone concentrations ranged from 0 pg/mL (not detected) to 4,546 pg/mL. No significant differences in the mean testosterone concentration per treatment (Table 3.11) were detected (Kruskal-Wallis, $H = 2.10$, $p = 0.349$, df = 2). The achieved power for this endpoint was 20%, and the sample size required to detect a significant difference from the Control treatment at 80% power was 12 (Table 3.11).

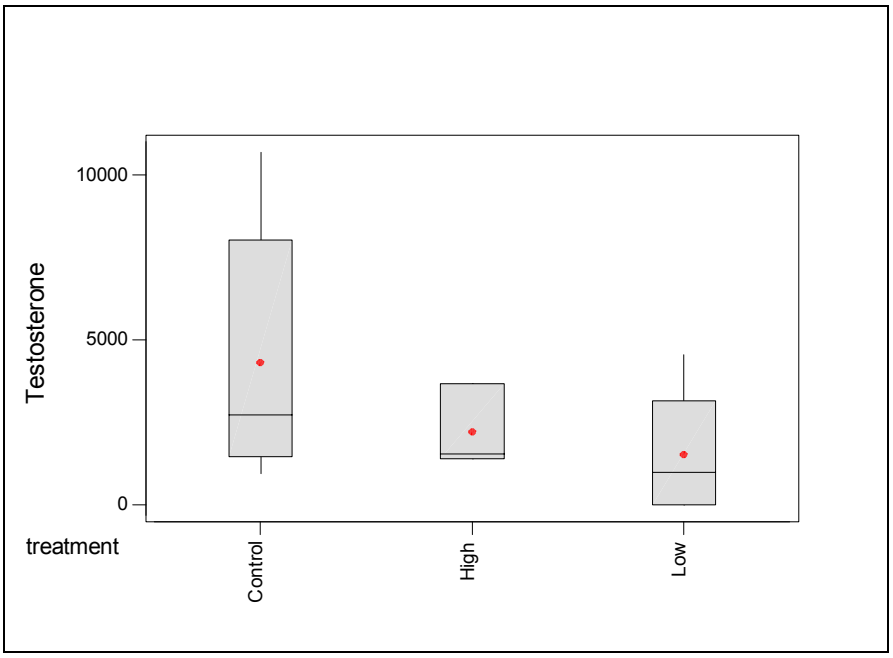

Figure 3.13. Boxplot of male testosterone concentration (pg/mL) by treatment for the EPA 14-Day Methoxychlor assay. The box represents the interquartile range, whiskers represent the data range, the horizontal line is the median value, and the circle is the mean value.

Table 3.11. Summary statistics and power estimates for male testosterone concentrations (pg/mL) for the EPA 14-Day Methoxychlor assay.

Level	N	Mean	Stdev	CV	Achieved Power [1]	Sample Size Required [2]
Control	6	4,307	3,854	89%	20%	12
Low	6	1,531	1,843	120%		
High	3	2,202	1,279	58%		

[1] Calculated from natural log transformed data; sample size = 3.

[2] Size required to detect a significant difference from Control treatment based on maximum achieved absolute difference; calculated on natural log transformed data.

11-Ketotestosterone—11-ketotesosterone was only detected in one of six Control-treatment females used during the EPA 14-Day Methoxychlor assay (Figure 3.14). Among females exposed to the two methoxychlor concentrations, 11-ketotesosterone was only detected in one individual from each treatment (four females analyzed for each treatment). No significant differences in the mean 11-ketotesosterone concentration per treatment (Table 3.12) were detected (Kruskal-Wallis, $H = 0.05$, $p = 0.977$, 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 691 (Table 3.12).

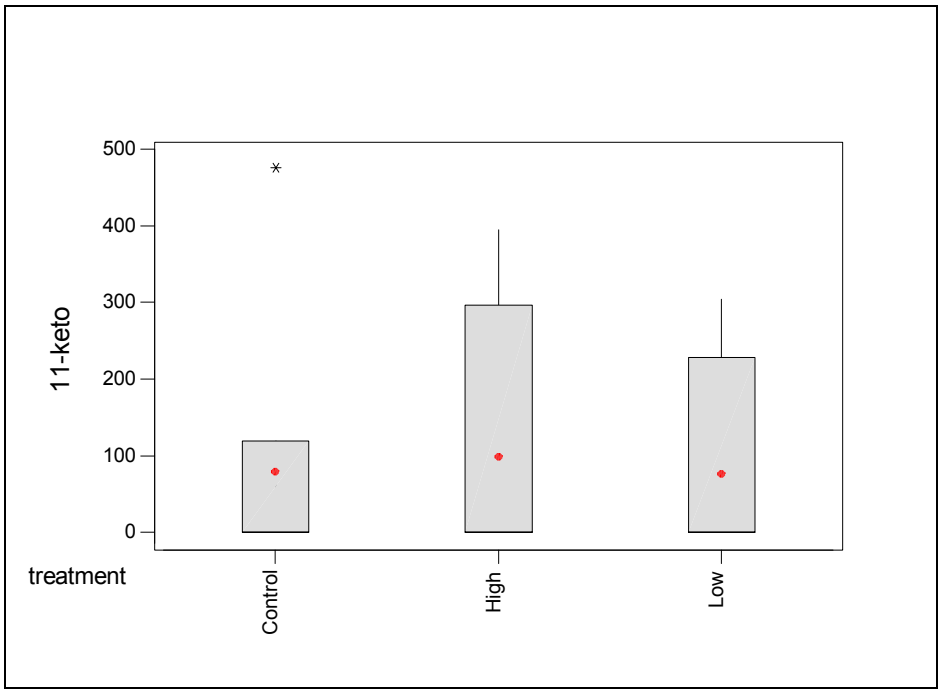

Figure 3.14. Boxplot of female 11-ketotesosterone concentration (pg/mL) by treatment for the EPA 14-Day Methoxychlor assay. The box represents the interquartile range, whiskers represent the data range, the horizontal line is the median value, the circle is the mean value, ad the asterisk represents a probable outlier.

Table 3.12. Summary statistics and power estimates for female 11-ketotesosterone concentrations (pg/mL) for the EPA 14-Day Methoxychlor assay.

Level	N	Mean	Stdev	CV	Achieved Power [1]	Sample Size Required [2]
Control	6	79	194	245%	5%	691
Low	4	76	152	200%		
High	4	99	197	200%		

[1] Calculated from natural log transformed data; sample size = 4.
[2] Size required to detect a significant difference from Control treatment based on maximum achieved absolute difference; calculated on natural log transformed data.

11-Ketotesosterone concentrations in Control-treatment males used during the EPA 14-Day Methoxychlor assay ranged from 7,660 pg/mL to 53,265 pg/mL (Figure 3.15). Among males exposed to the two methoxychlor concentrations, 11-ketotesosterone concentrations ranged from 1,660 pg/mL to 25,610 pg/mL. No significant differences in the mean 11-ketotesosterone concentration per treatment (Table 3.13) were detected (Kruskal-Wallis, $H = 1.51$, $p = 0.471$, df = 2). The achieved power for this endpoint was 14%, and the sample size required to detect a significant difference from the Control treatment at 80% power was 18 (Table 3.13).

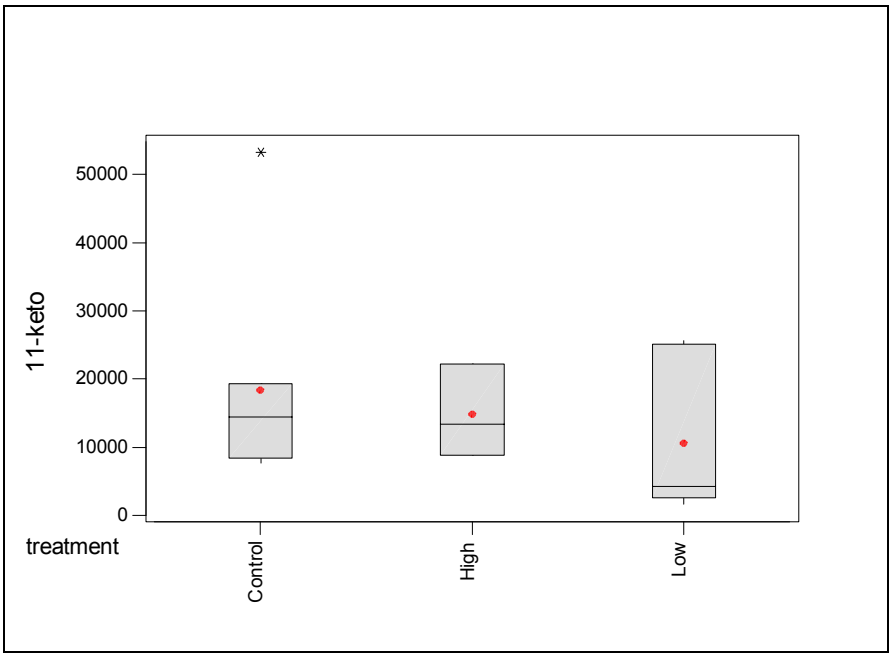

Figure 3.15. Boxplot of male 11-ketotesosterone concentration (pg/mL) by treatment for the EPA 14-Day Methoxychlor assay. The box represents the interquartile range, whiskers represent the data range, the horizontal line is the median value, the circle is the mean value, and the asterisk represents a probable outlier.

Table 3.13. Summary statistics and power estimates for male 11-ketotesosterone concentrations (pg/mL) for the EPA 14-Day Methoxychlor assay.

Level	N	Mean	Stdev	CV	Achieved Power [1]	Sample Size Required [2]
Control	8	18,346	14,922	81%	14%	18
Low	7	10,613	10,576	100%		
High	3	14,812	6,803	46%		

[1] Calculated from natural log transformed data; sample size = 3.
[2] Size required to detect a significant difference from Control treatment based on maximum achieved absolute difference; calculated on natural log transformed data.

3.1.8 Body Weight

The body weight of females used in the EPA 14-Day Methoxychlor assay ranged from 0.8 g to 2.3 g. There were no significant differences in mean body weight among treatments (Kruskal-Wallis, $H = 3.42$, $p = 0.181$, df = 2). The body weight of males used in the EPA 14-Day Methoxychlor assay ranged from 2.3 g to 5.3 g. There were no significant differences in mean body weight among treatments (Kruskal-Wallis, $H = 0.59$, $p = 0.744$, df = 2).

3.1.9 Fecundity

Total Fecundity—Variability among treatments in the total number of eggs produced during the EPA 14-Day Methoxychlor assay was very high (Figure 3.16). Total counts in the Control treatment ranged

more than five-fold, varying from 686 eggs to 3,852 eggs. Total counts for three replicates of the High methoxychlor concentration treatment were similar, ranging from 124 eggs to 311 eggs, whereas the total number of eggs produced in the fourth replicate was 1,360. Both males in two of the three High methoxychlor replicates with low total egg counts died after Day 6 or Day 7 and each tank was terminated. In the third replicate having low egg numbers, eggs were laid only on Day 2 and Day 6. There was about a three-fold difference between the minimum and maximum number of eggs produced among replicates in the Low methoxychlor concentration. No significant differences in the mean number of eggs (square-root transformed) produced per treatment were detected (1-way ANOVA, $F = 3.32$, $p = 0.083$, df = 2, 9) (Table 3.14). The achieved power for this assay was 39%, and the sample size required to detect a significant difference from the Control treatment at 80% power was 9 (Table 3.14).

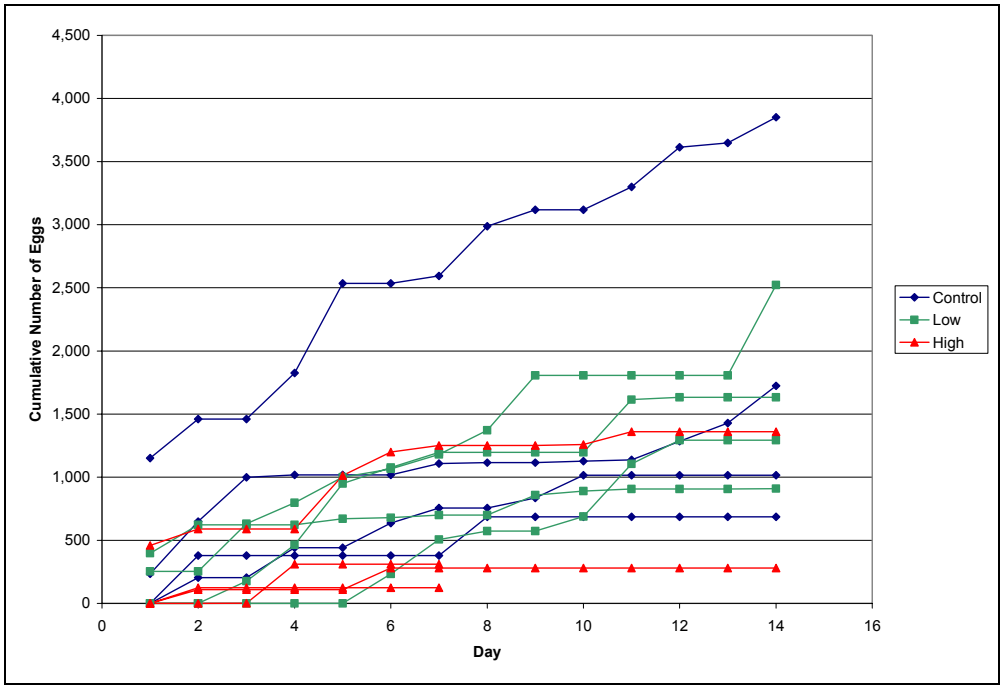

Figure 3.16. Total Egg Production by Replicate per Treatment for the EPA 14-Day Methoxychlor Assay

Table 3.14. Summary Statistics and Power Estimates for Fecundity Data for the EPA 14-Day Methoxychlor Assay

Level	N	Mean	SD	CV	Achieved Power [1]	Sample Size Required [2]
Control	4	1820	1423	78%	39%	9
Low	4	1590	689	43%		
High	4	519	567	109%		

[1] Calculated from arcsine square-root transformed data; sample size = 4.

[2] Required size to detect a significant difference from Control treatment based on maximum achieved absolute difference; calculated on arcsine square-root transformed data.

Fecundity per Female Reproductive Day—During the EPA 14-Day Methoxychlor assay, the maximum number of female reproductive days was achieved for the Low concentration, whereas 55.3 female reproductive days were achieved in the Control treatment and 40.0 female reproductive days (71% of the maximum) were achieved in the High concentration (Table 3.15). The number of eggs produced per female reproductive day in the Control treatment varied from 12.3 eggs to 68.8 eggs and from 16.4 eggs to 45.1 eggs in the Low concentration (Figure 3.17). For the High concentration, the number of eggs produced per female reproductive day ranged from 4.4 eggs to 24.7 eggs, with fish in three of the replicates producing fewer than 15 eggs per day (4.4, 5.3, 13.0). No significant difference in the number of eggs produced per female reproductive day among treatments were detected (Kruskal-Wallis, $H = 3.50$, $p = 0.174$, df = 2). The achieved power for this assay was 36%, and the sample size required to detect a significant difference from the Control treatment at 80% power was 10 (Table 3.15).

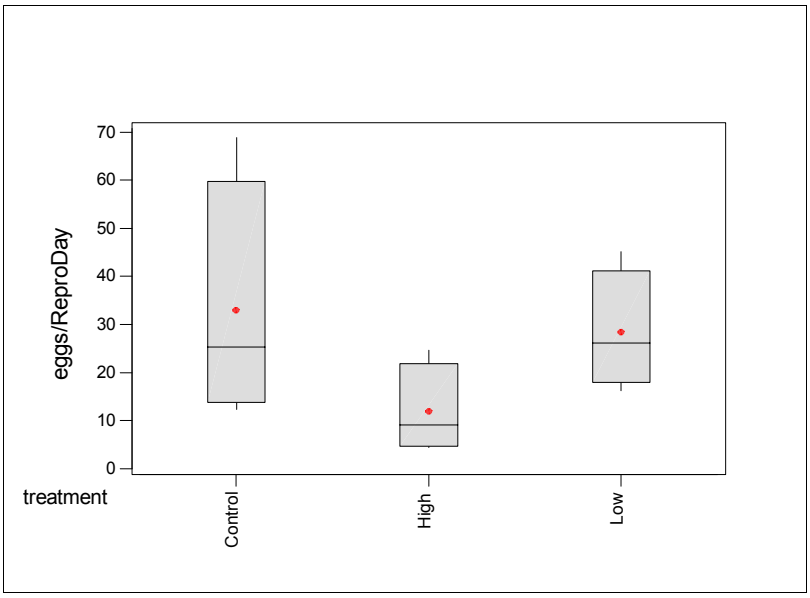

Figure 3.17. Boxplot of the Number of Eggs Produced per Female Reproductive Day by Treatment for the EPA 14-Day Methoxychlor Assay. The box represents the interquartile range, whiskers represent the data range, the horizontal line is the median value, and the circle is the mean value.

Table 3.15. Summary Statistics and Power Estimates for Fecundity per Female Reproductive Day for the EPA 14-Day Methoxychlor Assay

Level	Mean Number of Reproductive Days [1]	N	Mean	SD	CV	Achieved Power [2]	Sample Size Required [3]
Control	55.3	4	32.9	25.4	77%	36%	10
Low	56.0	4	28.4	12.3	43%		
High	40.0	4	11.9	9.4	79%		

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

Eggs on Tiles/Dishes—The mean number of eggs laid on the tiles among the treatments during the EPA 14-Day Methoxychlor assay varied from 442 eggs for the High concentration to 1,574 eggs for the Control treatment (Appendix E, Table 1.3). The number of eggs on dishes ranged from 77 eggs for the High concentration to 245 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. The proportional difference ranged from 0.25 (one High-concentration replicate) to 0.97 (one Control-treatment replicate) (Appendix E, Figure 1.2). There were no significant differences in this mean proportional difference among treatments (Kruskal-Wallis, $H = 0.73$, $p = 0.694$, df = 2).

3.1.10 Fertilization Success

Total Fertilization—The total (tiles and dishes) fertilization-success rates for most treatment replicates during the EPA 14-Day Methoxychlor assay were high, ranging from 0.932 (one Control-treatment replicate) to 1.00 (two High-concentration replicates, one Control-treatment replicate) (Figure 3.18). The fertilization-success rate for eggs in one High concentration was very low, 0.557. No significant differences in mean fertilization-success rates (Table 3.16) among treatments were detected (Kruskal-Wallis, $H = 0.47$, $p = 0.791$, df = 2). The achieved power for this assay was 8%, and the sample size required to detect a significant difference from the Control treatment at 80% power was 78 (Table 3.16).

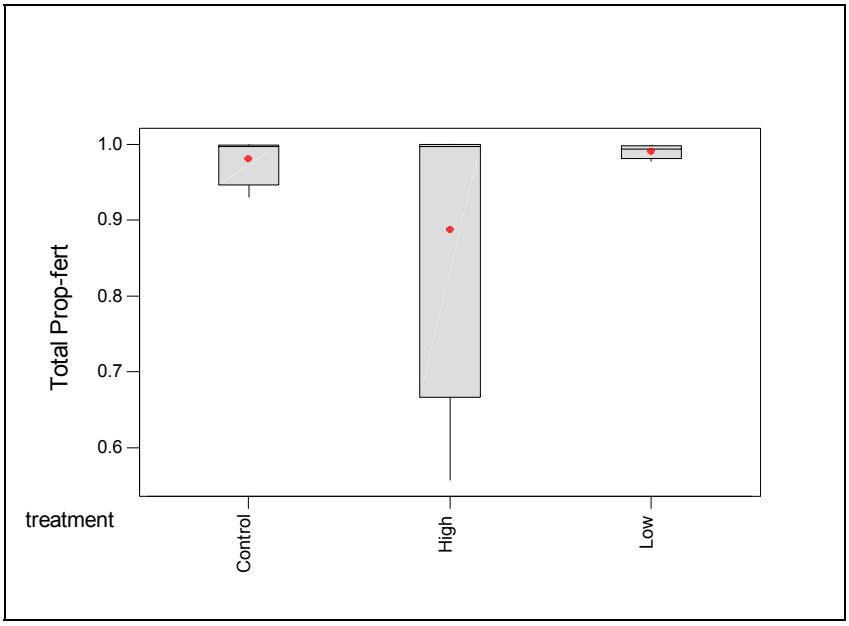

Figure 3.18. Boxplot of the Proportion of Eggs Fertilized by Treatment for the EPA 14-Day Methoxychlor Assay. The box represents the interquartile range, whiskers represent the data range, the horizontal line is the median value, and the circle is the mean value.

Table 3.16. Summary Statistics and Power Estimates for the Proportion of Eggs Fertilized for the EPA 14-Day Methoxychlor Assay

Level	N	Mean	SD	CV	Achieved Power [1]	Sample Size Required [2]
Control	4	0.981	0.034	3%	8%	78
Low	4	0.991	0.009	1%		
High	4	0.888	0.221	25%		

[1] Calculated from arcsine square-root transformed data; sample size = 4.

[2] Required size to detect a significant difference from Control treatment based on maximum achieved absolute difference; calculated on arcsine square-root transformed data.

Fertilization of Eggs on Tiles and Dishes— The fertilization-success rates for most treatment replicates for eggs laid on tiles during the EPA 14-Day Methoxychlor assay were high, ranging from 0.911 (one Control-treatment replicate) to 1.00 (two High-concentration replicates, one Control-treatment replicate) (Appendix E, Figure 1.3). One High-concentration replicate had a very low fertilization-success rate, 0.294. No significant differences in mean fertilization-success rates (Appendix E, Table 1.4) among treatments were detected (Kruskal-Wallis, $H = 0.27$, $p = 0.872$, df = 2). The fertilization-success rates for all treatment replicates for eggs laid on dishes during the assay were high, ranging from 0.908 (one High-concentration replicate) to 1.00 (several replicates; including all treatments) (Appendix E, Figure 1.4). No significant differences in mean fertilization-success rates (Appendix E, Table 1.4) among treatments were detected (Kruskal-Wallis, $H = 1.11$, $p = 0.573$, df = 2).

3.1.11 Hatchability and Larval Development

Eggs were collected during the pre-exposure period for the evaluation of hatchability. The proportion of fertilized eggs that hatched was 1.00 for all tanks in the Control treatment and Low concentration. The mean proportion of fertilized eggs that hatched in the High concentration was 0.99 (sd = 0.01). The proportion of fertilized eggs that hatched in the tanks evaluated during the pre-exposure period but not used in the 14-day assay was 1.00 in all tanks. There were no significant differences among treatments in the proportion of eggs that hatched (Kruskal-Wallis, $H = 2.75$, $p = 0.432$, df = 3).

Eggs were collected on Days 7 through 9 and Day 12 during the EPA 14-Day Methoxychlor assay for the evaluation of hatchability. The proportion of fertilized eggs that hatched ranged from 0.67 to 1.00 in the Control treatment and from 0.84 to 0.94 for the two test concentrations (Figure 3.19). There were no significant differences among treatments in the proportion of eggs that hatched (Kruskal-Wallis, $H = 1.68$, $p = 0.432$, df = 2). The achieved power for this endpoint was 7%, and the sample size required to detect a significant difference from the Control treatment at 80% power was 37 (Table 3.17).

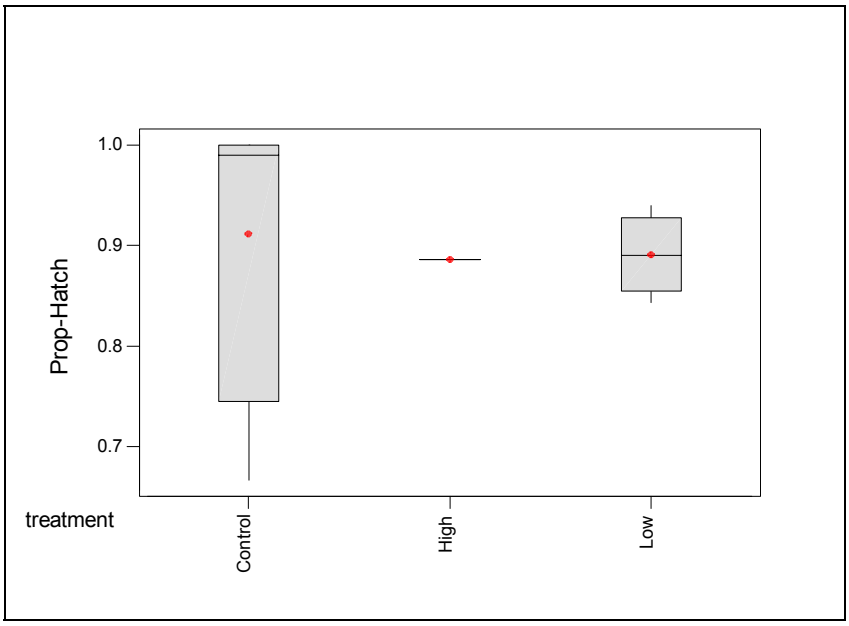

Figure 3.19. Boxplot of the proportion of fertile eggs that hatched by treatment for the EPA 14-Day Methoxychlor assay. The box represents the interquartile range, whiskers represent the data range, the horizontal line is the median value, and the circle is the mean value.

Table 3.17. Summary statistics and power estimates for the proportion of fertile eggs that hatched for the EPA 14-Day Methoxychlor assay

Level	N	Mean	SD	CV	Achieved Power [1]	Sample Size Required [2]
Control	4	0.91	0.16	18%	7%	37
Low	4	0.89	0.04	4%		
High	1	0.89	–	–		

[1] Calculated from arcsine square-root transformed data; sample size = 2.

[2] Required size to detect a significant difference from Control treatment based on maximum achieved absolute difference; calculated on arcsine square-root transformed data.

Eggs were collected during the pre-exposure period for the evaluation of larval development. The proportion of larvae that developed normally (i.e., that showed no morphological abnormalities) was 1.00 for all tanks in the Control treatment. The mean proportion of normal larvae in the remaining treatments was 1.00 (sd = 0) in the Low concentration and 0.99 (sd = 0.01) in the High concentration. The mean proportion of normal larvae in the tanks evaluated during the pre-exposure period but not used in the 14-day assay was 1.00 (sd = 0). There were no significant differences among treatments in the proportion of normal larvae (Kruskal-Wallis, $H = 2.46$, $p = 0.483$, df = 3).

Eggs were collected on Days 7 through 9 and Day 12 during the EPA 14-Day Methoxychlor assay for the evaluation of larval development. The proportion of larvae that developed normally (i.e., that showed no morphological abnormalities) was 1.00 for all replicates of the Control treatment and ranged from 0.93 to 1.00 for the two test concentrations (Figure 3.20). There were no significant differences among treatments in the proportion of normal larvae (Kruskal-Wallis, $H = 5.00$, $p = 0.082$, df = 2). The achieved power for this endpoint was 19%, and the sample size required to detect a significant difference from the Control treatment at 80% power was 6 (Table 3.18).

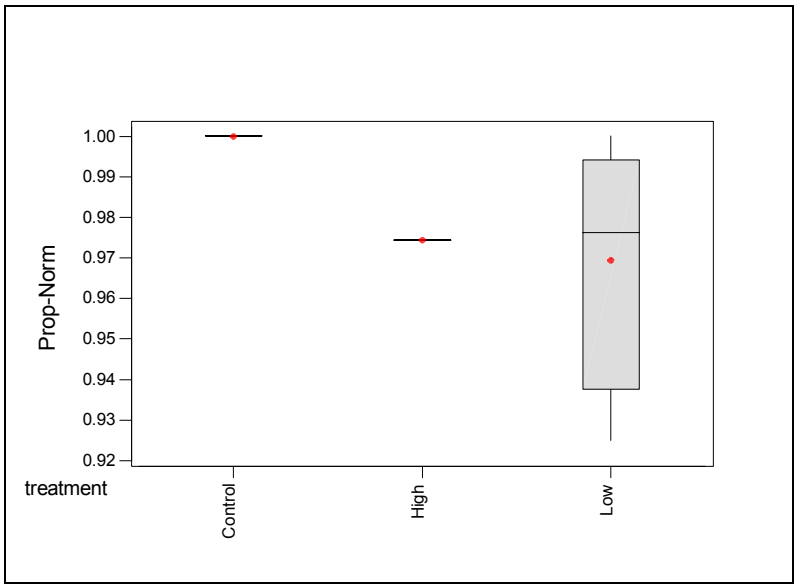

Figure 3.20. Boxplot of the proportion of normal larvae by treatment for the EPA 14-Day Methoxychlor assay. The box represents the interquartile range, whiskers represent the data range, the horizontal line is the median value, and the circle is the mean value.

Table 3.18. Summary statistics and power estimates for the proportion of normal larvae for the EPA 14-Day Methoxychlor assay

Level	N	Mean	SD	CV	Achieved Power [1]	Sample Size Required [2]
Control	4	1.00	0	0%	19%	6
Low	4	0.97	0.03	3%		
High	1	0.97	–	–		

[1] Calculated from arcsine square-root transformed data; sample size = 2.

[2] Required size to detect a significant difference from Control treatment based on maximum achieved absolute difference; calculated on arcsine square-root transformed data.

3.2 EPA 21-Day Assay for Methoxychlor

The EPA 21-Day Methoxychlor assay was conducted from September 29, 2002, to October 14, 2002 (pre-exposure assay), and from October 14, 2002, to November 4, 2002 (exposure assay).

3.2.1 Survival

All males and females in the Control treatment and Low concentration survived the EPA 21-Day Methoxychlor assay. High male mortality occurred in three replicates of the High methoxychlor concentration. Both males in these three replicates expired by Day 7, Day 14, or Day 15 of the exposure and each tank was terminated. One male in the fourth High-concentration tank died during the exposure. Female survival in the High concentration could not be evaluated because the three tanks in which both

males died were terminated before the end of the assay. All females in the one High-concentration tank that ran 21 days survived.

3.2.2 Vitellogenin

Vitellogenin concentrations in most Control-treatment females used during the EPA 21-Day Methoxychlor assay ranged from 107,850 ng/mL to 14,220,000 ng/mL (Figure 3.21). Among females exposed to the two methoxychlor concentrations, vitellogenin concentrations ranged from 373,200 ng/mL to 7,519,000 ng/mL. No significant differences in the mean vitellogenin concentration per treatment (Table 3.21) were detected (Kruskal-Wallis, $H = 5.29$, $p = 0.071$, df = 2). The achieved power for this endpoint was 12%, and the sample size required to detect a significant difference from the Control treatment at 80% power was 36 (Table 3.19).

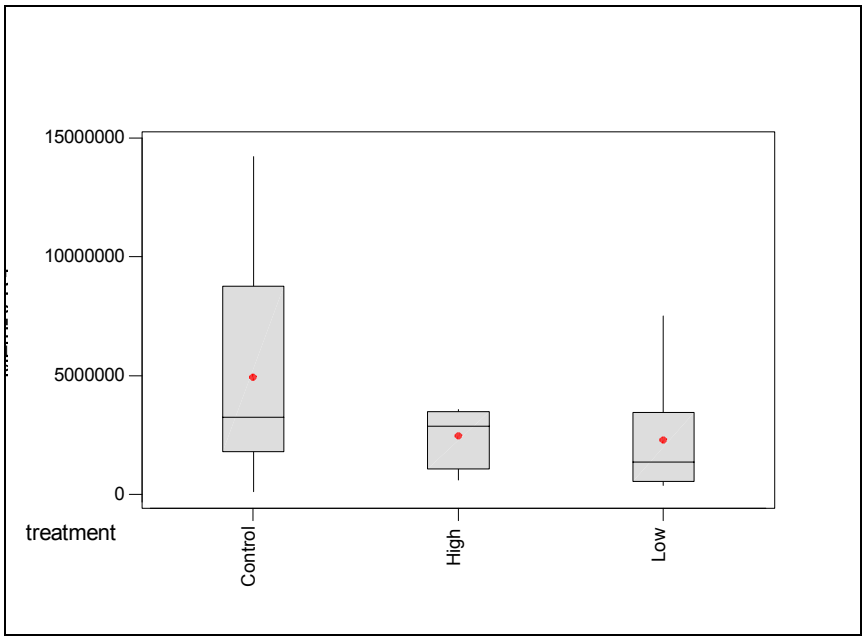

Figure 3.21. Boxplot of female vitellogenin concentration (ng/mL) by treatment for the EPA 21-Day Methoxychlor assay. The box represents the interquartile range, whiskers represent the data range, the horizontal line is the median value, and the circle is the mean value.

Table 3.19. Summary statistics and power estimates for female vitellogenin concentrations (ng/mL) for the EPA 21-Day Methoxychlor assay.

Level	N	Mean	Stdev	CV	Achieved Power [1]	Sample Size Required [2]
Control	16	4,928,594	4,073,229	83%	12%	36
Low	16	2,299,294	2,326,186	101%		
High	4	2,477,975	1,330,838	54%		

[1] Calculated from natural log transformed data; sample size = 4.
[2] Size required to detect a significant difference from Control treatment based on maximum achieved absolute difference; calculated on natural log transformed data.

Vitellogenin concentrations in Control-treatment males used during the EPA 21-Day Methoxychlor assay ranged from 0 ng/mL (not detected) to 366 ng/mL (Figure 3.22). Among males exposed to the Low-methoxychlor concentration, vitellogenin concentrations ranged from 0 ng/mL (not detected) to 3,395 ng/mL. Only one male from the High concentration was available for analysis. It had a vitellogenin concentration of 10,890,000 ng/mL. No significant differences in the mean vitellogenin concentration per treatment (Table 3.20) were detected (Kruskal-Wallis, $H = 5.52$, $p = 0.063$, df = 2). The achieved power for this endpoint was 71%, and the sample size required to detect a significant difference from the Control treatment at 80% power was 3 (Table 3.20).

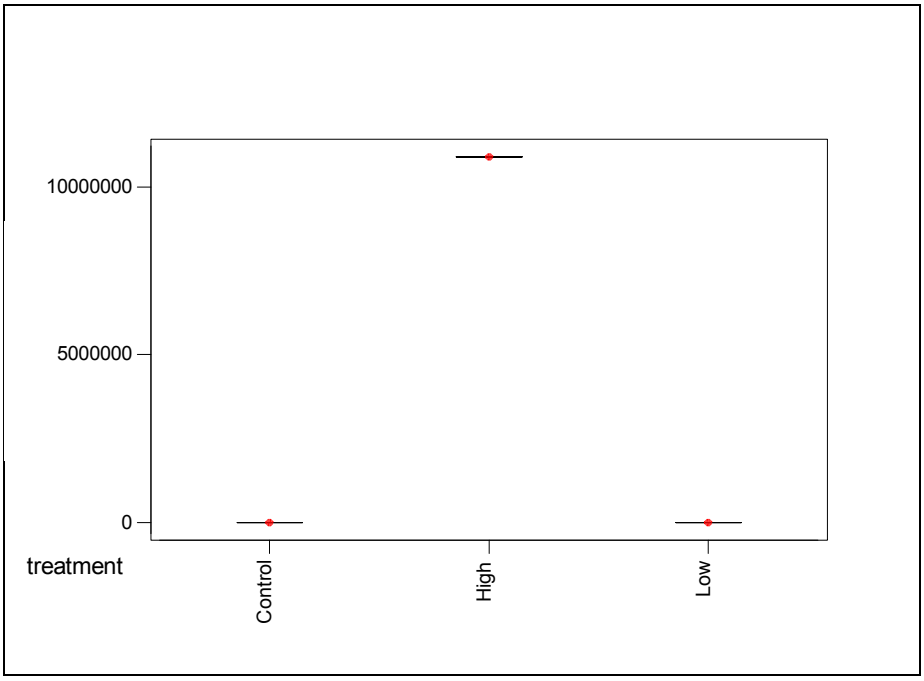

Figure 3.22. Boxplot of male vitellogenin concentration (ng/mL) by treatment for the EPA 21-Day Methoxychlor assay. The box represents the interquartile range, whiskers represent the data range, the horizontal line is the median value, and the circle is the mean value.

Table 3.20. Summary statistics and power estimates for male vitellogenin concentrations (ng/mL) for the EPA 21-Day Methoxychlor assay.

Level	N	Mean	Stdev	CV	Achieved Power [1]	Sample Size Required [2]
Control	7	112	157	140%	71%	3
Low	7	866	1,214	140%		
High	1	10,890,000	0	0%		

[1] Calculated from natural log transformed data; sample size = 2 (smallest allowable).

[2] Size required to detect a significant difference from Control treatment based on maximum achieved absolute difference; calculated on natural log transformed data.

3.2.3 Appearance / Secondary Sex Characteristics

All of the females used during the EPA 21-Day Methoxychlor assay exhibited typical female morphology (no fat pad, no tubercles, ovipositor present) except that two females from the Control treatment lacked ovipositors.

All males used during the EPA 21-Day Methoxychlor assay had typical male morphological features (tubercles, fat pads, vertical banding, no ovipositor).

3.2.4 Gonadosomatic Index

The range of GSI values calculated for females in all treatments varied from three- to five-fold (Figure 3.23), and the overall variability within the treatment was moderate to high (CVs = 41% to 86%) (Table 3.21). Several fish had unusually high GSI values. The highest value obtained for a female exposed to the High methoxychlor concentration had a GSI value of 51.1. The GSI values for the other three fish in this treatment ranged from 12.0 to 14.0. One female exposed to the Low concentration had a GSI of 37.8 and two control animals had GSI values of about 26. The total body weight of all four females were well within the normal range for the test animals, and it appears the high GSI value resulted from each fish having unusually high gonad weights. The gonad weights of these four fish ranged from 0.52 g to 0.82 g, well above the average values for the remaining fish (0.2 g). No significant differences in the mean GSI value per treatment were detected (Kruskal-Wallis, $H = 1.34$, $p = 0.512$, df = 2) (Table 3.21). 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 25 (Table 3.21).

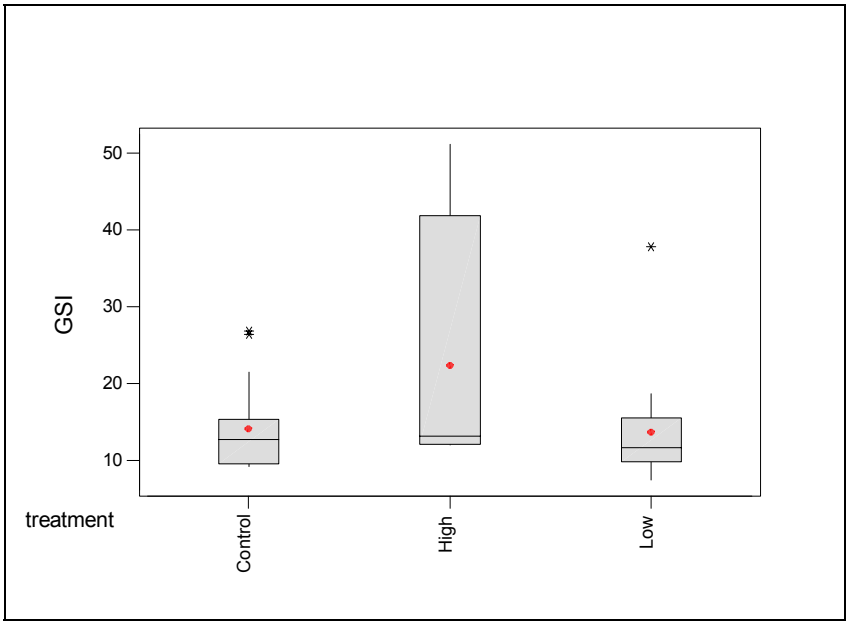

Figure 3.23. Boxplot of female GSI by treatment for the EPA 21-Day Methoxychlor assay. The box represents the interquartile range, whiskers represent the data range, the horizontal line is the median value, the circle is the mean value, and asterisks represent probable outliers.

Table 3.21. Summary Statistics and Power Estimates for Female Gonadosomatic Index Data for the EPA 21-Day Methoxychlor Assay

Level	N	Mean	SD	CV	Achieved Power [1]	Sample Size Required [2]
Control	16	14.1	5.8	41%	15%	25
Low	16	13.7	7.2	52%		
High	4	22.4	19.2	86%		

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

The range of most GSI values calculated for males during the EPA 21-Day Methoxychlor assay was small, ranging from 0.7 to 1.9 (Figure 3.24), which approximates the typical range for reproductively-active male fathead minnows. One male in the Low concentration had a GSI value of 2.6, attributable to its relatively high gonad weight of 0.06 g and small body size (2.4 g). There were no significant differences in mean GSI values among treatments (Kruskal-Wallis, $H = 1.58$, $p = 0.454$, df = 2) (Table 3.22). 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 203 (Table 3.22).

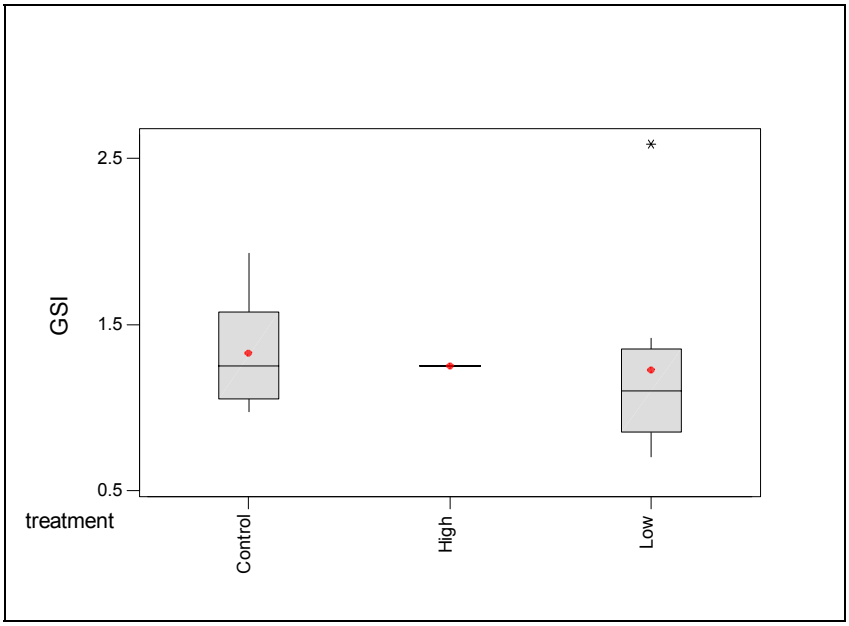

Figure 3.24. Boxplot of male GSI by treatment for the EPA 21-Day Methoxychlor assay. The box represents the interquartile range, whiskers represent the data range, the horizontal line is the median value, the circle is the mean value, and asterisks represent probable outliers.

Table 3.22. Summary Statistics and Power Estimates for Male Gonadosomatic Index Data for the EPA 21-Day Methoxychlor Assay

Level	N	Mean	SD	CV	Achieved Power [1]	Sample Size Required [2]
Control	8	1.33	0.33	25%	5%	203
Low	8	1.23	0.59	48%		
High	1	1.25	—	—		

[1] Calculated from arcsine square-root transformed data; sample size = 2.

[2] Required size to detect a significant difference from Control treatment based on maximum achieved absolute difference; calculated on arcsine square-root transformed data.

3.2.5 Female Gonad Histology

Histological analyses were conducted on the ovaries of 36 females exposed to methoxychlor during the EPA 21-day assay. Of these 36 fish, 10 were observed to have moderate-to-diffuse macrophage infiltration into the ovaries. Tissues from three fish with this condition were evaluated with a Gram stain and acid-fast stain that demonstrated acid-fast staining structures consistent with mycobacteria. All other fish with a similar macrophage infiltration in the ovaries were also presumed to have mycobacteriosis. At the time of the histological examination, it could not be determined whether the condition was exacerbated by the chemical exposure. Because Control-treatment fish were infected, it was clear that the infection was distributed throughout the population. To determine whether the infection affected the results, the analyses were conducted once with all fish included and then were repeated with the infected fish excluded. The results of the second set of analyses (with infected fish removed) did not change the pattern of statistical significance obtained for the analyses that included all fish.

General Ovary Staging—Statistical analysis of the mean ovarian staging from 12 microscopic fields per fish revealed no significant differences among treatments (Kruskal-Wallis, $H = 3.97$, $p = 0.137$, df = 2).

Quantitative Ovarian Staging— One hundred cells in each of three sections per female were examined to quantitatively determine the developmental stage of the ovaries. Ova from fish in all treatments ranged from Stage 1a to Stage 5 (see Section 2: Methods, for a description of the stages) (Figure 3.25). Variability within treatments for each stage was very high, as indicated by CVs that ranged as high as 400% (Table 3.23). Although statistical analyses showed that there was a significant difference among treatments in the proportion of cells in developmental Stage 2, there were no significant differences among treatments in the proportion of cells in developmental Stages 1a, 1b, 3, 4, and 5 (Table 3.23). Therefore, there was no consistent pattern of significant difference associated with methoxychlor dose.

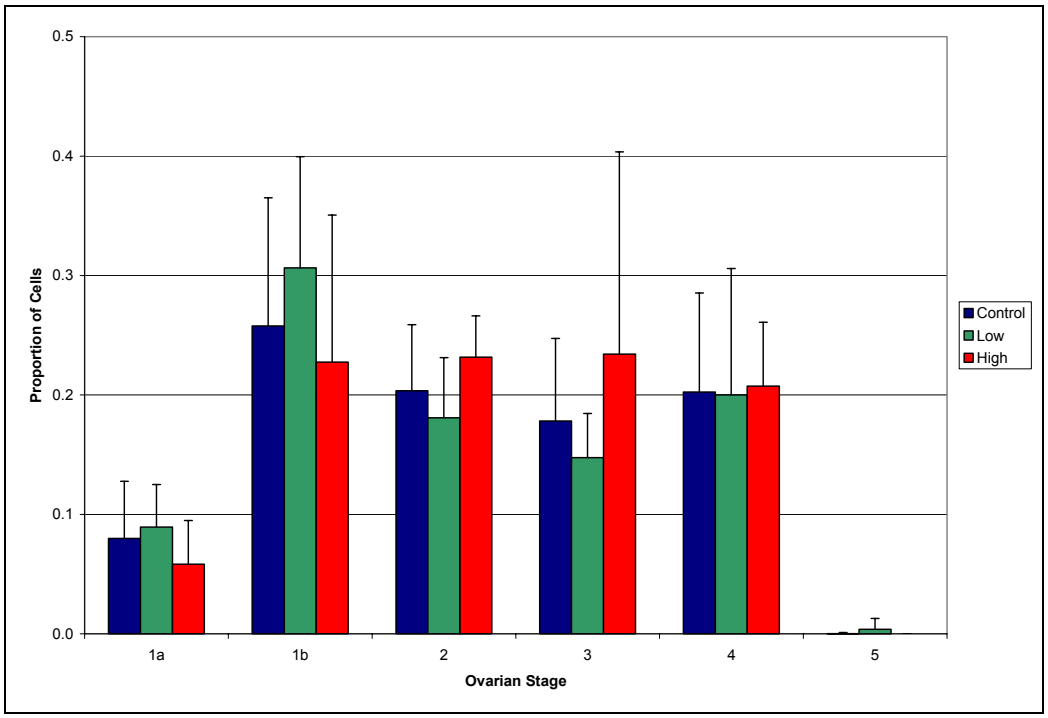

Figure 3.25. Frequency histogram showing the quantitative developmental staging of ovaries for each treatment of the EPA 21-Day Methoxychlor assay. For each treatment, the columns represent the grand mean proportion of cells in each stage and the bars represent the standard deviation.

Table 3.23. Descriptive Statistics of the Proportion of Ovarian Cells in each Developmental Stage for Females from the EPA 21-Day Methoxychlor Assay and Results of the Kruskal-Wallis Test (df = 2) Comparing Treatments

	Control (n = 16)			Low (n = 16)			High (n = 4)			Kruskal-Wallis	
Stage	**Mean**	**SD**	**CV**	**Mean**	**SD**	**CV**	**Value**	**SD**	**CV**	***H***	***p***
1a	0.080	0.048	60%	0.089	0.036	40%	0.058	0.037	63%	2.05	0.359
1b	0.258	0.107	42%	0.306	0.093	30%	0.228	0.123	54%	2.98	0.226
2	0.204	0.055	27%	0.181	0.051	28%	0.232	0.035	15%	6.23	0.044*
3	0.178	0.069	39%	0.148	0.037	25%	0.234	0.169	72%	1.60	0.450
4	0.203	0.083	41%	0.200	0.106	53%	0.208	0.053	26%	0.62	0.732
5	0.000	0.001	400%	0.004	0.009	245%	0	0	–	1.98	0.371

* $p < 0.05$

Atretic Follicles—The mean proportion of atretic follicles per 300 follicles ranged from 0.02 for females in the High concentration to 0.06 for females in the Control treatment (Figure 3.26). There were no significant differences in the proportions of atretic follicles among treatments (Kruskal-Wallis, $H = 0.08$, $p = 0.963$, df = 2).

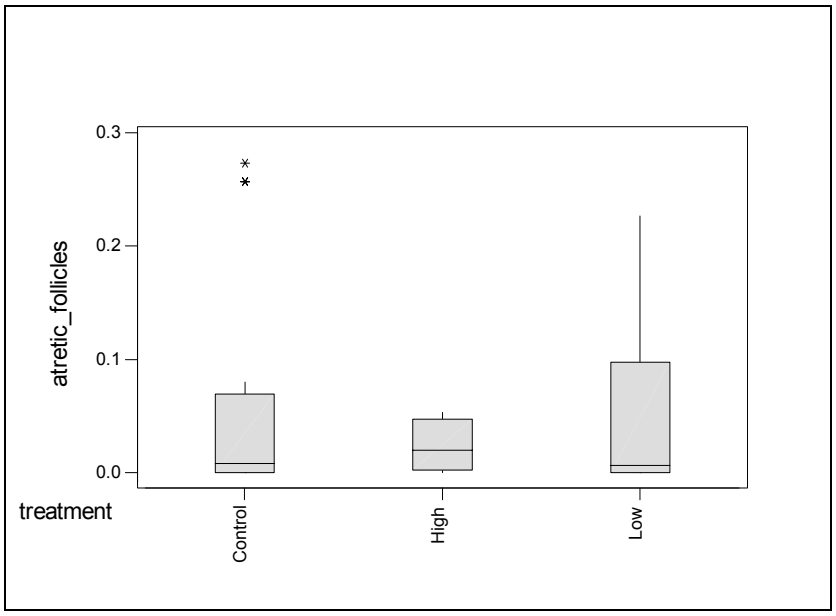

Figure 3.26. Boxplot of the proportion of atretic follicles per 300 follicles by treatment for the EPA 21-Day Methoxychlor assay. The box represents the interquartile range, whiskers represent the data range, the horizontal line is the median value, the circle is the mean value, and asterisks represent probable outliers.

Corpora Lutea—The mean proportion of corpora lutea per 300 follicles (counted per fish) ranged from 0.016 for females in the Control treatment to 0.019 for females in the Low concentration (Figure 3.27). There were no significant differences in the proportions of corpora lutea among treatments (Kruskal-Wallis, $H = 0.12$, $p = 0.941$, df = 2).

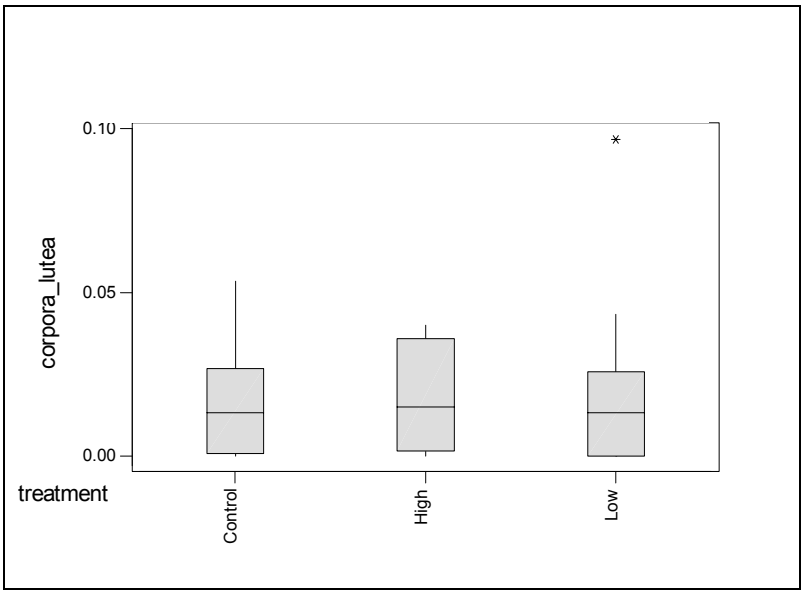

Figure 3.27. Boxplot of the proportion corpora lutea per 300 follicles by treatment for the EPA 21-Day Methoxychlor assay. The box represents the interquartile range, whiskers represent the data range, the horizontal line is the median value, the circle is the mean value, and asterisks represent probable outliers.

3.2.6 Male Gonad Histology

Testes Staging by Microscopic Field—Testes from males exposed to methoxychlor during the EPA 21-Day Methoxychlor assay were examined to determine their general developmental condition. Males in all treatments had well-developed testes with most showing Stage 4 and Stage 5 development (see Section 2: Methods, for description of developmental stages). Ninety-four of the 96 microscopic fields examined in the 8 Control-treatment males showed Stage 4 (80 fields) or Stage 5 (14 fields) development. Two microscopic fields showed Stage 3 development. All of the 96 fields examined in the 8 Low-concentration treatment males showed Stage 4 (86 fields) or Stage 5 (10 fields) development. In the single High-concentration male available for examination, all 12 microscopic fields showed Stage 4 development. Statistical analysis of the mean staging from 12 sections per fish revealed no significant differences among treatments (Kruskal-Wallis, $H = 0.46$, $p = 0.795$, 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 of the Control-treatment testes ranged from Stage 2b to Stage 5, whereas testes in males from the Low- and High-concentration treatments showed Stage 2a to Stage 5 development (Figure 3.28). Variability within treatments for each stage was very high, as indicated by CVs that ranged as high as 113% (Table 3.24). Although statistical analyses showed that there was a significant difference among treatments in the proportion of cells in developmental Stage 3b, there were no significant differences among treatments in the proportion of cells in developmental Stages 2a, 2b, 3a, 4, and 5 (Table 3.13). Therefore, there was no consistent pattern of significant difference associated with methoxychlor dose.

Table 3.24. Descriptive Statistics of the Proportion of Testes Cells in Each Developmental Stage for Males from the EPA 21-Day Methoxychlor Assay and Results of the Kruskal-Wallis Test (df = 2) Comparing Treatments

	Control (n = 8)			Low (n = 8)			High (n = 1)			Kruskal-Wallis	
Stage	**Mean**	**SD**	**CV**	**Mean**	**SD**	**CV**	**Value**	**SD**	**CV**	***H***	***p***
1	0	0	0%	0	0	0%	0	–	–	–	–
2a	0.006	0.006	104%	0.002	0.002	107%	0	–	–	4.18	0.124
2b	0.015	0.011	75%	0.013	0.015	113%	0.017	–	–	0.62	0.734
3a	0.105	0.060	57%	0.118	0.042	36%	0.333	–	–	2.80	0.247
3b	0.243	0.105	43%	0.329	0.086	26%	0.163	–	–	7.04	0.030 *
4	0.147	0.118	81%	0.198	0.100	50%	0.277	–	–	1.97	0.374
5	0.484	0.226	47%	0.340	0.162	48%	0.210	–	–	3.10	0.212

* $p < 0.05$

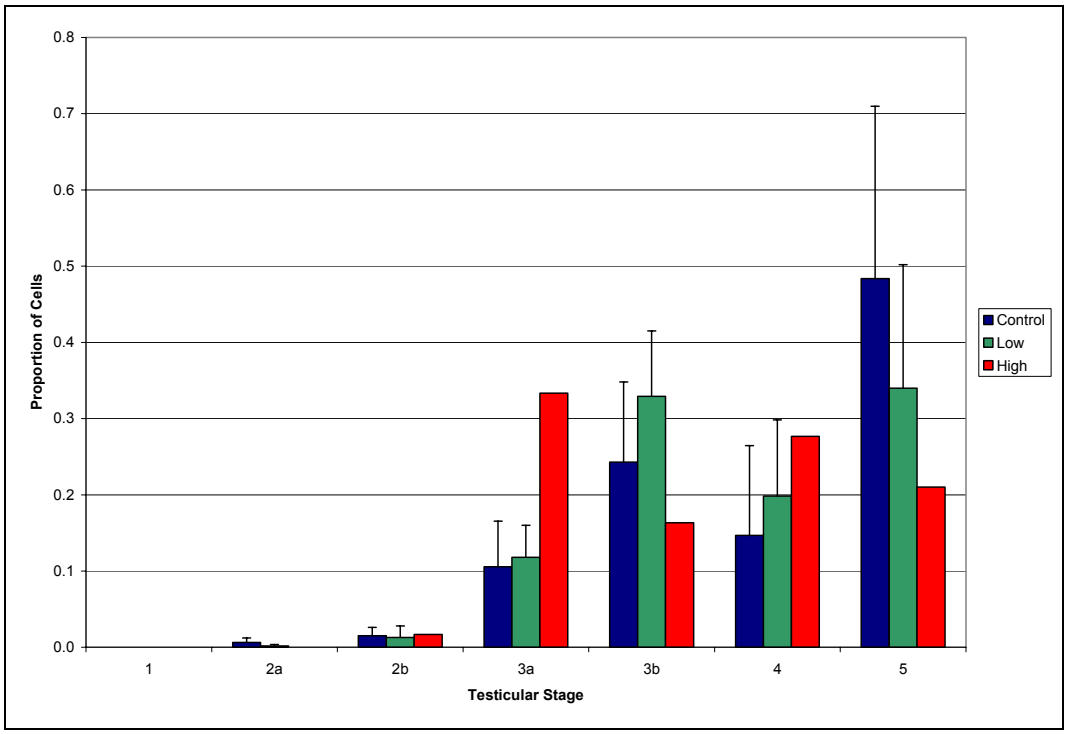

Figure 3.28. Frequency Histogram Showing the Quantitative Developmental Staging of Testes for Each Treatment of the EPA 21-Day Methoxychlor Assay. For each treatment, the columns represent the grand mean proportion of cells in each stage and the bars represent the standard deviation.

Tubule Diameter—The average diameter of the seminiferous tubules of males from the Control treatment ranged from 97.5 µm to 176.7 µm (Figure 3.29). Tubule diameters of males from the two test concentrations ranged from 97.8 µm to 157.8 µm. No significant differences in the mean tubule diameter per treatment were detected (Kruskal-Wallis, $H = 2.29$, $p = 0.318$, df = 2) (Table 3.25). The achieved power for this endpoint was 10%, and the sample size required to detect a significant difference from the Control treatment at 80% power was 14 (Table 3.25).

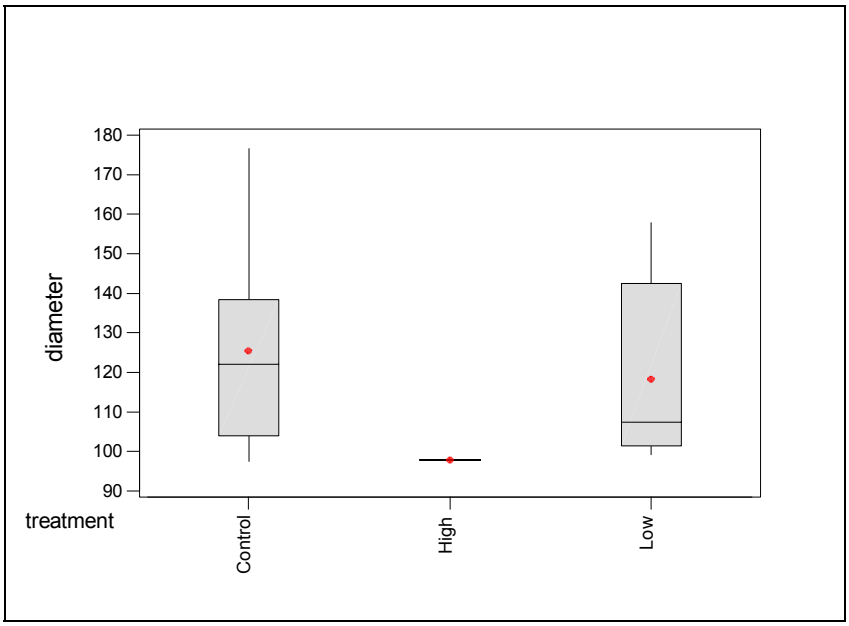

Figure 3.29. Boxplot of male seminiferous tubule diameter (μm) by treatment for the EPA 21-Day Methoxychlor assay. The box represents the interquartile range, whiskers represent the data range, the horizontal line is the median value, and the circle is the mean value.

Table 3.25. Summary Statistics and Power Estimates for Male Seminiferous Tubule Diameter Data for the EPA 21-Day Methoxychlor Assay

Level	N	Mean	SD	CV	Achieved Power [1]	Sample Size Required [2]
Control	8	125.4	25.6	20%	10%	14
Low	8	118.3	23.0	19%		
High	1	97.8	–	–		

[1] Calculated from natural log transformed data; sample size = 2 (smallest allowable).

[2] Required size to detect a significant difference from Control treatment based on maximum achieved absolute difference; calculated on natural log transformed data.

Observations—One male in the Low-concentration treatment showed interstitial Sertoli cell proliferation and one showed multifocal areas of Leydig cell proliferation. One male from the High-concentration treatment showed mild Leydig cell proliferation. No testicular atrophy was recorded, and no ovatestes were observed for any treatment.

3.2.7 Plasma Steroid Concentrations

Estradiol—Estradiol concentrations in Control-treatment females used during the EPA 21-Day Methoxychlor assay ranged from 292 pg/mL to 6,208 pg/mL (Figure 3.30). Among females exposed to the two methoxychlor concentrations, estradiol concentrations ranged from 0 pg/mL (not detected) to 6,362 pg/mL. No significant differences in the mean estradiol concentration per treatment (Table 3.26) were detected (Kruskal-Wallis, $H = 5.64$, $p = 0.060$, df = 2). The achieved power for this endpoint was

8%, and the sample size required to detect a significant difference from the Control treatment at 80% power was 72 (Table 3.26).

Table 3.26 Summary statistics and power estimates for female estradiol concentrations (pg/mL) for the EPA 21-Day Methoxychlor assay.

Level	N	Mean	Stdev	CV	Achieved Power [1]	Sample Size Required [2]
Control	16	2,681	1,861	69%	8%	72
Low	16	1,371	1,639	120%		
High	4	3,927	2,854	73%		

[1] Calculated from natural log transformed data; sample size = 4.
[2] Size required to detect a significant difference from Control treatment based on maximum achieved absolute difference; calculated on natural log transformed data.

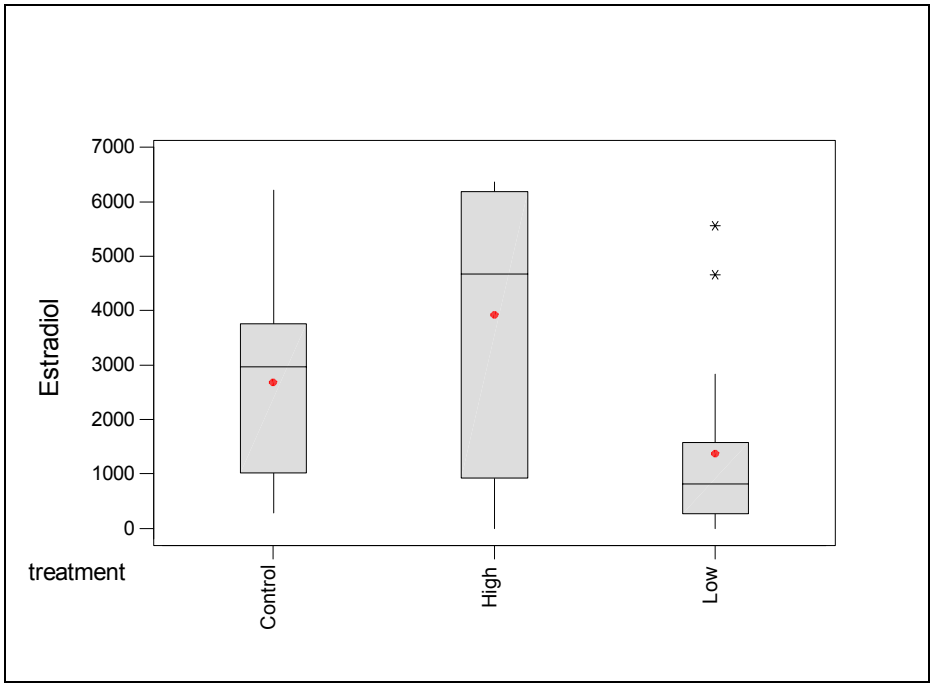

Figure 3.30. Boxplot of female estradiol concentration (pg/mL) by treatment for the EPA 21-Day Methoxychlor assay. The box represents the interquartile range, whiskers represent the data range, the horizontal line is the median value, the circle is the mean value, and the asterisks represent probable outliers.

Estradiol concentrations in Control-treatment males used during the EPA 21-Day Methoxychlor assay ranged from 125 pg/mL to 420 pg/mL (Figure 3.31). Among males exposed to the two methoxychlor concentrations, estradiol concentrations ranged from 62 pg/mL to 666 pg/mL. No significant differences in the mean estradiol concentration per treatment (Table 3.27) were detected (Kruskal-Wallis, $H = 4.77$, $p = 0.092$, 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 171 (Table 3.27).

Table 3.27. Summary statistics and power estimates for male estradiol concentrations (pg/mL) for the

EPA 21-Day Methoxychlor assay.

Level	N	Mean	Stdev	CV	Achieved Power [1]	Sample Size Required [2]
Control	7	306	96	31%	5%	171
Low	7	428	191	45%		
High	1	263	–	–		

[1] Calculated from natural log transformed data; sample size = 2 (smallest allowable).
[2] Size required to detect a significant difference from Control treatment based on maximum achieved absolute difference; calculated on natural log transformed data.

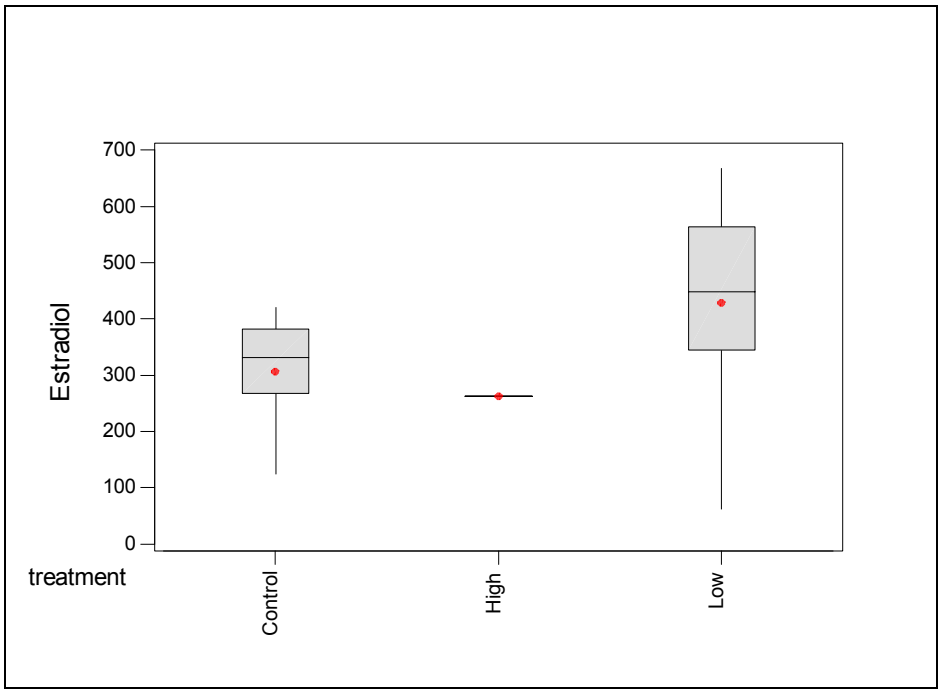

Figure 3.31. Boxplot of male estradiol concentration (pg/mL) by treatment for the EPA 21-Day Methoxychlor assay. The box represents the interquartile range, whiskers represent the data range, the horizontal line is the median value, and the circle is the mean value.

Testosterone—Testosterone concentrations in most Control-treatment females used during the EPA 21-Day Methoxychlor assay ranged from 113 pg/mL to 6,035 pg/mL (Figure 3.32). One Control-treatment female had a testosterone concentration of 27,568 pg/mL. Because this value was substantially greater than any other female testosterone value, the statistical analyses were performed including and excluding this female. Among females exposed to the two methoxychlor concentrations, testosterone concentrations ranged from 0 pg/mL (not detected) to 9,356 pg/mL. No significant differences in the mean testosterone concentration per treatment (Table 3.28) were detected when all measured testosterone values were included in the analysis (Kruskal-Wallis, $H = 5.71$, $p = 0.058$, df = 2). The calculated probability value was slightly greater than the critical limit of 0.050. The achieved power for this endpoint when all measured testosterone values were included in the analysis was 12%, and the sample size required to detect a significant difference from the Control treatment at 80% power was 36 (Table

3.28). When the Control-treatment female that had a High-testosterone concentration was excluded from the analysis, no significant differences in the mean testosterone concentration per treatment were detected when all measured testosterone values were included in the analysis (Kruskal-Wallis, $H = 5.33$, $p = 0.070$, df = 2). The achieved power for this endpoint in this case was 9%, and the sample size required to detect a significant difference from the Control treatment at 80% power was 53.

Table 3.28. Summary statistics and power estimates for female testosterone concentrations (pg/mL) for the EPA 21-Day Methoxychlor assay with all females having a measured testosterone value included.

Level	N	Mean	Stdev	CV	Achieved Power [1]	Sample Size Required [2]
Control	12	4,335	7,479	173%	12%	36
Low	14	1,688	2,361	140%		
High	4	3,964	3,310	84%		

[1] Calculated from natural log transformed data; sample size = 4.
[2] Size required to detect a significant difference from Control treatment based on maximum achieved absolute difference; calculated on natural log transformed data.

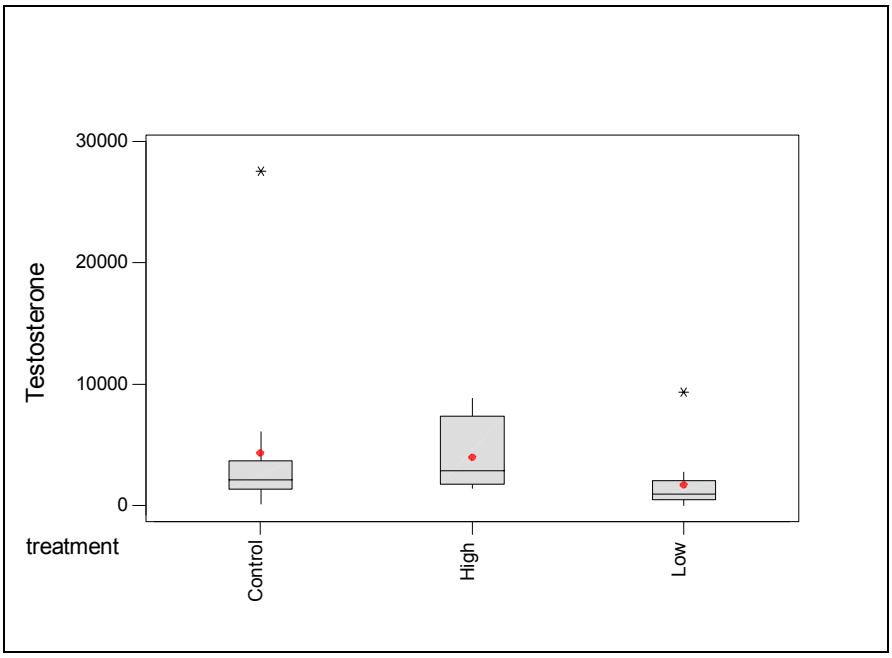

Figure 3.32. Boxplot of female testosterone concentration (pg/mL) by treatment for the EPA 21-Day Methoxychlor assay. The box represents the interquartile range, whiskers represent the data range, the horizontal line is the median value, the circle is the mean value, and the asterisks represent probable outliers.

Testosterone concentrations in Control-treatment males used during the EPA 21-Day Methoxychlor assay ranged from 908 pg/mL to 8,956 pg/mL (Figure 3.33). Among males exposed to the two methoxychlor concentrations, testosterone concentrations ranged from 1,288 pg/mL to 9,012 pg/mL. No significant differences in the mean testosterone concentration per treatment (Table 3.29) were detected (Kruskal-

Wallis, $H = 1.44$, $p = 0.487$, 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 17 (Table 3.29).

Table 3.29. Summary statistics and power estimates for male testosterone concentrations (pg/mL) for the EPA 21-Day Methoxychlor assay.

Level	N	Mean	Stdev	CV	Achieved Power [1]	Sample Size Required [2]
Control	8	5,209	3,008	58%	9%	17
Low	7	6,377	3,100	49%		
High	1	1,741	–	–		

[1] Calculated from natural log transformed data; sample size = 2 (smallest allowable).
[2] Size required to detect a significant difference from Control treatment based on maximum achieved absolute difference; calculated on natural log transformed data.

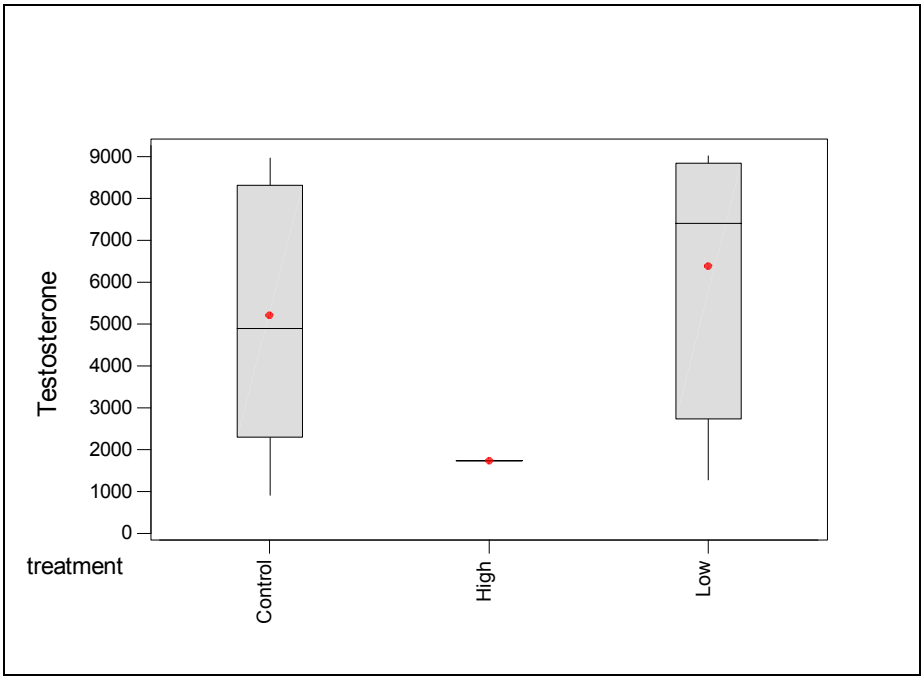

Figure 3.33. Boxplot of male testosterone concentration (pg/mL) by treatment for the EPA 21-Day Methoxychlor assay. The box represents the interquartile range, whiskers represent the data range, the horizontal line is the median value, and the circle is the mean value.

11-ketotestosterone—11-ketotesosterone concentrations in Control-treatment females used during the EPA 21-Day Methoxychlor assay ranged from 528 pg/mL to 1,152 pg/mL (Figure 3.34). Among females exposed to the two methoxychlor concentrations, 11-ketotesosterone concentrations ranged from 0 pg/mL (not detected) to 1,497 pg/mL. Significant differences in the mean 11-ketotesosterone concentration per treatment (Table 3.30) were detected (Kruskal-Wallis, $H = 7.93$, $p = 0.019$, df = 2). The mean concentration of 11-ketotestosterone was lower in females exposed to the Low concentration than in females from the High concentration and the Control treatment. The achieved power for this endpoint was 44%, and the sample size required to detect a significant difference from the Control treatment at 80% power was 3 (Table 3.30).

Table 3.30. Summary statistics and power estimates for female 11-ketotesosterone concentrations (pg/mL) for the EPA 21-Day Methoxychlor assay.

Level	N	Mean	Stdev	CV	Achieved Power [1]	Sample Size Required [2]
Control	2	840	441	53%	44%	3
Low	11	162	451	279%		
High	3	675	172	25%		

[1] Calculated from natural log transformed data; sample size = 2.
[2] Size required to detect a significant difference from Control treatment based on maximum achieved absolute difference; calculated on natural log transformed data.

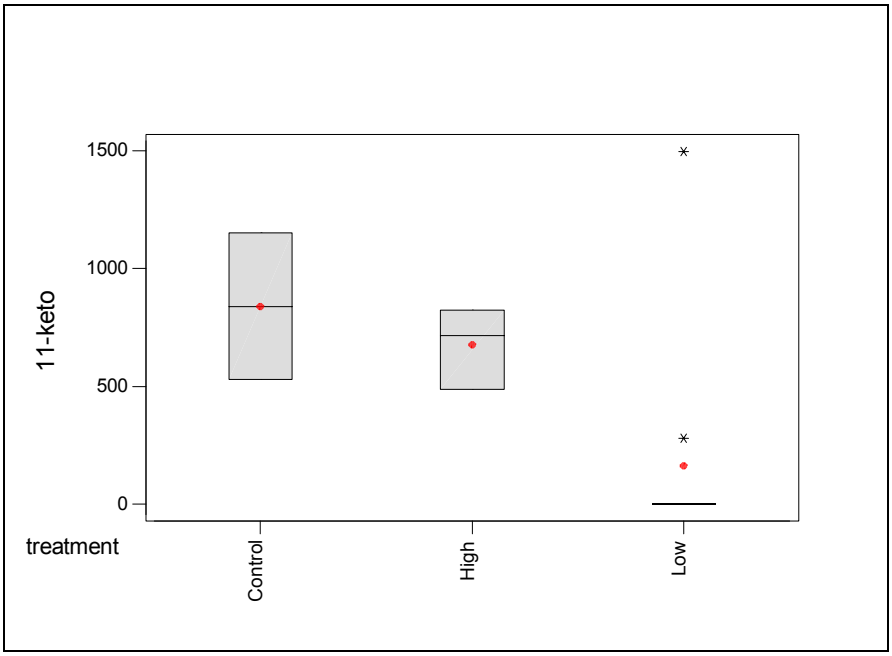

Figure 3.34. Boxplot of female 11-ketotesosterone concentration (pg/mL) by treatment for the EPA 21-Day Methoxychlor assay. The box represents the interquartile range, whiskers represent the data range, the horizontal line is the median value, the circle is the mean value, and the asterisks represent probable outliers.

11-ketotesosterone concentrations in Control-treatment males used during the EPA 21-Day Methoxychlor assay ranged from 14,531 pg/mL to 58,088 pg/mL (**Figure 3.35**). Among males exposed to the two methoxychlor concentrations, 11-ketotesosterone concentrations ranged from 4,481 pg/mL to 88,994 pg/mL. No significant differences in the mean 11-ketotesosterone concentration per treatment (**Table 3.31**) were detected (Kruskal-Wallis, $H = 3.34$, $p = 0.188$, 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 18 (**Table 3.31**).

Table 3.31. Summary statistics and power estimates for male 11-ketotesosterone concentrations (pg/mL) for the EPA 21-Day Methoxychlor assay.

Level	N	Mean	Stdev	CV	Achieved Power [1]	Sample Size Required [2]
Control	8	35,211	16,809	48%	9%	18
Low	8	50,059	31,324	63%		
High	1	12,764				

[1] Calculated from natural log transformed data; sample size = 2 (smallest allowable).
[2] Size required to detect a significant difference from Control treatment based on maximum achieved absolute difference; calculated on natural log transformed data.

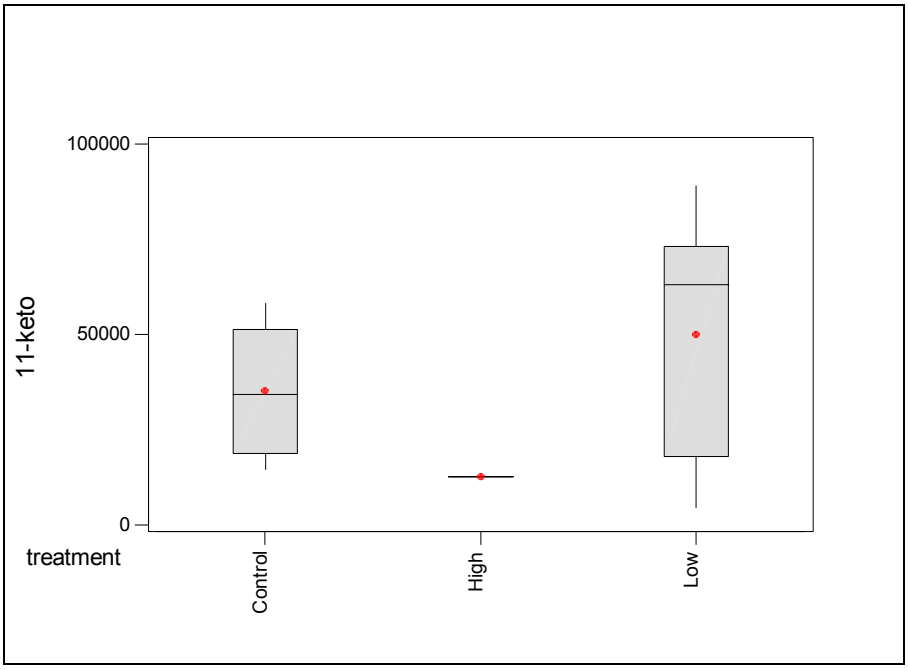

Figure 3.35. Boxplot of male 11-ketotesosterone concentration (pg/mL) by treatment for the EPA 21-Day Methoxychlor assay. The box represents the interquartile range, whiskers represent the data range, the horizontal line is the median value, and the circle is the mean value.

3.2.8 Body Weight

The body weight of females used in the EPA 21-Day Methoxychlor assay ranged from 1.0 g to 2.2 g. There were no significant differences in mean body weight among treatments (Kruskal-Wallis, $H = 2.53$, $p = 0.282$, df = 2). The body weight of males used in the EPA 21-Day Methoxychlor assay ranged from 2.3 g to 5.3 g. There were no significant differences in mean body weight among treatments (Kruskal-Wallis, $H = 1.83$, $p = 0.400$, df = 2).

3.2.9 Fecundity

Total Fecundity—Two pre-exposure evaluations of total egg production were performed. One evaluation examined the total egg production at 15 days, which is within the assay guidelines; the other evaluated egg production at Day 7 of the pre-exposure assay to determine whether or not the shorter pre-exposure period would be sufficient to evaluate the potential reproductive success of the test fish. Total 15-day counts among the tanks that were eventually used for the three treatments in the exposure assay (individual tank values summed for each treatment) ranged from about 7,000 eggs to 11,000 eggs (Figure 3.36). No significant differences in the mean 15-day egg production among the groups of replicates that would be used in the methoxychlor exposure assay were detected (1-way ANOVA, $F = 2.39$, $p = 0.124$, df = 3, 11). Production at Day 7 of the pre-exposure assay among the 12 replicates that were eventually used in the exposure assay ranged from 503 eggs to 1923 eggs. No significant differences in the mean 7-day egg production among the groups of replicates that would be used in the methoxychlor exposure assay were detected (1-way ANOVA, $F = 1.97$, $p = 0.178$, df = 3, 11). No significant differences were detected in the mean number of eggs laid per day per replicate group at 7 days versus 15 days (Two-Sample *t*-Test, $t = -0.34$, $p = 0.368$, df = 27). However, daily within-treatment variation (indicated by fluctuating coefficient of variation [CV] values) was high through Day 9. Therefore, variability during a 7-day pre-exposure assay is likely to be much greater than that during a pre-exposure assay that lasts longer than 9 days, which reduces the likelihood of reliably choosing replicates with successful reproduction histories for use in the exposure assay.

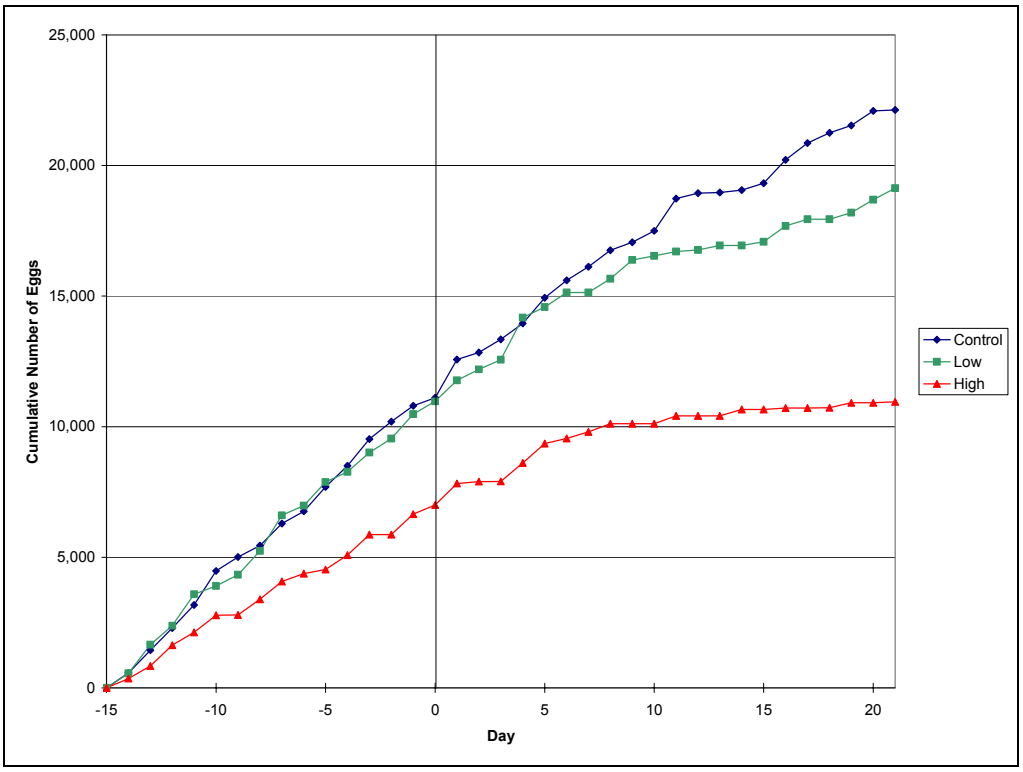

Figure 3.36. Total egg production per treatment during the pre-exposure and exposure periods of the EPA 21-Day Methoxychlor assay. The vertical line at Day 0 denotes the start of the exposure period.

During the EPA 21-Day Methoxychlor assay, total counts in the Control treatment were reasonably consistent among replicates, varying from 2437 eggs to 2910 eggs (Figure 3.37). Variability in total egg

production among Low-concentration replicates was somewhat greater, ranging from 1381 eggs to 3055 eggs. Total counts among the High-concentration replicates varied about nine-fold, from 185 eggs to 1744 eggs. Statistical analysis of square-root transformed egg counts showed significant among-treatment differences (1-way ANOVA, $F = 7.29$, $p = 0.013$, df = 2, 9) in mean total numbers of eggs produced (Table 3.32). Tukey's pairwise comparison identified significant differences in mean egg production between the High concentration and the Control treatment, but not between the High and Low concentrations or between the Low concentration and the Control treatment. Daily within-treatment variation (indicated by fluctuating coefficient of variation values) was much reduced after Day 9 through Day 11, except for the High concentration. The achieved power for this assay was 81%, and the sample size required to detect a significant difference from the Control treatment at 80% power was 4 (Table 3.32).

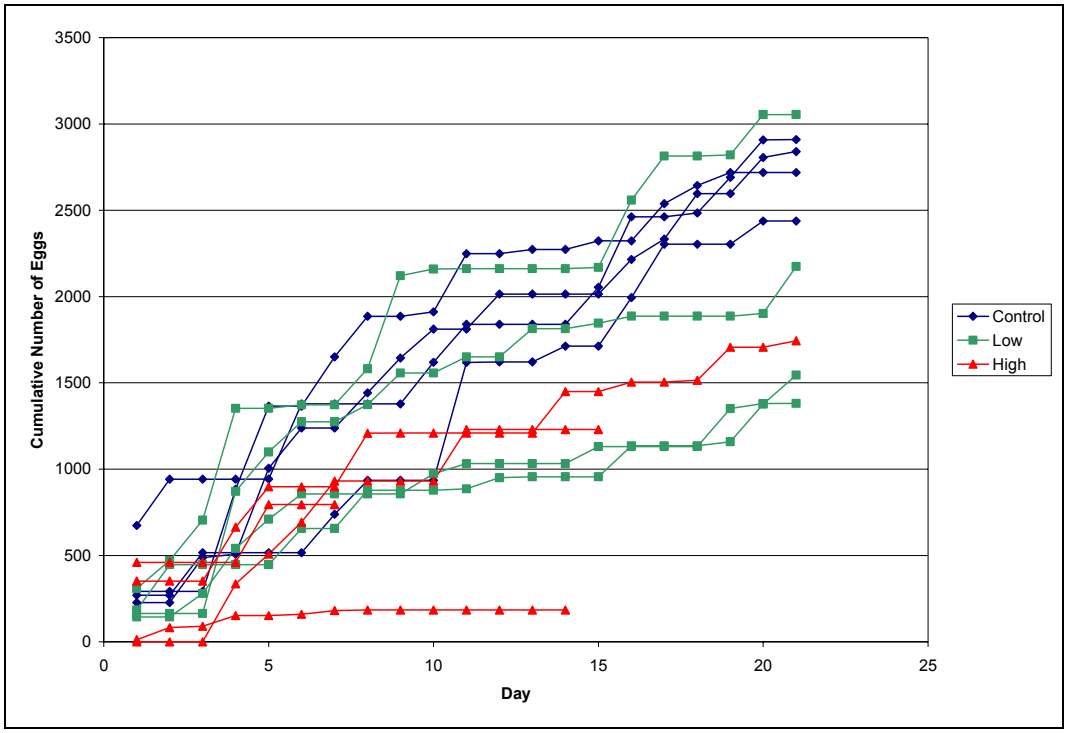

Figure 3.37. Total egg production by replicate per treatment during the EPA 21-Day Methoxychlor assay.

Table 3.32. Summary Statistics and Power Estimates for Total Fecundity Data for the EPA 21-Day Methoxychlor Assay

Level	N	Mean	SD	CV	Achieved Power [1]	Sample Size Required [2]
Control	4	2727	209	8%	81%	4
Low	4	2039	759	37%		
High	4	989	661	67%		

[1] Calculated from square-root transformed data; sample size = 4.

[2] Required size to detect a significant difference from Control treatment based on maximum achieved absolute difference, calculated on square-root transformed data.

Fecundity per Female Reproductive Day—During the 15-day pre-exposure evaluation, the mean number of eggs produced per female reproductive day ranged from 28.0 eggs/day for the tanks that would be used for the High-concentration treatment to 46.6 eggs/day for the tanks that would be used for the Low concentration during the 21-day exposure assay. There were no significant differences among treatments in the numbers of eggs produced per reproductive day during the pre-exposure period (Kruskal-Wallis, $H = 6.06$, $p = 0.109$, df = 3).

During the EPA 21-Day Methoxychlor assay, the maximum number of female reproductive days (84) was achieved for the Control and Low-concentration treatments, whereas only 55 female reproductive days (65% of the maximum) were achieved in the High concentration (Table 3.33). The number of eggs produced per female reproductive day varied from 29.0 eggs to 34.6 eggs in the Control treatment and from 16.4 to 36.4 in the Low concentration (Figure 3.38). For the High concentration, the number of eggs produced per female reproductive day ranged from 4.2 eggs to 24.8 eggs, although fish in three of the replicates yielded more than 20 eggs per day (20.5, 20.8, 24.8). No significant difference in the number of eggs produced per female reproductive day among treatments were detected (Kruskal-Wallis, $H = 4.77$, $p = 0.092$, df = 2). The achieved power for this assay was 35%, and the sample size required to detect a significant difference from the Control treatment at 80% power was 10 (Table 3.33).

Table 3.33. Summary Statistics and Power Estimates for Fecundity per Female Reproductive Day for the EPA 21-Day Methoxychlor Assay

Level	Mean Number of Reproductive Days [1]	N	Mean	SD	CV	Achieved Power [2]	Sample Size Required [3]
Control	84.0	4	32.5	2.5	8%	35%	10
Low	84.0	4	24.3	9.1	37%		
High	55.0	4	17.6	9.1	52%		

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

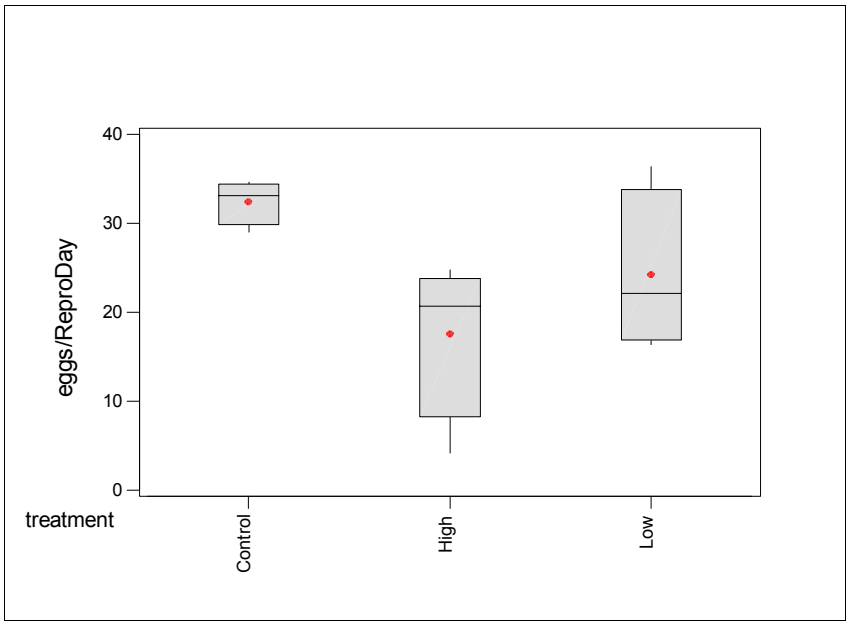

Figure 3.38. Boxplot of the number of eggs produced per female reproductive day by treatment for the EPA 21-Day Methoxychlor assay. The box represents the interquartile range, whiskers represent the data range, the horizontal line is the median value, and the circle is the mean value.

Eggs on Tiles/Dishes—The mean number of eggs laid on the tiles during the 15-day pre-exposure assay varied from 1424 eggs for the tanks that would be used for the High concentration to 2612 eggs for the tanks that would be used for the Control treatment. The mean number of eggs laid on the dishes during the 15-day pre-exposure assay varied from 166 eggs for the tanks that would be used for the Control treatment to 360 eggs for the tanks that would be used for the Low concentration. Because of the variability in the total number of eggs laid per treatment, the proportional difference in the number of eggs on dishes versus those on tiles (1–[# eggs on dishes ÷ # eggs on tiles]) was calculated. There were no significant differences in the mean proportional difference among treatments during the 15-day pre-exposure assay (Kruskal-Wallis, $H = 2.33$, $p = 0.508$, df = 3).

The mean number of eggs laid on the tiles among the treatments during the EPA 21-Day Methoxychlor assay varied from 844 eggs for the High concentration to 2,468 eggs for the Control treatment (Appendix E, Table 1.7). The number of eggs on dishes ranged from 145 eggs for the High concentration to 259 eggs for the Control treatment. The proportional difference ranged from 0.27 (one High-concentration replicate) to 0.95 to 0.96 (also High-concentration replicates) (Appendix E, Figure 1.6). There were no significant differences in this mean proportional difference among treatments (Kruskal-Wallis, $H = 0.35$, $p = 0.841$, df = 2).

3.2.10 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 0.998 (sd = 0.001), 0.995 (sd = 0.006) in the Low concentration, and 0.997 (sd = 0.002) in the High concentration.

The mean proportion of eggs fertilized in the replicates that were not used in the 21-day validation assay was 0.990 (sd = 0.015). There were no significant differences among treatments in the proportion of eggs that hatched (Kruskal-Wallis, $H = 0.71$, $p = 0.870$, df = 3).

The total (tiles + dishes) fertilization-success rates for all treatment replicates during the EPA 21-Day Methoxychlor assay were high, ranging from 0.987 (one Low-concentration replicate) to 1.00 (one High-concentration replicate) (Figure 3.39). No significant differences in mean fertilization-success rates (Table 3.34) among treatments were detected (Kruskal-Wallis, $H = 0.73$, $p = 0.694$, df = 2). The achieved power for this assay was 10%, and the sample size required to detect a significant difference from the Control treatment at 80% power was 46 (Table 3.34).

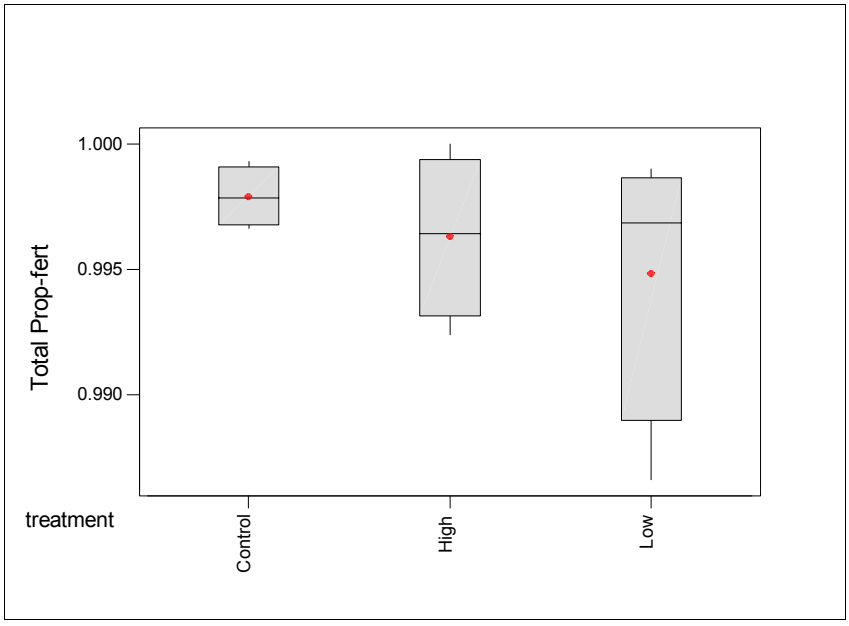

Figure 3.39. Boxplot of the proportion of eggs fertilized by treatment for the EPA 21-Day Methoxychlor assay. The box represents the interquartile range, whiskers represent the data range, the horizontal line is the median value, and the circle is the mean value.

Table 3.34. Summary Statistics and Power Estimates for the Proportion of Eggs Fertilized for the EPA 21-Day Methoxychlor Assay

Level	N	Mean	SD	CV	Achieved Power [1]	Sample Size Required [2]
Control	4	0.998	0.001	0%	10%	46
Low	4	0.995	0.006	1%		
High	4	0.996	0.003	0%		

[1] Calculated from arcsine square-root transformed data; sample size = 4.

[2] Required size to detect a significant difference from Control treatment based on maximum achieved absolute difference; calculated on arcsine square-root transformed data.

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 in tiles (Kruskal-Wallis, $H = 0.68$, $p = 0.879$, df = 3) or on dishes (Kruskal-Wallis, $H = 0.35$, $p = 0.950$, df = 3). The fertilization-success rates for all treatment replicates for eggs laid on tiles during the EPA 21-Day Methoxychlor assay were high, ranging from 0.984 (one Low-concentration replicate) to 1.00 (two High-concentration replicates) (Appendix E, Figure 1.8). No significant differences in mean fertilization-success rates (Appendix E, Table 1.9) among treatments were detected (Kruskal-Wallis, $H = 0.46$, $p = 0.793$, df = 2).

The fertilization-success rates for all treatment replicates for eggs laid on dishes during the assay were high, ranging from 0.945 (one High-concentration replicate) to 1.00 (several replicates; including all treatments) (Appendix E, Figure 1.9). No significant differences in mean fertilization-success rates (Appendix E, Table 1.9) among treatments were detected (Kruskal-Wallis, $H = 0.67$, $p = 0.716$, df = 2).

3.2.11 Hatchability and Larval Development

Eggs were collected during the pre-exposure period for the evaluation of hatchability. The proportion of fertilized eggs that hatched was 1.00 for all tanks in the Control treatment, Low concentration, and from three tanks in the High concentration. The proportion of fertilized eggs that hatched in the fourth High-concentration replicate was 0.98. The mean proportion of fertilized eggs that hatched in the tanks evaluated during the pre-exposure period but not used in the 21-day assay was 0.97 (sd = 0.07). There were no significant differences among treatments in the proportion of eggs that hatched (Kruskal-Wallis, $H = 2.15$, $p = 0.542$, df = 3).

Eggs were collected between Days 7 through 10, Days 12 through 16, and on Day 21 during the EPA 21-Day Methoxychlor assay for the evaluation of hatchability. The proportion of fertilized eggs that hatched ranged from 0.75 to 0.96 in the Control treatment and from 0.48 to 0.98 for the two test concentrations (Figure 3.40). There were no significant differences among treatments in the proportion of eggs that hatched (Kruskal-Wallis, $H = 1.48$, $p = 0.476$, df = 2). The achieved power for this endpoint was 8%, and the sample size required to detect a significant difference from the Control treatment at 80% power was 25 (Table 3.35).

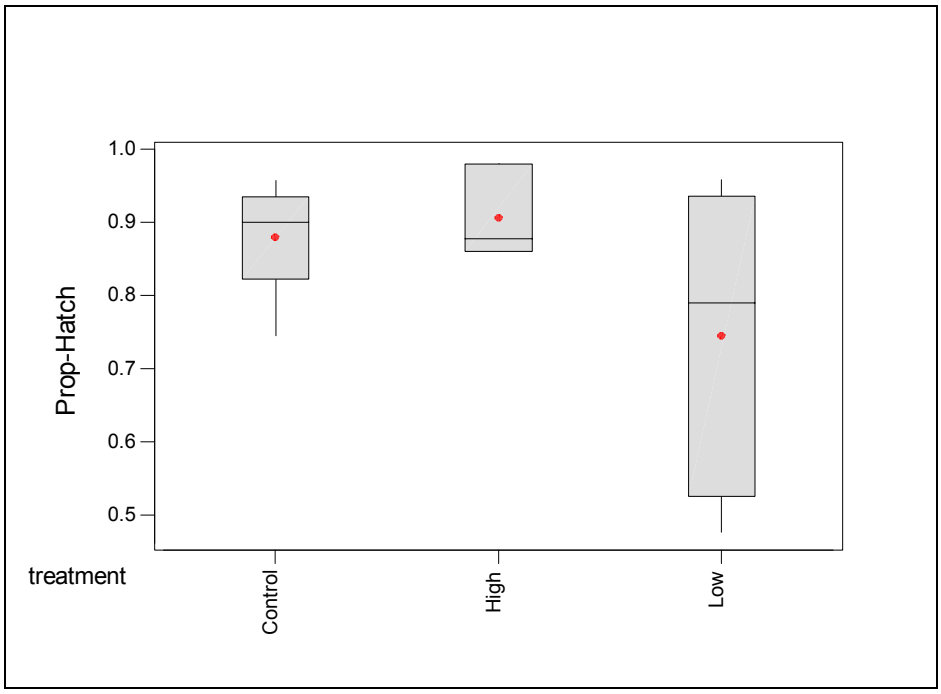

Figure 3.40. Boxplot of the proportion of fertile eggs that hatched by treatment for the EPA 21-Day Methoxychlor assay. The box represents the interquartile range, whiskers represent the data range, the horizontal line is the median value, and the circle is the mean value.

Table 3.35. Summary Statistics and Power Estimates for the Proportion of Fertile Eggs that Hatched for the EPA 21-Day Methoxychlor Assay

Level	N	Mean	SD	CV	Achieved Power [1]	Sample Size Required [2]
Control	8	0.88	0.07	8%	8%	25
Low	8	0.74	0.20	27%		
High	3	0.91	0.06	7%		

[1] Calculated from arcsine square-root transformed data; sample size = 3.

[2] Required size to detect a significant difference from Control treatment based on maximum achieved absolute difference; calculated on arcsine square-root transformed data.

Eggs were collected during the pre-exposure period for the evaluation of larval development. The proportion of larvae that developed normally (i.e., that showed no morphological abnormalities) was 1.00 for all tanks in the Control treatment. The mean proportion of normal larvae in the remaining treatments was 0.98 (sd = 0.04) in the Low concentration and 0.995 (sd = 0.01) in the High concentration. The mean proportion of normal larvae in the tanks evaluated during the pre-exposure period but not used in the 21-day assay, was 0.95 (sd = 0.07). There were no significant differences among treatments in the proportion of normal larvae (Kruskal-Wallis, $H = 3.37$, $p = 0.337$, df = 3).

Eggs were collected between Days 7 through 10, Days 12 through 16, and on Day 21 during the EPA 21-Day Methoxychlor assay for the evaluation of larval development. The proportion of larvae that

developed normally (i.e., that showed no morphological abnormalities) ranged from 0.97 to 1.00 in the Control treatment and from 0.96 to 1.00 for the two test concentrations (Figure 3.41). There were no significant differences among treatments in the proportion of normal larvae (Kruskal-Wallis, $H = 0.88$, $p = 0.643$, 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 49 (Table 3.36).

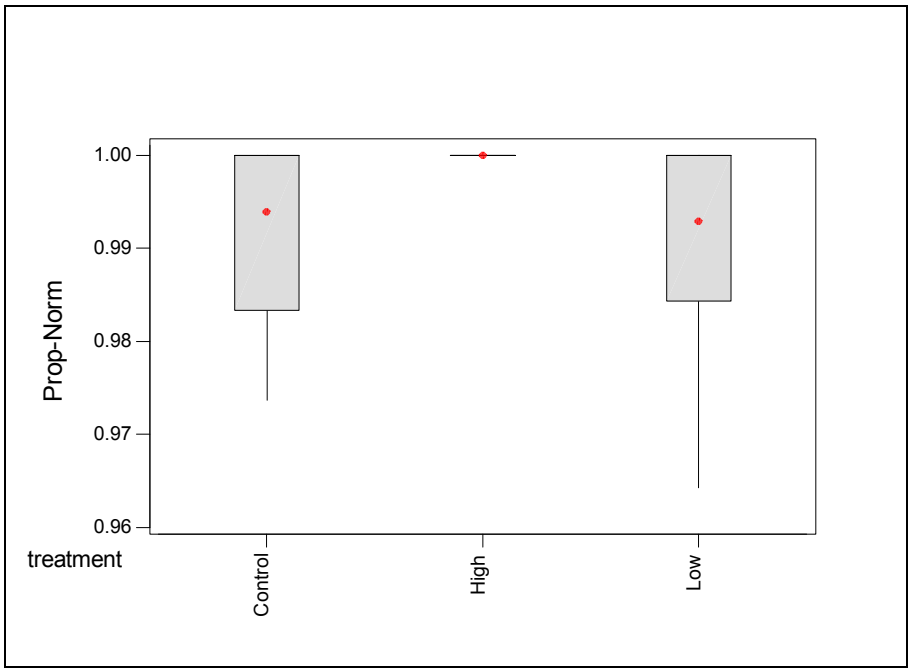

Figure 3.41. Boxplot of the proportion of normal larvae by treatment for the EPA 21-Day Methoxychlor assay. The box represents the interquartile range, whiskers represent the data range, the horizontal line is the median value, and the circle is the mean value.

Table 3.36. Summary Statistics and Power Estimates for the Proportion of Normal Larvae for the EPA 21-Day Methoxychlor Assay

Level	N	Mean	SD	CV	Achieved Power [1]	Sample Size Required [2]
Control	8	0.994	0.011	1%	6%	49
Low	8	0.994	0.014	1%		
High	3	1.000	0	0%		

[1] Calculated from arcsine square-root transformed data; sample size = 3.
[2] Size required to detect a significant difference from Control treatment based on maximum achieved absolute difference; calculated on arcsine square-root transformed data.

3.3 Non-spawning Adult 14-day Assay for Methoxychlor

The non-spawning adult 14-Day Methoxychlor assay was conducted from October 14, 2002, to October 28, 2002 (exposure assay).

3.3.1 Survival

Total survival in the Control treatment during the non-spawning adult 14-Day Methoxychlor assay was 100%. Among the three concentration treatments, total survival ranged from 90% (High concentration) to 100% (Low concentration). One female died in the High and Medium concentrations and one male died in the High concentration.

3.3.2 Vitellogenin

Vitellogenin concentrations in Control-treatment females used during the Non-spawning Adult 14-Day Methoxychlor assay ranged from 78,344 ng/mL to 1,620,000 ng/mL (Figure 3.42). Among females exposed to the three methoxychlor concentrations, vitellogenin concentrations ranged from 13,512 ng/mL to 30,970,000 ng/mL. No significant differences in the mean vitellogenin concentration among treatments (Table 3.37) were detected (Kruskal-Wallis, $H = 1.52$, $p = 0.677$, df = 2). The achieved power for this endpoint was 98%, and the sample size required to detect a significant difference from the Control treatment at 80% power was 5 (Table 3.37).

Table 3.37. Summary statistics and power estimates for female vitellogenin concentrations (ng/mL) for the Non-spawning Adult 14-Day Methoxychlor assay.

Level	N	Mean	Stdev	CV	Achieved Power [1]	Sample Size Required [2]
Control	10	6,959,184	4,999,771	72%	11%	91
Low	10	9,617,600	9,591,390	100%		
Medium	9	8,056,562	7,644,691	95%		
High	9	10,758,278	7,481,935	70%		

[1] Calculated from natural log transformed data; sample size = 9.

[2] Size required to detect a significant difference from Control treatment based on maximum achieved absolute difference; calculated on natural log transformed data.

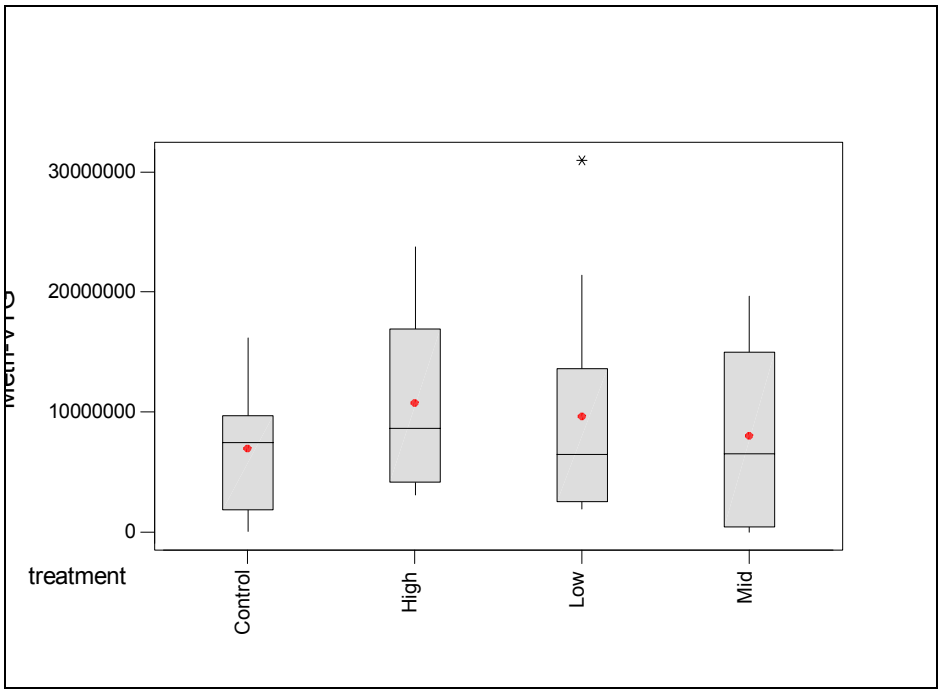

Figure 3.42. Boxplot of female vitellogenin concentration (ng/mL) by treatment for the Non-spawning Adult 14-Day Methoxychlor assay. The box represents the interquartile range, whiskers represent the data range, the horizontal line is the median value, the circle is the mean value, the asterisk represents a probable outlier.

Vitellogenin concentrations in Control-treatment males used during the Non-spawning Adult 14-Day Methoxychlor assay ranged from 0 ng/mL (not detected) to 1,818 ng/mL (Figure 3.43). Among males exposed to the three methoxychlor concentrations, vitellogenin concentrations ranged from 0 ng/mL (not detected) to 517,020 ng/mL. No significant differences in the mean vitellogenin concentration per treatment (Table 3.38) were detected (Kruskal-Wallis, $H = 2.48$, $p = 0.478$, df = 2). The achieved power for this endpoint was 18%, and the sample size required to detect a significant difference from the Control treatment at 80% power was 47 (Table 3.38).

Table 3.38. Summary statistics and power estimates for male vitellogenin concentrations (ng/mL) for the Non-spawning Adult 14-Day Methoxychlor assay.

Level	N	Mean	Stdev	CV	Achieved Power [1]	Sample Size Required [2]
Control	10	229	563	246%	18%	47
Low	10	1,566	3,242	207%		
Medium	10	505	1,213	240%		
High	9	158,488	219,547	139%		

[1] Calculated from natural log transformed data; sample size = 9.

[2] Size required to detect a significant difference from Control treatment based on maximum achieved absolute difference; calculated on natural log transformed data.

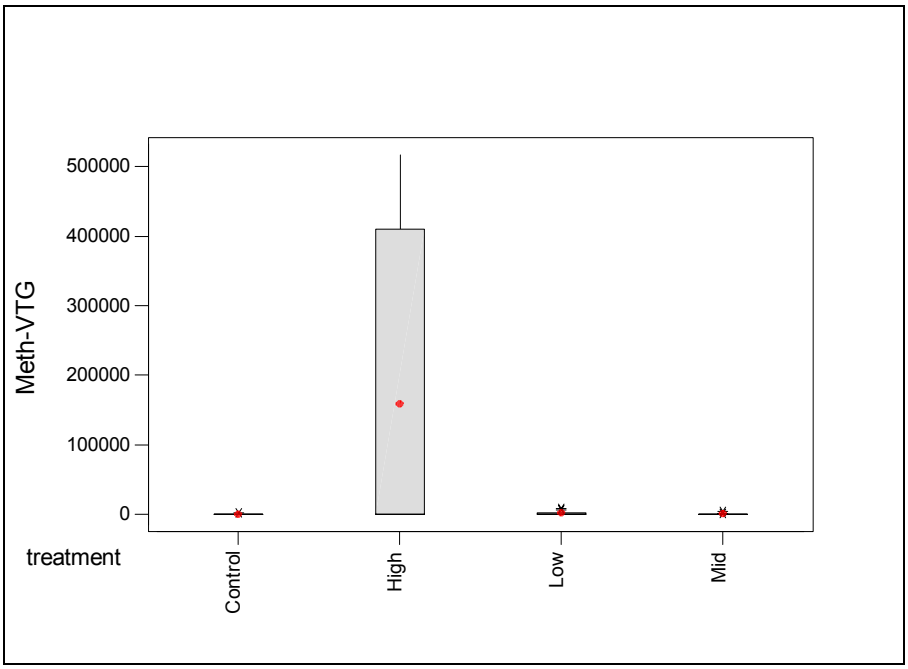

Figure 3.43. Boxplot of male vitellogenin concentration (ng/mL) by treatment for the Non-spawning Adult 14-Day Methoxychlor assay. The box represents the interquartile range, whiskers represent the data range, the horizontal line is the median value, the circle is the mean value, and asterisks represent probable outliers.

3.3.3 Appearance / Secondary Sex Characteristics

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

Morphological development among 39 males used during the methoxychlor Non-spawning Adult 14-day assay varied among treatments (Figure 3.44). Four males used during the assay lacked tubercles. Six males lacked a dorsal fat pad. Thirty-six males lacked vertical banding. There was no consistent dose-related pattern to these variations in morphology.

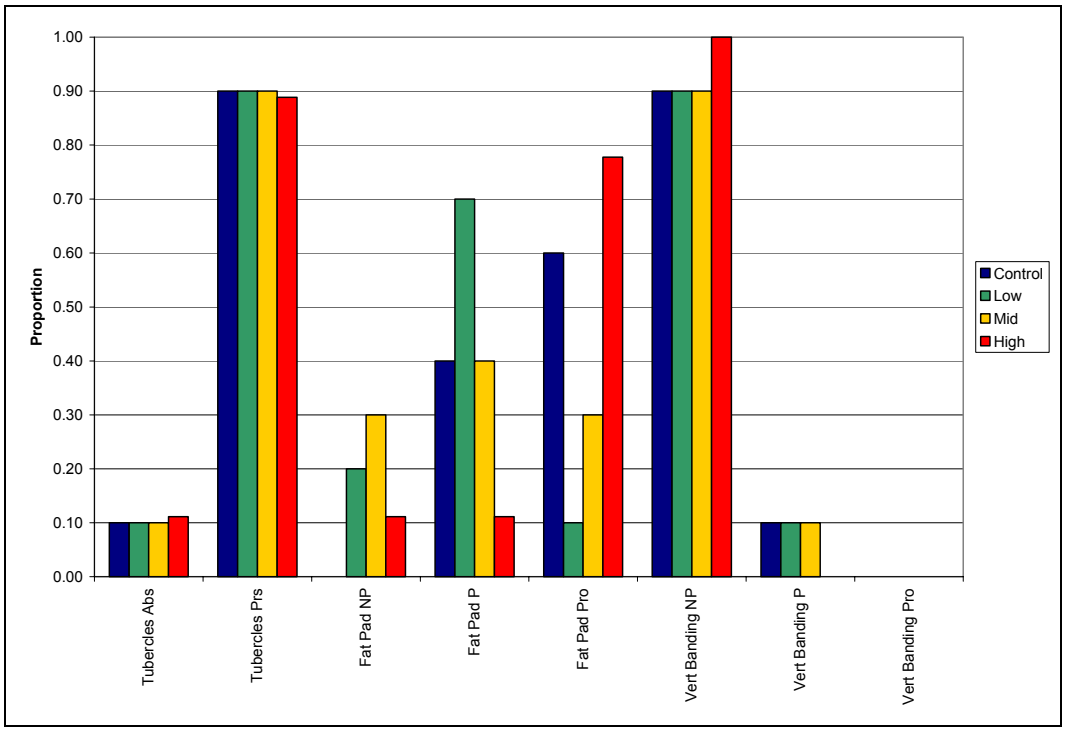

Figure 3.44. Secondary sex characteristics of males used during the methoxychlor Non-spawning Adult 14-Day assay.

3.3.4 Gonadosomatic Index

The range of GSI values calculated for females in the all treatments varied from two- to four-fold (Figure 3.45), and the overall within-treatment variability was moderate (CVs = 21% to 38%) (Table 3.39). The highest female GSI value was 34.6 (one fish in the Control treatment), but several fish had GSI values >20. There were no significant differences in mean GSI values among treatments (Kruskal-Wallis, H = 0.91, p = 0.822, df = 3) (Table 3.39). The achieved power for this endpoint was 7%, and the sample size required to detect a significant difference from the Control treatment at 80% power was 222 (Table 3.39).

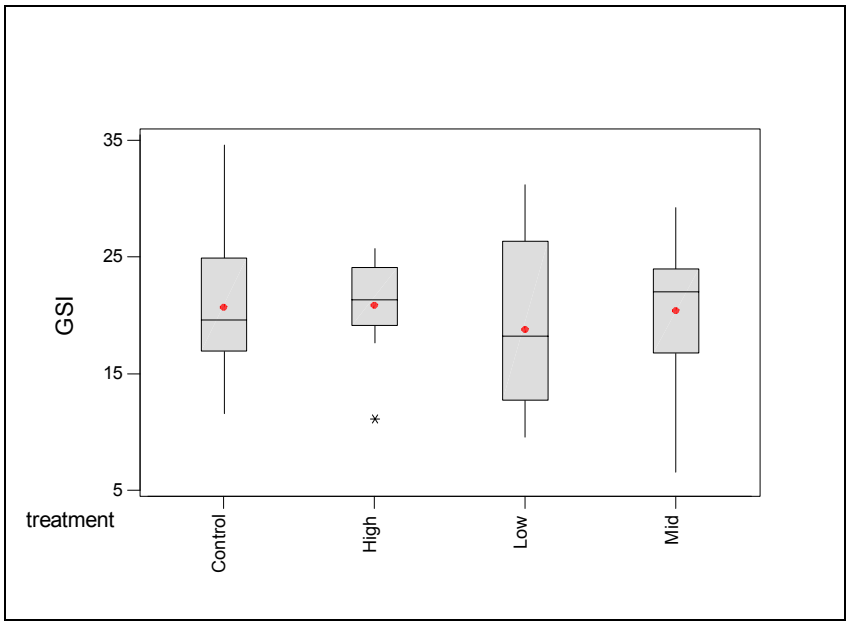

Figure 3.45. Boxplot of female GSI by treatment for the Non-spawning Adult 14-Day Methoxychlor assay. The box represents the interquartile range, whiskers represent the data range, the horizontal line is the median value, the circle is the mean value, and asterisks represent probable outliers.

Table 3.39. Summary statistics and power estimates for female gonadosomatic index data for the Non-spawning Adult 14-Day Methoxychlor assay

Level	N	Mean	SD	CV	Achieved Power [1]	Sample Size Required [2]
Control	10	20.7	6.4	31%	7%	222
Low	10	18.8	7.2	38%		
Medium	9	20.4	6.5	32%		
High	9	20.9	4.4	21%		

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

The range of most GSI values calculated for males during the non-spawning adult 14-Day Methoxychlor assay was large, ranging from 0.8 to 2.0 (Figure 3.46), which approximates the typical range for reproductively-active male fathead minnows. Overall within-treatment variability was moderate to high (CVs = 18% to 67%) (Table 3.40). The highest and lowest male GSI values were 2.4 to 2.7 (for two fish in the Medium concentration) and 0.3 to 0.6 (four fish in the Medium concentration), respectively. However, there were no significant differences (with $\alpha = 0.05$) in mean GSI values among treatments (Kruskal-Wallis, $H = 7.20$, $p = 0.066$, df = 3) (Table 3.40). The achieved power for this endpoint was 33%, and the sample size required to detect a significant difference from the Control treatment at 80% power was 26 (Table 3.40).

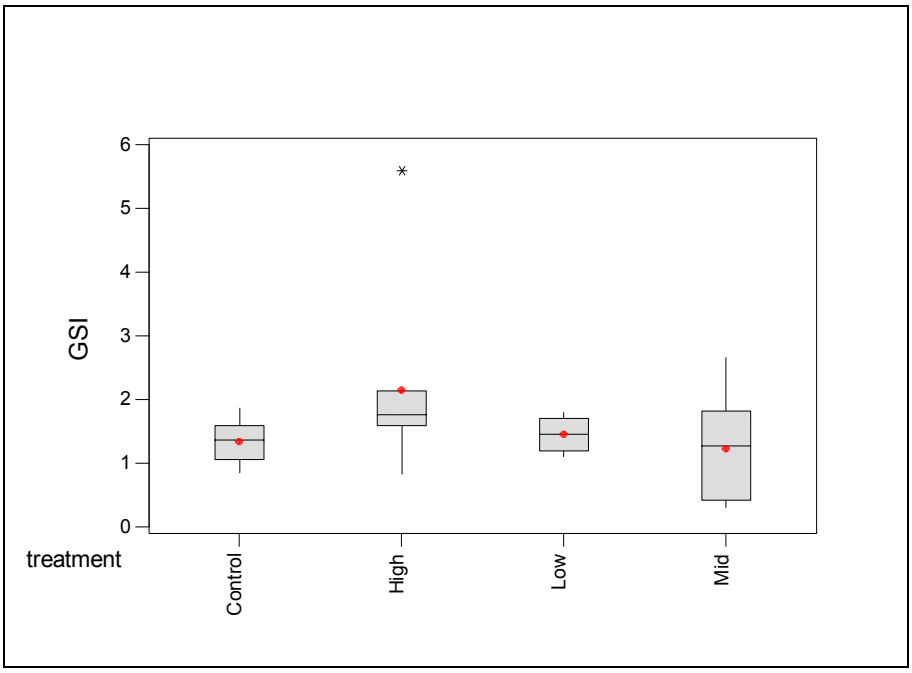

Figure 3.46. Boxplot of male GSI by treatment for the Non-spawning Adult 14-Day Methoxychlor assay. The box represents the interquartile range, whiskers represent the data range, the horizontal line is the median value, the circle is the mean value, and asterisks represent probable outliers.

Table 3.40. Summary statistics and power estimates for male gonadosomatic index data for the Non-spawning Adult 14-Day Methoxychlor assay

Level	N	Mean	SD	CV	Achieved Power [1]	Sample Size Required [2]
Control	10	1.34	0.33	25%	33%	26
Low	10	1.46	0.26	18%		
Medium	10	1.23	0.82	67%		
High	9	2.15	1.35	63%		

[1] Calculated from arcsine square-root transformed data; sample size = 9.

[2] Required size to detect a significant difference from Control treatment based on maximum achieved absolute difference; calculated on arcsine square-root transformed data.

3.3.5 Female Gonad Histology

A histological analysis was performed on the ovaries of 39 females exposed to methoxychlor during the non-spawning adult 14-day assay. Of these 35 fish, 8 were observed to have moderate-to-diffuse macrophage infiltration into the ovaries. Tissues from three fish with this condition were evaluated with a Gram stain and an acid-fast stain that demonstrated acid-fast-staining structures consistent with mycobacteria. All other fish with a similar macrophage infiltration in the ovaries were also presumed to have mycobacteriosis. At the time of the histological examination, it could not be determined whether the condition was exacerbated by the chemical exposure. Because one Control-treatment fish was infected, it was clear that the infection was distributed throughout the population. To determine whether the

infection affected the results, the analyses were conducted once with all fish included and then repeated with the infected fish excluded. The results of the second set of analyses (with infected fish removed) were reported only if they resulted in a change in the pattern of statistical significance obtained for the analyses that included all fish.

General Ovary Staging—Statistical analysis of the mean ovarian staging from 12 microscopic fields per fish revealed no significant differences among treatments (Kruskal-Wallis, $H = 5.46$, $p = 0.141$, df = 3). There was no change in this pattern of statistical significance when the infected fish were excluded from the analysis.

Quantitative Ovarian Staging—One hundred cells in each of three sections per female were examined to quantitatively determine the developmental stage of the ovaries. Ova from fish in all treatments ranged from Stage 1a to Stage 5 (see Section 2: Methods for a description of the stages) (Figure 3.47). Variability within treatments for each stage was very high, as indicated by CVs that ranged as high as 316% (Table 3.41). Although statistical analyses showed that there was a significant difference among treatments in the proportion of cells in developmental Stages 1a and 4, there were no significant differences among treatments in the proportion of cells in the developmental Stages 1b, 2, 3, and 5 (Table 3.41). The proportion of cells in developmental Stage 1a in the Medium concentration was significantly lower than those in the High- and Low-concentration treatments. The proportion of cells in developmental Stage 4 in the Control treatment was significantly lower than those in the Medium- and High-concentration treatments. There was no consistent trend of significant difference associated with the methoxychlor dose. When infected fish were excluded from the analyses, there were no significant differences in any of the developmental stages among treatments (Kruskal-Wallis, Stage 1a–$H = 5.18$, $p = 0.159$, df = 3; Stage 4–$H = 7.17$, $p = 0.067$, df = 3).

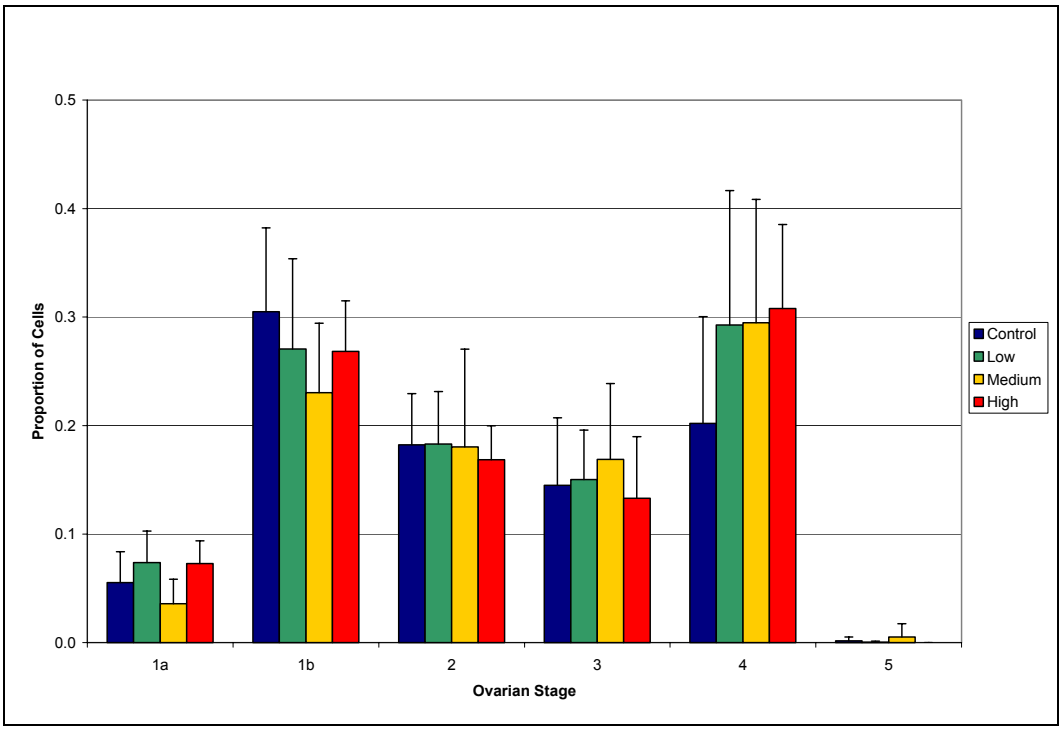

Figure 3.47. Frequency histogram showing the quantitative developmental staging of ovaries for each treatment of the Non-spawning Adult 14-Day Methoxychlor assay. For each treatment, the columns represent the grand mean proportion of cells in each stage and the bars represent the standard deviation.

Table 3.41. Descriptive statistics of the proportion of ovarian cells in each developmental stage for females from the Non-spawning Adult 14-Day Methoxychlor assay and results of the Kruskal-Wallis Test (df = 2) comparing treatments

	Control (n = 10)			Low (n = 10)			Medium (n = 9)			High (n = 9)			Kruskal-Wallis	
Stage	**Mean**	**SD**	**CV**	**Mean**	**SD**	**CV**	**Mean**	**SD**	**CV**	**Mean**	**SD**	**CV**	***H***	***p***
1a	0.055	0.028	51%	0.074	0.029	39%	0.036	0.023	63%	0.073	0.021	28%	10.14	0.017*
1b	0.305	0.077	25%	0.271	0.083	31%	0.230	0.064	28%	0.269	0.046	17%	3.70	0.296
2	0.182	0.047	26%	0.183	0.048	26%	0.180	0.090	50%	0.169	0.031	19%	1.27	0.736
3	0.145	0.062	43%	0.150	0.046	30%	0.169	0.070	41%	0.133	0.057	43%	1.28	0.735
4	0.202	0.098	49%	0.293	0.124	42%	0.295	0.114	39%	0.308	0.077	25%	9.52	0.023*
5	0.002	0.004	216%	0.000	0.001	316%	0.005	0.012	236%	0	0	–	2.68	0.443

* $p < 0.05$

Atretic Follicles—The mean proportion of atretic follicles per 300 follicles (counted per fish) ranged from 0.003 for females in the Kiwconcentration to 0.10 for females in the Control treatment (Figure 3.48). There was a significant difference in the proportions of atretic follicles among treatments (Kruskal-Wallis, $H = 8.89$, $p = 0.031$, df = 3). The values for females in the Low concentration was lower than those for females in the Control treatment. However, there was no consistent pattern of significant difference associated with the methoxychlor dose. There was no change in this pattern of statistical significance when the infected fish were excluded from the analysis.

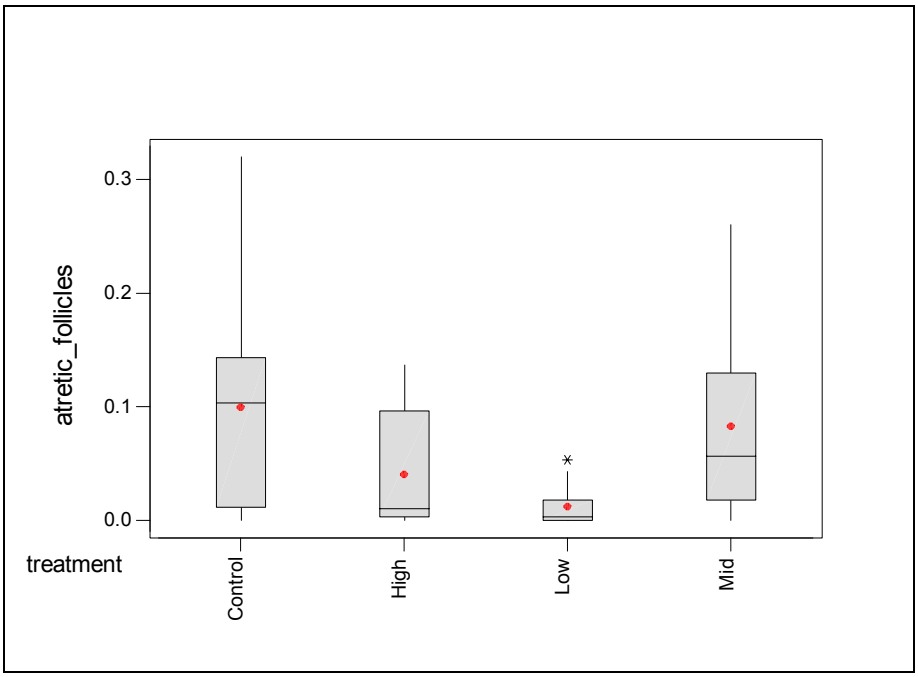

Figure 3.48. Boxplot of the proportion of atretic follicles per 300 follicles by treatment for the Non-spawning Adult 14-Day Methoxychlor assay. The box represents the interquartile range, whiskers represent the data range, the horizontal line is the median value, the circle is the mean value, and the asterisk represents a probable outlier.

Corpora Lutea—The mean proportion of corpora lutea per 300 follicles (counted per fish) ranged from 0.002 for females in the Medium concentration to 0.017 for females in the Low concentration (Figure 3.49). There were no significant differences in the proportions of corpora lutea among treatments (Kruskal-Wallis, $H = 6.19$, $p = 0.103$, df = 3). There was no change in this pattern of statistical significance when the infected fish were excluded from the analysis.

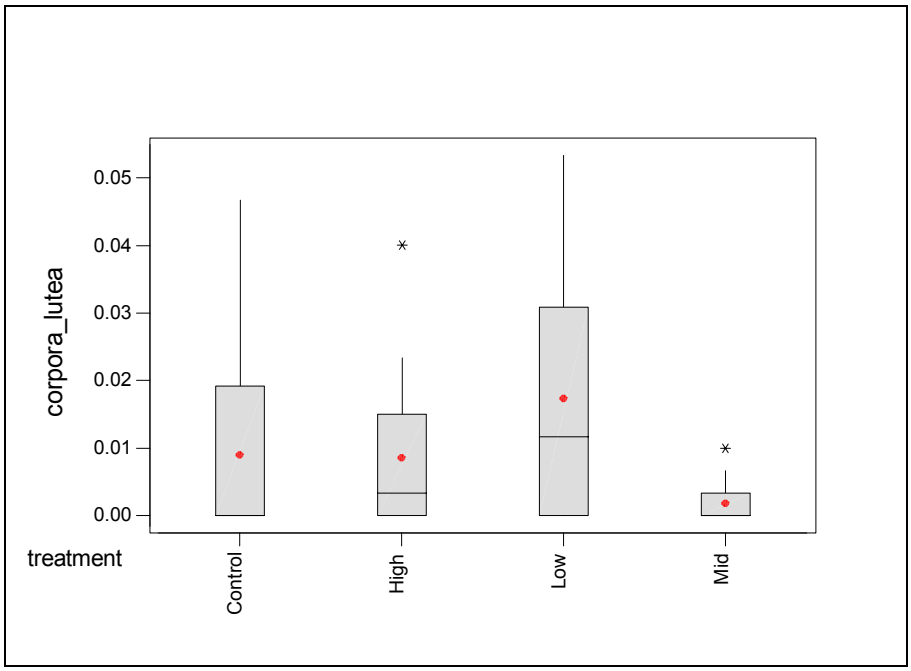

Figure 3.49. Boxplot of the proportion of corpora lutea per 300 follicles by treatment for the Non-spawning Adult 14-Day Methoxychlor assay. The box represents the interquartile range, whiskers represent the data range, the horizontal line is the median value, the circle is the mean value, asterisks represent probable outliers.

3.3.6 Male Gonad Histology

Testes Staging by Microscopic Field —Testes from males exposed to methoxychlor during the non-spawning adult 14-Day Methoxychlor 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 Section 2: Methods for description of developmental stages). All of the 120 microscopic fields examined in the 10 Control-treatment males showed Stage 4 (23 fields) or Stage 5 (97 fields) development. All of the 120 microscopic fields examined in the 10 Low-concentration treatment males showed Stage 4 (60 fields) or Stage 5 (60 fields) development. In the 8 Medium-concentration males available for examination, all of the 96 microscopic fields showed Stage 4 (58 fields) or Stage 5 (38 fields) development. In the 8 High-concentration males available for examination, all of the 96 microscopic fields showed Stage 4 (38 fields) or Stage 5 (58 fields) development. Statistical analysis of the mean staging from 12 microscopic fields per fish revealed that there was a significant difference among treatments (Kruskal-Wallis, $H = 10.54$, $p = 0.014$, df = 3). The mean testes stage for males in the Control treatment (4.81, sd = 0.21) was significantly greater than that in the Medium-concentration (4.40, sd = 0.37) and Low-concentration treatment (4.49, sd = 0.18), but not greater than that in the High-concentration treatment.

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 of the testes from all treatments ranged from Stage 2a to Stage 5 (Figure 3.50). Variability within treatments for each stage was very high, as indicated by CVs that ranged as high as 186% (Table 3.42). Although statistical analyses showed that there was a significant difference among treatments in the proportion of cells in developmental Stage 2b, there were no significant differences among treatments in the proportion

of cells in developmental Stages 2a, 3a, 3b, 4, and 5 (Table 3.42). Therefore, there was no consistent pattern of significant difference associated with methoxychlor dose.

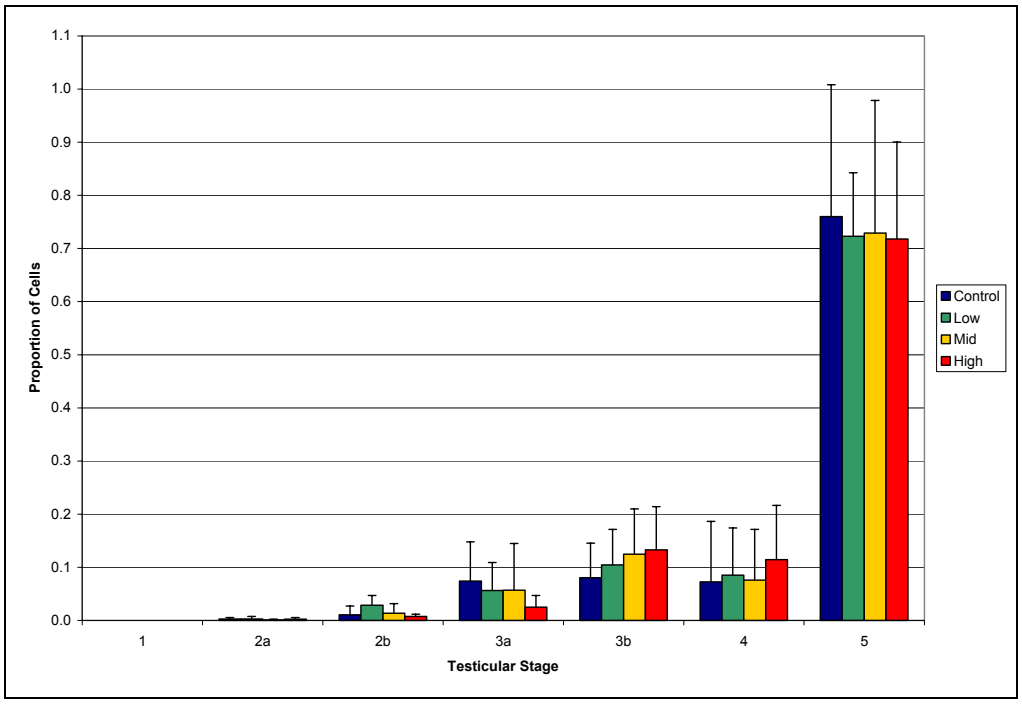

Figure 3.50. Frequency histogram showing the quantitative developmental staging of testes for each treatment of the Non-spawning Adult 14-Day Methoxychlor assay. For each treatment, the columns represent the grand mean proportion of cells in each stage and the bars represent the standard deviation.

Table 3.42. Descriptive statistics of the proportion of testes cells in each developmental stage for males from the Non-spawning Adult 14-Day Methoxychlor assay and results of the Kruskal-Wallis Test (df = 2) comparing treatments

	Control (n = 10)			Low (n = 10)			Medium (n = 8)			High (n = 8)			Kruskal-Wallis	
Stage	**Mean**	**SD**	**CV**	**Mean**	**SD**	**CV**	**Mean**	**SD**	**CV**	**Mean**	**SD**	**CV**	***H***	***p***
1	0	–	–	0	–	–	0	–	–	0	–	–	–	–
2a	0.003	0.003	99%	0.003	0.005	175%	0.001	0.002	186%	0.003	0.003	118%	2.41	0.491
2b	0.010	0.017	162%	0.028	0.018	65%	0.013	0.018	137%	0.008	0.004	57%	8.94	0.030*
3a	0.074	0.074	100%	0.056	0.053	94%	0.057	0.088	155%	0.025	0.022	87%	2.42	0.490
3b	0.080	0.065	81%	0.104	0.067	64%	0.125	0.085	68%	0.133	0.081	61%	3.23	0.358
4	0.073	0.114	156%	0.085	0.089	104%	0.076	0.095	126%	0.114	0.102	89%	1.79	0.617
5	0.760	0.248	33%	0.723	0.120	17%	0.729	0.250	34%	0.718	0.183	25%	1.98	0.576

* $p < 0.05$

Tubule Diameter—The diameter of the seminiferous tubules of males from the Control treatment ranged from 120.0 µm to 189.2 µm (Figure 3.51). Tubule diameters of males from the three test concentrations ranged from 89.2 µm to 208.6 µm. No significant differences in the mean tubule diameter per treatment were detected (Kruskal-Wallis, $H = 4.98$, $p = 0.173$, df = 3) (Table 3.43). The achieved power for this

endpoint was 26%, and the sample size required to detect a significant difference from the Control treatment at 80% power was 29 (Table 3.43).

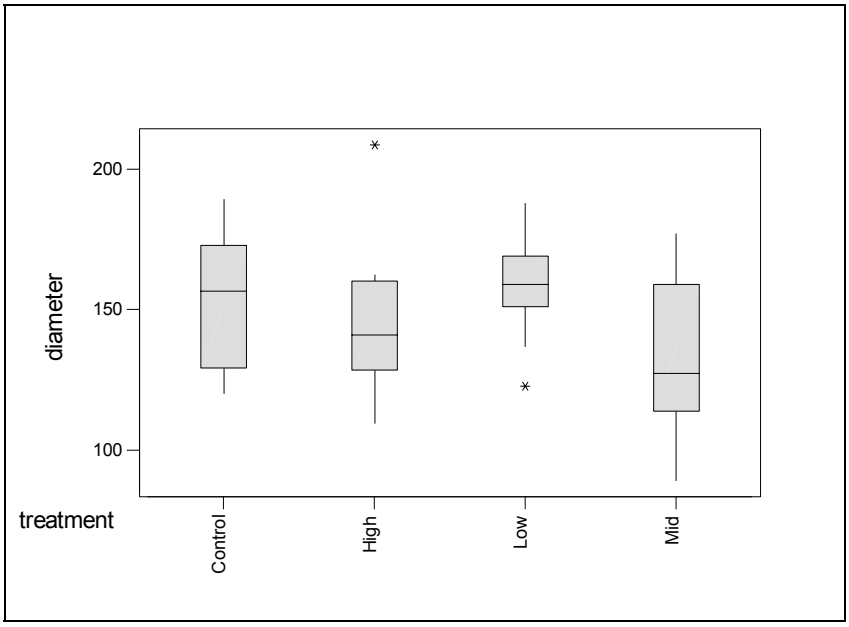

Figure 3.51. Boxplot of seminiferous tubule diameter (μm) by treatment for the Non-spawning Adult 14-Day Methoxychlor assay. The box represents the interquartile range, whiskers represent the data range, the horizontal line is the median value, the circle is the mean value, and asterisks represent probable outliers.

Table 3.43. Summary statistics and power estimates for male seminiferous tubule diameter data for the Non-spawning Adult 14-Day Methoxychlor assay

Level	N	Mean	SD	CV	Achieved Power [1]	Sample Size Required [2]
Control	10	154.1	23.2	15%	26%	29
Low	10	158.3	18.1	11%		
Medium	8	133.1	28.4	21%		
High	8	147.3	29.6	20%		

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

Observations— One male in the Medium-concentration treatment showed interstitial Sertoli cell proliferation and one showed multiple foci of inflammatory cells in the testes. No testicular atrophy was recorded and no ovatestes were observed for any treatment.

3.3.7 Plasma Steroid Concentrations

Estradiol—Estradiol concentrations in Control-treatment females used during the Non-spawning Adult 14-Day Methoxychlor assay ranged from 306 pg/mL to 3,953 pg/mL (Figure 3.52). Among females exposed to the two methoxychlor concentrations, estradiol concentrations ranged from 0 pg/mL (not detected) to 6,336 pg/mL. No significant differences in the mean estradiol concentration per treatment (Table 3.44) were detected (Kruskal-Wallis, $H = 5.52$, $p = 0.137$, df = 2). The achieved power for this endpoint was 32%, and the sample size required to detect a significant difference from the Control treatment at 80% power was 20 (Table 3.44).

Table 3.44. Summary statistics and power estimates for female estradiol concentrations (pg/mL) for the Non-spawning Adult 14-Day Methoxychlor assay.

Level	N	Mean	Stdev	CV	Achieved Power [1]	Sample Size Required [2]
Control	7	1,732	1,375	79%	32%	20
Low	10	517	480	93%		
Medium	7	2,611	2,417	93%		
High	8	1,183	1,953	165%		

[1] Calculated from natural log transformed data; sample size = 7.
[2] Size required to detect a significant difference from Control treatment based on maximum achieved absolute difference; calculated on natural log transformed data.

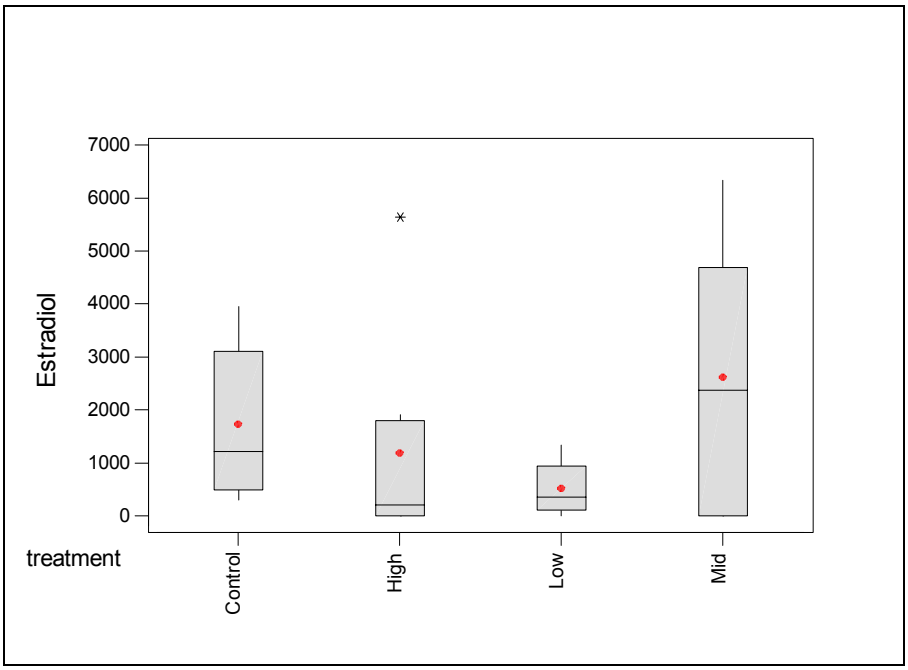

Figure 3.52. Boxplot of female estradiol concentration (pg/mL) by treatment for the Non-spawning Adult 14-Day Methoxychlor assay. The box represents the interquartile range, whiskers represent the data range, the horizontal line is the median value, the circle is the mean value, and the asterisk represents a probable outlier.

Estradiol concentrations in Control-treatment males used during the Non-spawning Adult 14-Day Methoxychlor assay ranged from 135 pg/mL to 312 pg/mL (Figure 3.53). Estradiol was not detected in males exposed to the three methoxychlor concentrations (Table 3.45). Achieved power was not calculated for this endpoint.

Table 3.45. Summary statistics and power estimates for male estradiol concentrations (pg/mL) for the Non-spawning Adult 14-Day Methoxychlor assay.

Level	N	Mean	Stdev	CV	Achieved Power [1]	Sample Size Required [1]
Control	8	229	64	28%	–	–
Low	6	0	0	–		
Medium	5	0	0	–		
High	5	0	0	–		

[1] Not calculated for this endpoint.

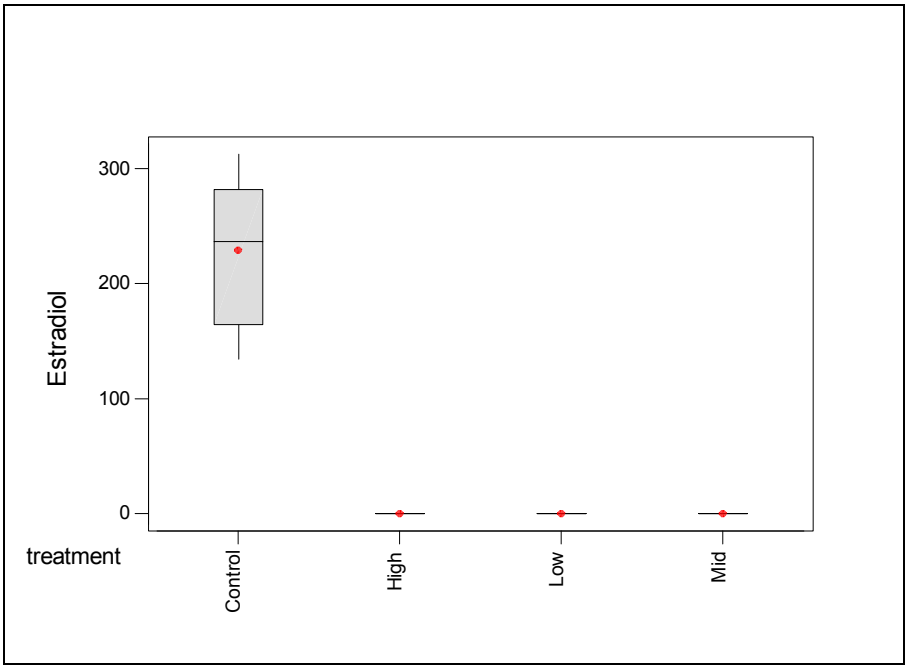

Figure 3.53. Boxplot of male estradiol concentration (pg/mL) by treatment for the Non-spawning Adult 14-Day Methoxychlor assay. The box represents the interquartile range, whiskers represent the data range, the horizontal line is the median value, and the circle is the mean value.

Testosterone—Testosterone concentrations in Control-treatment females used during the Non-spawning Adult 14-Day Methoxychlor assay ranged from 0 pg/mL (not detected) to 7,781 pg/mL (Figure 3.54). Among females exposed to the three methoxychlor concentrations, testosterone concentrations ranged from 0 pg/mL (not detected) to 9,649 pg/mL. A significant difference in the mean testosterone concentration per treatment (Table 3.46) was detected (Kruskal-Wallis, $H = 8.23$, $p = 0.041$, df = 2). The mean concentration of testosterone in females from the Medium concentration was greater than that in

females from the Low concentration. The achieved power for this endpoint was 7%, and the sample size required to detect a significant difference from the Control treatment at 80% power was 38 (Table 3.46).

Table 3.46. Summary statistics and power estimates for female testosterone concentrations (pg/mL) for the Non-spawning Adult 14-Day Methoxychlor assay.

Level	N	Mean	Stdev	CV	Achieved Power [1]	Sample Size Required [2]
Control	7	3,063	2,795	91%	7%	38
Low	7	789	564	71%		
Medium	4	5,379	3,168	59%		
High	2	1,929	2,228	116%		

[1] Calculated from natural log transformed data; sample size = 2.
[2] Size required to detect a significant difference from Control treatment based on maximum achieved absolute difference; calculated on natural log transformed data.

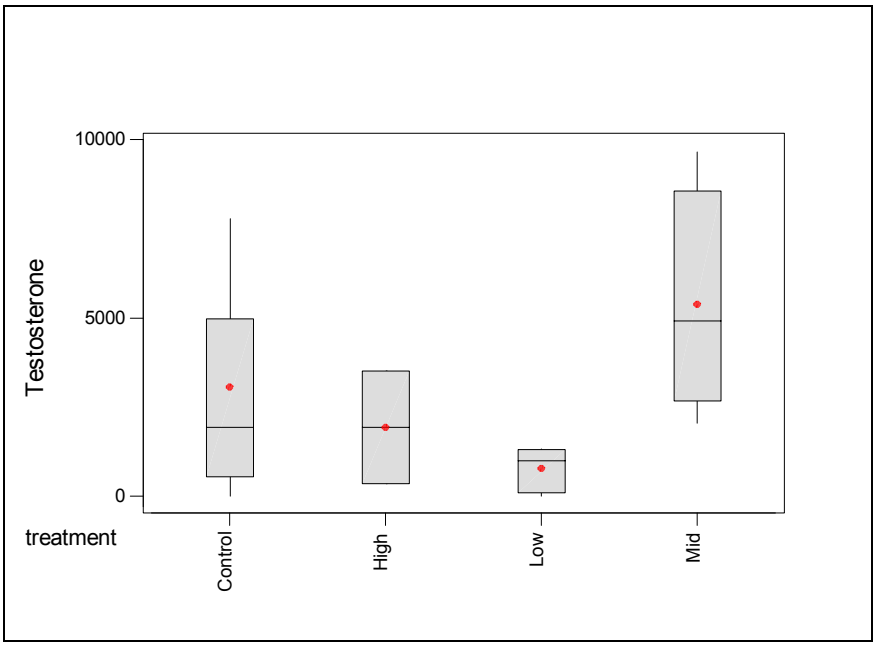

Figure 3.54. Boxplot of female testosterone concentration (pg/mL) by treatment for the Non-spawning Adult 14-Day Methoxychlor assay. The box represents the interquartile range, whiskers represent the data range, the horizontal line is the median value, and the circle is the mean value.

Testosterone concentrations in Control-treatment males used during the Non-spawning Adult 14-Day Methoxychlor assay ranged from 132 pg/mL to 5,170 pg/mL (Figure 3.55). Among males exposed to the three methoxychlor concentrations, testosterone concentrations ranged from 295 pg/mL to 7,802 pg/mL. No significant differences in the mean testosterone concentration per treatment (Table 3.47) were detected (Kruskal-Wallis, $H = 5.45$, $p = 0.142$, df = 2). The achieved power for this endpoint was 14%, and the sample size required to detect a significant difference from the Control treatment at 80% power was 33 (Table 3.47).

Table 3.47. Summary statistics and power estimates for male testosterone concentrations (pg/mL) for the Non-spawning Adult 14-Day Methoxychlor assay.

Level	N	Mean	Stdev	CV	Achieved Power [1]	Sample Size Required [2]
Control	8	2,247	1,855	83%	14%	33
Low	5	2,026	1,375	68%		
Medium	7	703	635	90%		
High	7	3,195	2,774	87%		

[1] Calculated from natural log transformed data; sample size = 5.
[2] Size required to detect a significant difference from Control treatment based on maximum achieved absolute difference; calculated on natural log transformed data.

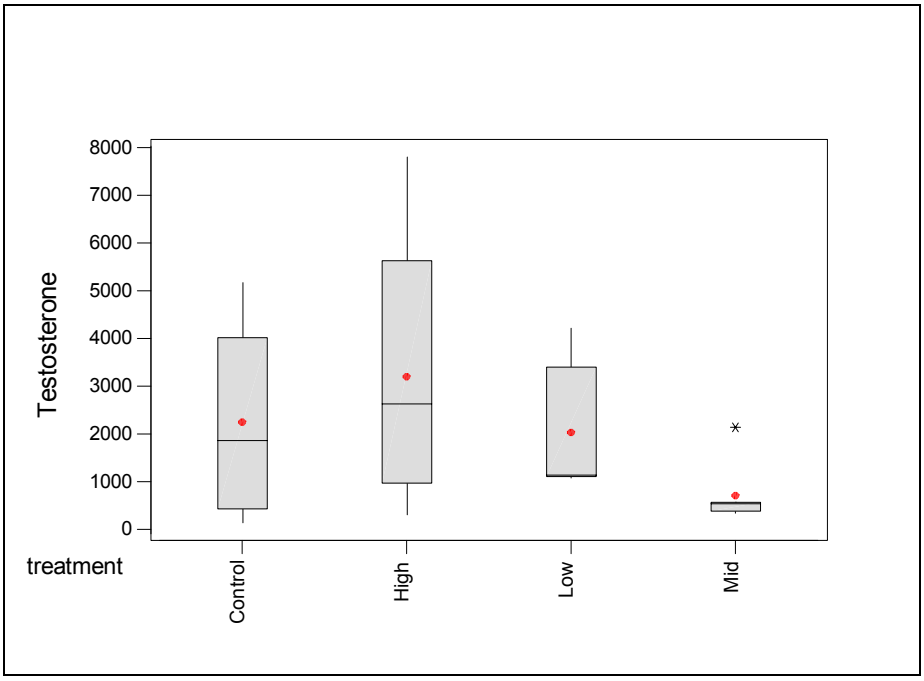

Figure 3.55. Boxplot of male testosterone concentration (pg/mL) by treatment for the Non-spawning Adult 14-Day Methoxychlor assay. The box represents the interquartile range, whiskers represent the data range, the horizontal line is the median value, the circle is the mean value, and the asterisk represents a probable outlier.

11-ketotestosterone—11-ketotesosterone was only detected in one of five Control-treatment females used during the Non-spawning Adult 14-Day Methoxychlor assay (Figure 3.56). Among females exposed to the three methoxychlor concentrations, 11-ketotesosterone was only detected in one individual from the Low and Medium concentrations (four and three females analyzed for each treatment, respectively). 11-ketotesosterone was not detected in the single female from the High concentration available for analysis. No significant differences in the mean 11-ketotesosterone concentration per treatment (Table 3.48) were detected (Kruskal-Wallis, $H = 0.44$, $p = 0.932$, 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 128 (Table 3.48).

Table 3.48. Summary statistics and power estimates for female 11-ketotesosterone concentrations (pg/mL) for the Non-spawning Adult 14-Day Methoxychlor assay.

Level	N	Mean	Stdev	CV	Achieved Power [1]	Sample Size Required [2]
Control	5	186	415	224%	5%	128
Low	4	80	159	200%		
Medium	3	260	450	173%		
High	1	0	0	–		

[1] Calculated from natural log transformed data; sample size = 2 (smallest allowable).
[2] Size required to detect a significant difference from Control treatment based on maximum achieved absolute difference; calculated on natural log transformed data.

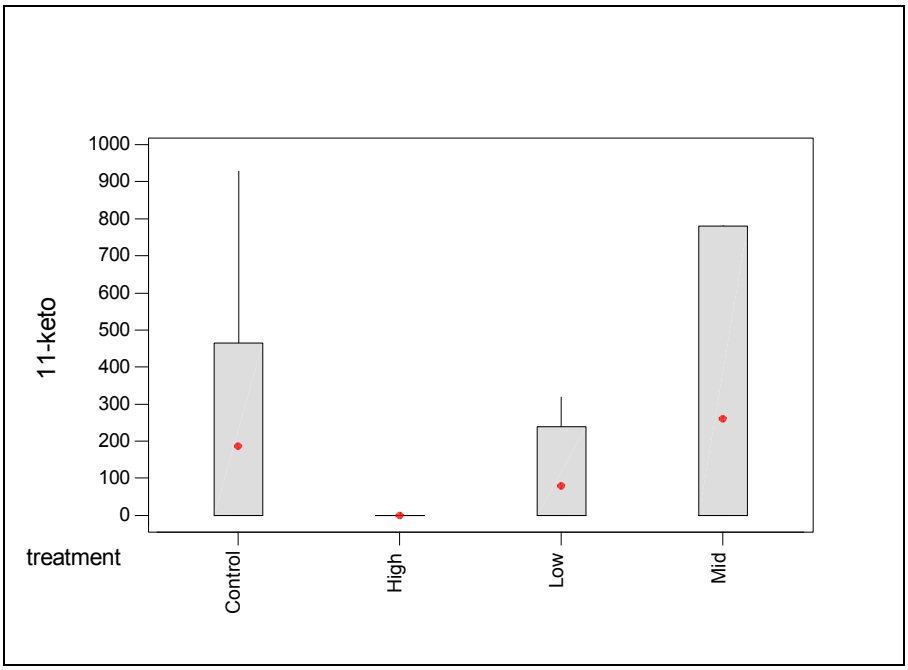

Figure 3.56. Boxplot of female 11-ketotesosterone concentration (pg/mL) by treatment for the Non-spawning Adult 14-Day Methoxychlor assay. The box represents the interquartile range, whiskers represent the data range, the horizontal line is the median value, and the circle is the mean value.

11-ketotesosterone concentrations in Control-treatment males used during the Non-spawning Adult 14-Day Methoxychlor assay ranged from 999 pg/mL to 40,100 pg/mL (Figure 3.57). Among males exposed to the three methoxychlor concentrations, 11-ketotesosterone concentrations ranged from 0 pg/mL (not detected) to 159,600 pg/mL. No significant differences in the mean 11-ketotesosterone concentration per treatment (Table 3.49) were detected (Kruskal-Wallis, $H = 6.15$, $p = 0.105$, 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 25 (Table 3.49).

Table 3.49. Summary statistics and power estimates for male 11-ketotesosterone concentrations (pg/mL)

for the Non-spawning Adult 14-Day Methoxychlor assay.

Level	N	Mean	Stdev	CV	Achieved Power [1]	Sample Size Required [2]
Control	8	14,552	13,669	94%	29%	25
Low	9	13,965	14,896	107%		
Medium	10	4,257	4,364	103%		
High	9	36,396	50,102	138%		

[1] Calculated from natural log transformed data; sample size = 8.

[2] Size required to detect a significant difference from Control treatment based on maximum achieved absolute difference; calculated on natural log transformed data.

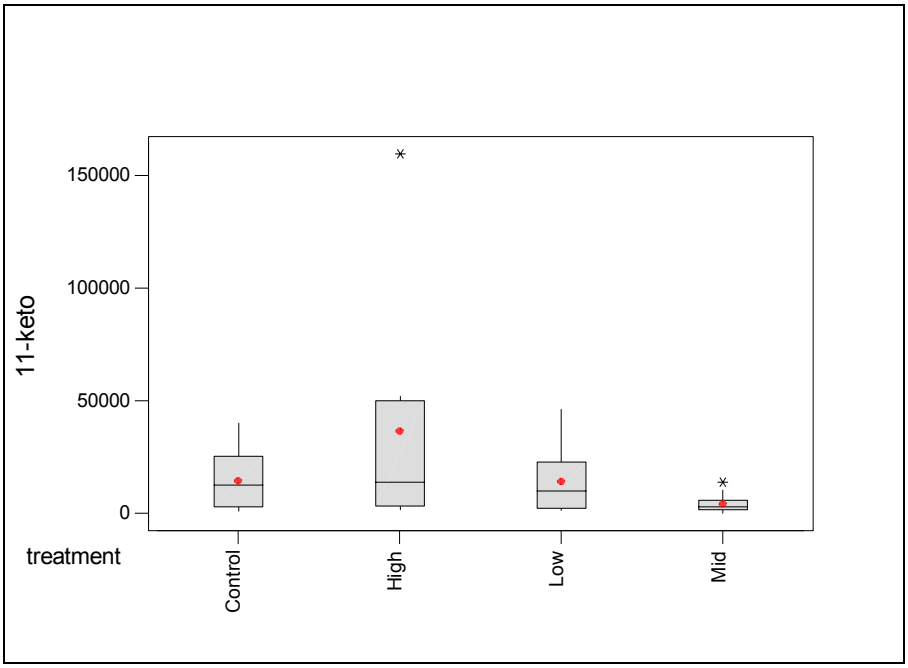

Figure 3.57. Boxplot of male 11-ketotesosterone concentration (pg/mL) by treatment for the Non-spawning Adult 14-Day Methoxychlor assay. The box represents the interquartile range, whiskers represent the data range, the horizontal line is the median value, the circle is the mean value, and the asterisks represent probable outliers.

3.3.8 Body Weight and Length

The body weight of females used in the non-spawning adult 14-Day Methoxychlor assay ranged from 1.3 g to 3.1 g (Figure 3.58). There were no significant differences in mean body weight (natural log transformed) among treatments (Kruskal-Wallis, $H = 6.30$, $p = 0.098$, df = 3). The achieved power for this endpoint was 41%, and the sample size required to detect a significant difference from the Control treatment at 80% power was 20 (Table 3.50).

The body (fork) length of females used in the non-spawning adult 14-Day Methoxychlor assay ranged from 40 mm to 55 mm (Figure 3.59). There were no significant differences in mean length among treatments (Kruskal-Wallis, $H = 1.51$, $p = 0.679$, df = 3). The achieved power for this endpoint was 11%,

and the sample size required to detect a significant difference from the Control treatment at 80% power was 93 (Table 3.51).

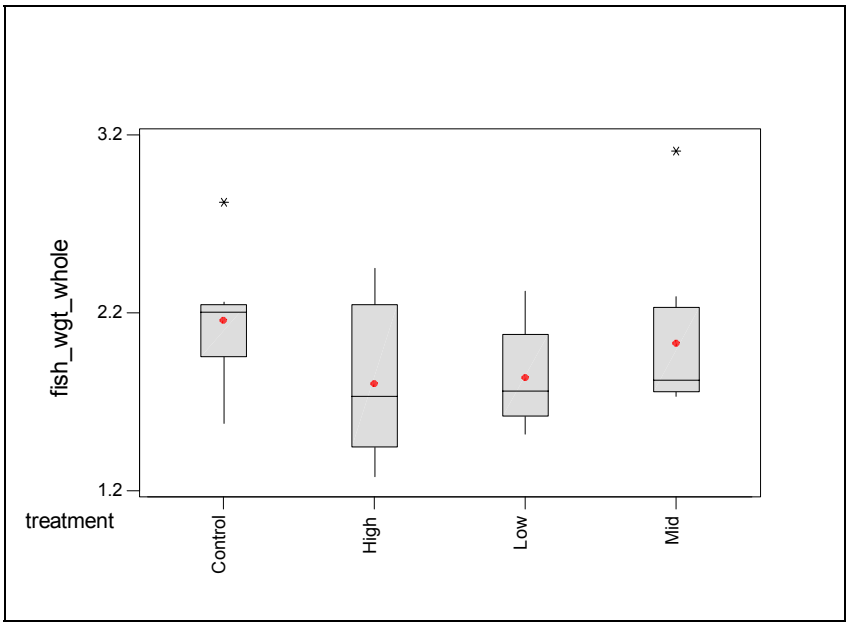

Figure 3.58. Boxplot of female body weight (g) by treatment for the Non-spawning Adult 14-Day Methoxychlor assay. The box represents the interquartile range, whiskers represent the data range, the horizontal line is the median value, the circle is the mean value, and asterisks represent probable outliers.

Table 3.50. Summary statistics and power estimates for female body weight (g) data for the Non-spawning Adult 14-Day Methoxychlor assay

Level	N	Mean	SD	CV	Achieved Power [1]	Sample Size Required [2]
Control	10	2.2	0.3	15%	41%	20
Low	10	1.8	0.3	15%		
Medium	9	2.0	0.5	22%		
High	9	1.8	0.4	24%		

[1] Calculated from natural log transformed data; sample size = 9.

[2] Required size to detect a significant difference from Control treatment based on maximum achieved absolute difference; calculated on natural log transformed data.

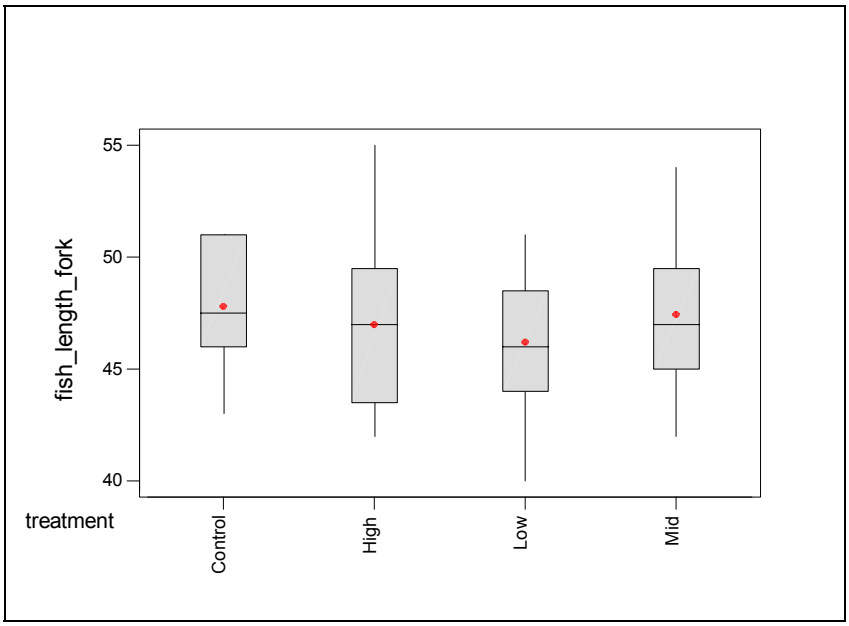

Figure 3.59. Boxplot of female body length (mm) by treatment for the Non-spawning Adult 14-Day Methoxychlor assay. The box represents the interquartile range, whiskers represent the data range, the horizontal line is the median value, and the circle is the mean value.

Table 3.51. Summary statistics and power estimates for female body length (mm) data for the Non-spawning Adult 14-Day Methoxychlor assay

Level	N	Mean	SD	CV	Achieved Power [1]	Sample Size Required [2]
Control	10	47.8	2.6	5%	11%	93
Low	10	46.2	3.2	7%		
Medium	9	47.4	3.5	7%		
High	9	47.0	4.2	9%		

[1] Calculated from natural log transformed data; sample size = 9.

[2] Required size to detect a significant difference from Control treatment based on maximum achieved absolute difference; calculated on natural log transformed data.

The body weight of males used in the non-spawning adult 14-Day Methoxychlor assay ranged from 1.6 g to 5.5 g (Figure 3.60). There were no significant differences in mean body weight among treatments (Kruskal-Wallis, $H = 3.80$, $p = 0.284$, 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 27 (Table 3.52).

The body length of males used in the Non-spawning adult 14-Day Methoxychlor assay ranged from 52 mm to 70 mm (Figure 3.61). There were no significant differences in mean body length among treatments (Kruskal-Wallis, $H = 1.73$, $p = 0.630$, df = 3). The achieved power for this endpoint was 16%, and the sample size required to detect a significant difference from the Control treatment at 80% power was 56 (Table 3.52).

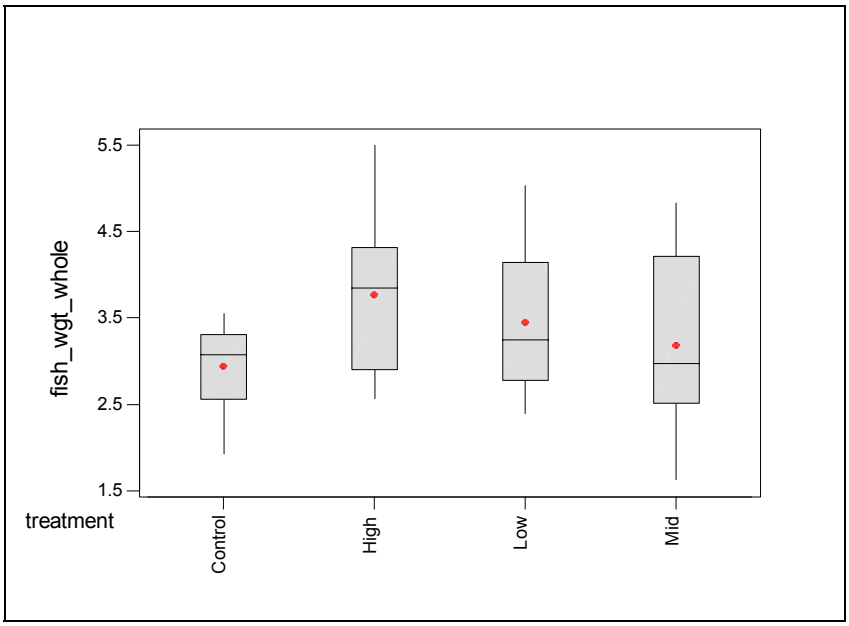

Figure 3.60. Boxplot of male body weight (g) by treatment for the Non-spawning Adult 14-Day Methoxychlor assay. The box represents the interquartile range, whiskers represent the data range, the horizontal line is the median value, and the circle is the mean value.

Table 3.52. Summary statistics and power estimates for male body weight (g) data for the Non-spawning Adult 14-Day Methoxychlor assay

Level	N	Mean	SD	CV	Achieved Power [1]	Sample Size Required [2]
Control	10	2.9	0.5	17%	30%	27
Low	10	3.4	0.8	24%		
Medium	10	3.2	1.1	33%		
High	9	3.8	0.9	25%		

[1] Calculated from natural log transformed data; sample size = 9.

[2] Required size to detect a significant difference from Control treatment based on maximum achieved absolute difference; calculated on natural log transformed data.

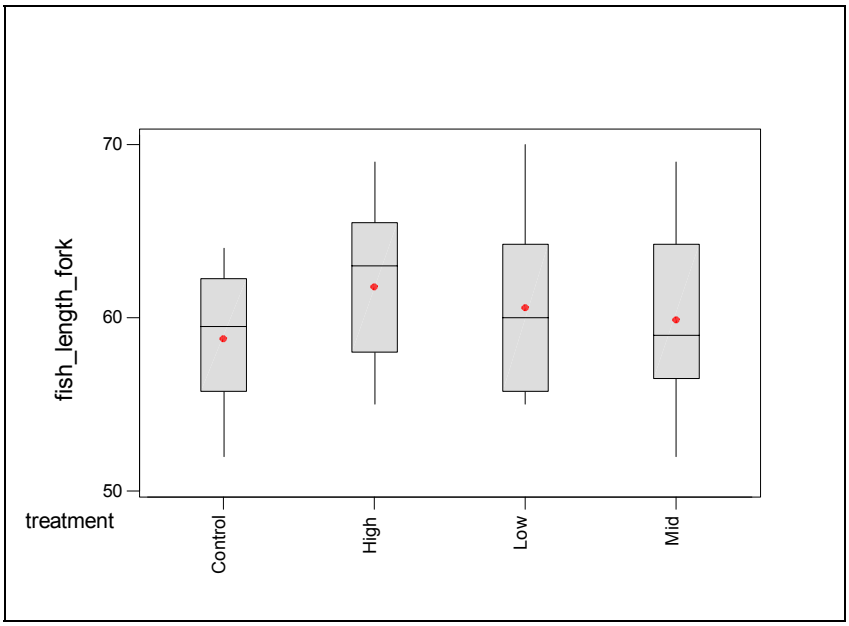

Figure 3.61. Boxplot of male body length (mm) by treatment for the Non-spawning Adult 14-Day Methoxychlor assay. The box represents the interquartile range, whiskers represent the data range, the horizontal line is the median value, and the circle is the mean value.

Table 3.53. Summary statistics and power estimates for male body length (mm) data for the Non-spawning Adult 14-Day Methoxychlor assay

Level	N	Mean	SD	CV	Achieved Power [1]	Sample Size Required [2]
Control	10	58.8	3.9	7%	16%	56
Low	10	60.6	4.8	8%		
Medium	10	59.9	5.4	9%		
High	9	61.8	4.5	7%		

[1] Calculated from natural log transformed data; sample size = 9.

[2] Required size to detect a significant difference from Control treatment based on maximum achieved absolute difference; calculated on natural log transformed data.