

**Hylebos Waterway Fish Injury Studies  
Individual Data and Quality Assurance Results  
CASE NARRATIVE**

**Reproductive Toxicology in Flatfish**

**Reproductive Steroid RIAs-Plasma 17-b Estradiol Analyses**

Added explanation for changes in standard curve range for all test dates to the case narrative.

Added explanations for the following test dates to the case narrative:

Date: 6/27/95; Analyzed by: SS;

Date: 7/6/95; Analyzed by: LC;

Date: 7/18/95; Analyzed by: SS.

Tables:

Cleaned up tables, comments and significant digits are now more consistent.

Changes made to Table 3:

specimen# 3406: Non-target species, removed from the table;

specimen# 3528: Non-target species, removed from the table;

specimen# 3638: Non-target species, removed from the table;

specimen# 3721: Non-target species, removed from the table;

specimen# 3594A: Non-target species, removed from the table;

specimen#: 3271, test date 6/22/95:                    Changed E2 final from 858.03 to 875.73(pg/ml);

specimen#: 3532, test date 6/22/95:                    Changed E2 final from 131.04 to 146.75(pg/ml);

specimen# 3540, test date 7/18/95:                    Changed E2 final from 3520.18 to 3726.95(pg/ml);

specimen# 3568, test date 7/10/95:                    13% avg 48.33 changed to 31.86;

specimen# 3501, test date 7/14/95:                    This specimen# tested on 6/22/95 did not meet the QA criteria. It was rerun on 7/14/95, however, not enough plasma was available for triplicate test. The value previously reported, 393.55pg/ml, was deleted because it was calculated based on a single analysis.

**Hylebos Waterway Fish Injury Studies  
Individual Data and Quality Assurance Results  
CASE NARRATIVE**

**Reproductive Toxicology in Flatfish**

**Reproductive Steroid RIAs-Plasma--17- $\beta$  Estradiol Analyses**

**Calibrations**

Standard curve: As stated in the SAP, standards for this assay typically range from 0 - 2000 pg estradiol 17-b/100 $\mu$ l; to reflect the typical range of the sample concentrations, the standard curve was modified to range from 0 to 500pg/estradiol 17-b/100 $\mu$ l. Therefore, all final estradiol concentrations are slightly modified from those originally submitted to DARC on 5/30/1996.

The data used to quantitate plasma 17-b estradiol (Table 1) met the calibration criteria outlined in the Hylebos SAP, Table 4, except as following:

Date: 6/22/95

Analyzed by:S.S.

Two of the triplicate NSB samples analyzed were outside the range typically observed, and deleted. RSD for 250pg/100ml standard (20.62%) exceeded maximum RSD of 200% outlined in the SAP.

Date: 6/27/95

Analyzed by:S.S.

Radioactive Liquid scintillation counter (LSC) broke down in the middle of the count. Remaining samples including standards were recounted on another LSC, and plasma estradiol concentrations of the samples were calculated on the new standard curve generated.

Date: 7/6/95

Analyzed by: LC

RSD for 250pg/100ml standard (22.95%) exceeded maximum RSD Of 20% outlined in the SAP.

Date: 7/10/95

Analyzed by: LC

Duplicate rather than triplicate samples were used to calculate NSB for the standard curve.

Date 7/18/95

Analyzed by SS

3.9pg/100ml standards were not used in the calculation of standard curve because the values obtained were outside the range typically observed.

Date: 3/13/96

Analyzed by: B.B.

The standard curve regression coefficient, 0.94, was lower than the acceptable limit, 0.95, outlined in the SAP. Nevertheless, other criterias outlined in the SAP were satisfied.

An assay performed on 6/22/95, and two assays performed on 7/10/95 had lower  $B_0$  than the acceptable range outlined on Hylebos SAP, Table 4. However, these values were accepted because the binding percentage of the standard curve and samples' (B%) are calculated based on the  $B_0$ . The change in  $B_0$  shifts the effective range of the assay, not the plasma estradiol concentrations. The change in  $B_0$  produced only minor changes in the effective range of the assay, therefore, the assays were not rerun.

### **Quality Control Sample**

All quality, control samples tested (Table 2) met the criteria outlined in the Hylebos SAP, Table 4.

### **Sample Triplicate and Performance Evaluation**

All samples were analyzed in triplicate. Samples in which the estradiol concentration was below effective concentration of the assay ( $B\% > 80\%$ ) were retested at a higher concentration (i.e. maximum plasma volume of 100 $\mu$ I of plasma/tube), or given a value equal to half of lower detection limit. Samples in which the estradiol concentrations was above effective concentration of the assay ( $B\% < 20\%$ ) were retested at a lower concentration (i.e. minimum volume of 5 $\mu$ I of plasma/tube). A few samples were deleted because reliable measurement could not be obtained with amount of samples available. Samples that did not meet the criteria outlined in the Hylebos SAP, Table 4, i.e. RSD greater than 20%, were reanalyzed, or the outlier of the triplicate was removed. However, some samples in which B% was slightly higher than 80% (i.e. 80.41), and or, RSD slightly higher than 20% (i.e. 21.40) were accepted.

### **Reanalysis**

Samples that were reanalyzed are noted in Table 3.

## Reproductive Toxicology in Flatfish

### Reproductive Steroid RIAs-Plasma 17:β Estradiol Analyses Table 1 Notes

Total: counts per minute (cpm) of tritiated 17-β estradiol (label) added to each tubes.

NSB (non specific binding): binding to label in the absence of antigen and steroid.

0 standard: binding to label and antigen, no steroid present.

Bo: binding efficiency of the antibody to labeled antigen.  
 $((0 \text{ standard (cpm)} - \text{NSB(cpm)}) / (\text{Total(cpm)} - \text{NSB(cpm)})) \times 100$ .

B%: binding percentage of sample and standards (3.9 - 500pg/ml 17-β estradiol) relative to Bo.

$$\left( \frac{((\text{sample(cpm)} - \text{NSB(cpm)}) / \text{Total(cpm)} - \text{NSB(cpm)})}{((0 \text{ standard(cpm)} - \text{NSB(cpm)}) / (\text{Total(cpm)} - \text{NSB(cpm)})} \right)$$

EC80: Lower limit of the assay, where B% of 80 occurs.

EC20: Upper limit of the assay, where B% of 20 occurs.

RSD (relative standard deviation): standard deviation (SD) of each triplicate analyses divided by mean of each triplicate multiplied by 100.

## Reproductive Toxicology in Flatfish

### **Reproductive Steroid RIAs-Plasma 17-β Estradiol Analyses**

#### **Table 2- Notes**

B%: binding percentage of sample relative to total, NSB and 0 standard.

Total: counts per minute (cpm) of tritiated 17-β estradiol (label) added to each tubes.

NSB (nonspecific binding): binding to label in the absence of antigen and steroid.

0 standard: binding to label and antigen, no steroid present.

B% is calculated as following:

$$\left( \frac{((\text{sample}(cpm) - NSB(cpm)) / \text{Total}(cpm) - NSB(cpm)))}{((\text{0 standard}(cpm) - NSB(cpm)) / (\text{Total}(cpm) - NSB(cpm)))} \right)$$

Plasma 17-β estradiol concentrations (pg/ml plasma) are generated by automatic interpolation from the standard curve fit of the mean B% of protein standards (logistic regression).

RSD (relative standard deviation), unless otherwise noted, is standard deviation (SD) of each triplicate analyses divided by mean of each triplicate multiplied by 100.

## Reproductive' Toxicology in Flatfish

### Rel2rodugtive Steroid RIAs-Plasma 17-β Estradiol Analyses Table 3 Notes

B%: binding percentage of sample relative to total, NSB and 0 standard.

Total: counts per minute (cpm) of tritiated 17-β estradiol (label) added to each tubes.

NSB (non specific binding): binding to label in the absence of antigen and steroid.

0 standard: binding to label and antigen, no steroid present.

13% is calculated as following:

$$\left( \frac{((sample(cpm) - NSB(cpm)) / Total(cpm) - NSB(cpm)))}{((0s\ tan\ dard(cpm) - NSB(cpm)) / (Total(cpm) - NSB(cpm)))} \right)$$

Plasma 17-β estradiol concentrations (pg/ml plasma) are generated by automatic interpolation from the standard curve fit of the mean B% of protein standards (logistic regression).

RSD (relative standard deviation), unless otherwise noted, is standard deviation (SD) of each triplicate analyses divided by mean of each triplicate multiplied by 100.

Intra assay RSD is RSD within a run.

Inter assay RSD is RSD between runs.

**Table 1. Standard Curve Calibration for 17- $\beta$  Estradiol RIA.  
Hylebos Waterway Reproductive Toxicology in Flatfish**

| test date | tested by | Standard pg/ml | CPM   | B%    | logit B% | Log Std | RSD  |        |      |      |                  |           |          |       |
|-----------|-----------|----------------|-------|-------|----------|---------|------|--------|------|------|------------------|-----------|----------|-------|
| 6/20/95   | SS        | 3.9            | 2404  | 83.91 | 1.65     | 0.59    | 0.83 | Total= | 9455 | 9244 | R <sup>2</sup> = | 0.99      |          |       |
|           |           | 3.9            | 2443  | 85.44 | 1.77     | 0.59    |      |        | 9243 |      |                  |           |          |       |
|           |           | 3.9            | 2415  | 84.36 | 1.68     | 0.59    |      |        | 9035 |      |                  |           | Slope b= | -0.48 |
|           |           | 7.8            | 2341  | 81.49 | 1.48     | 0.89    | 2.75 |        |      |      |                  |           |          |       |
|           |           | 7.8            | 2234  | 77.30 | 1.23     | 0.89    |      | NSB=   | 238  | 243  | Y int=           | 1.52      |          |       |
|           |           | 7.8            | 2345  | 81.65 | 1.49     | 0.89    |      |        | 252  |      |                  |           |          |       |
|           |           | 15.6           | 1906  | 64.57 | 0.60     | 1.19    | 3.11 |        | 239  |      |                  | EC 20=    | 155.23   |       |
|           |           | 15.6           | 2026  | 69.23 | 0.81     | 1.19    |      |        |      |      |                  | EC 50=    | 33.06    |       |
|           |           | 15.6           | 1948  | 66.20 | 0.67     | 1.19    |      | 0 STD= | 2868 | 2818 | EC 80=           | 7.04      |          |       |
|           |           | 31.3           | 1553  | 50.86 | 0.03     | 1.50    | 6.08 |        | 2827 |      |                  |           |          |       |
|           |           | 31.3           | 1722  | 57.43 | 0.30     | 1.50    |      |        | 2759 |      |                  | Bind Eff. |          |       |
|           |           | 31.3           | 1552  | 50.84 | 0.03     | 1.50    |      |        |      |      |                  | Bo=       | 28.61    |       |
|           |           | 62.5           | 1214  | 37.71 | -0.50    | 1.80    | 0.72 |        |      |      |                  |           |          |       |
|           |           | 62.5           | 1218  | 37.87 | -0.50    | 1.80    |      |        |      |      |                  |           |          |       |
|           |           | 62.5           | 1202  | 37.22 | -0.52    | 1.80    |      |        |      |      |                  |           |          |       |
|           |           | 125            | 845   | 23.39 | -1.19    | 2.10    | 3.01 |        |      |      |                  |           |          |       |
|           |           | 125            | 898   | 25.42 | -1.08    | 2.10    |      |        |      |      |                  |           |          |       |
|           |           | 125            | 867   | 24.23 | -1.14    | 2.10    |      |        |      |      |                  |           |          |       |
|           |           | 250            | 585   | 13.29 | -1.88    | 2.40    | 4.08 |        |      |      |                  |           |          |       |
|           |           | 250            | 591   | 13.50 | -1.86    | 2.40    |      |        |      |      |                  |           |          |       |
|           |           | 250            | 630   | 15.04 | -1.73    | 2.40    |      |        |      |      |                  |           |          |       |
|           |           | 500            | 413   | 6.60  | -2.65    | 2.70    | 3.72 |        |      |      |                  |           |          |       |
|           |           | 500            | 440   | 7.66  | -2.49    | 2.70    |      |        |      |      |                  |           |          |       |
| 500       | 442       | 7.71           | -2.48 | 2.70  |          |         |      |        |      |      |                  |           |          |       |
| 6/21/95   |           | 3.9            | 2796  | 89.42 | 2.13     | 0.59    | 0.11 | Total= | 9358 | 9295 | R <sup>2</sup> = | 1.00      |          |       |
|           |           | 3.9            | 2790  | 89.21 | 2.11     | 0.59    |      |        | 9174 |      |                  |           |          |       |
|           |           | 3.9            | 2793  | 89.31 | 2.12     | 0.59    |      |        | 9352 |      |                  |           | Slope b= | -0.47 |
|           |           | 7.8            | 2436  | 76.86 | 1.20     | 0.89    | 3.46 |        |      |      |                  |           |          |       |
|           |           | 7.8            | 2409  | 75.94 | 1.15     | 0.89    |      | NSB=   | 233  | 231  | Y int=           | 1.55      |          |       |
|           |           | 7.8            | 2569  | 81.50 | 1.48     | 0.89    |      |        | 218  |      |                  |           |          |       |
|           |           | 15.6           | 2166  | 67.46 | 0.73     | 1.19    | 1.64 |        | 244  |      |                  | EC 20=    | 160.31   |       |
|           |           | 15.6           | 2132  | 66.28 | 0.68     | 1.19    |      |        |      |      |                  | EC 50=    | 35.22    |       |
|           |           | 15.6           | 2096  | 65.02 | 0.62     | 1.19    |      | 0 STD= | 3109 | 3100 | EC 80=           | 7.74      |          |       |
|           |           | 31.3           | 1745  | 52.79 | 0.11     | 1.50    | 1.32 |        | 3100 |      |                  |           |          |       |
|           |           | 31.3           | 1739  | 52.56 | 0.10     | 1.50    |      |        | 3090 |      |                  | Bind Eff. |          |       |
|           |           | 31.3           | 1782  | 54.05 | 0.16     | 1.50    |      |        |      |      |                  | Bo=       | 31.65    |       |
|           |           | 62.5           | 1310  | 37.59 | -0.51    | 1.80    | 2.07 |        |      |      |                  |           |          |       |
|           |           | 62.5           | 1262  | 35.95 | -0.58    | 1.80    |      |        |      |      |                  |           |          |       |
|           |           | 62.5           | 1308  | 37.54 | -0.51    | 1.80    |      |        |      |      |                  |           |          |       |
|           |           | 125            | 1009  | 27.12 | -0.99    | 2.10    | 5.47 |        |      |      |                  |           |          |       |
|           |           | 125            | 956   | 25.27 | -1.08    | 2.10    |      |        |      |      |                  |           |          |       |
|           |           | 125            | 905   | 23.48 | -1.18    | 2.10    |      |        |      |      |                  |           |          |       |

\* Outlier removed

Hylebos Waterway Reproductive Toxicology in Flatfish - Reproductive Steroid RIAs

**Table 1. Standard Curve Calibration for 17- $\beta$  Estradiol RIA.  
Hylebos Waterway Reproductive Toxicology in Flatfish**

| test date | tested by | Standard pg/ml | CPM   | B %   | logit B % | Log Std | RSD   |        |      |      |                  |        |
|-----------|-----------|----------------|-------|-------|-----------|---------|-------|--------|------|------|------------------|--------|
|           |           | 250            | 608   | 13.12 | -1.89     | 2.40    | 4.29  |        |      |      |                  |        |
|           |           | 250            | 640   | 14.25 | -1.79     | 2.40    |       |        |      |      |                  |        |
|           |           | 250            | 588   | 12.44 | -1.95     | 2.40    |       |        |      |      |                  |        |
|           |           | 500            | 487   | 8.90  | -2.33     | 2.70    | 1.83  |        |      |      |                  |        |
|           |           | 500            | 470   | 8.31  | -2.40     | 2.70    |       |        |      |      |                  |        |
|           |           | 500            | 474   | 8.46  | -2.38     | 2.70    |       |        |      |      |                  |        |
| 6/22/95   | BB        | 3.9            | 2515  | 90.22 | 2.22      | 0.59    | 2.42  | Total= | 9170 | 9051 | R <sup>2</sup> = | 1.00   |
|           |           | 3.9            | 2449  | 87.55 | 1.95      | 0.59    |       |        | 9009 |      |                  |        |
|           |           | 3.9            | 2397  | 85.46 | 1.77      | 0.59    |       |        | 8975 |      | Slope b=         | -0.45  |
|           |           | 7.8            | 2264  | 80.12 | 1.39      | 0.89    | 0.55  |        |      |      |                  |        |
|           |           | 7.8            | 2288  | 81.11 | 1.46      | 0.89    |       | NSB=   | 257  | 269  | Y int=           | 1.51   |
|           |           | 7.8            | 2280  | 80.77 | 1.44      | 0.89    |       |        | 278  |      |                  |        |
|           |           | 15.6           | 1923  | 66.45 | 0.68      | 1.19    | 1.18  |        | 273  |      | EC 20=           | 135.96 |
|           |           | 15.6           | 1879  | 64.66 | 0.60      | 1.19    |       |        |      |      | EC 50=           | 32.28  |
|           |           | 15.6           | 1896  | 65.34 | 0.63      | 1.19    |       | 0 STD= | 2750 | 2759 | EC 80=           | 7.67   |
|           |           | 31.3           | 1463  | 47.94 | -0.08     | 1.50    | 3.41  |        | 2749 |      |                  |        |
|           |           | 31.3           | 1534  | 50.79 | 0.03      | 1.50    |       |        | 2777 |      | Bind Eff.        |        |
|           |           | 31.3           | 1564  | 52.00 | 0.08      | 1.50    |       |        |      |      | Bo=              | 28.35  |
|           |           | 62.5           | 1101  | 33.39 | -0.69     | 1.80    | 4.24  |        |      |      |                  |        |
|           |           | 62.5           | 1157  | 35.65 | -0.59     | 1.80    |       |        |      |      |                  |        |
|           |           | 62.5           | 1198  | 37.30 | -0.52     | 1.80    |       |        |      |      |                  |        |
|           |           | 125            | 870   | 24.13 | -1.15     | 2.10    | 1.24  |        |      |      |                  |        |
|           |           | 125            | 849   | 23.28 | -1.19     | 2.10    |       |        |      |      |                  |        |
|           |           | 125            | 856   | 23.58 | -1.18     | 2.10    |       |        |      |      |                  |        |
|           |           | 250            | 540   | 10.86 | -2.10     | 2.40    | 3.88  |        |      |      |                  |        |
|           |           | 250            | 567   | 11.96 | -2.00     | 2.40    |       |        |      |      |                  |        |
|           |           | 250            | 583   | 12.60 | -1.94     | 2.40    |       |        |      |      |                  |        |
|           |           | 500            | 411   | 5.69  | -2.81     | 2.70    | 3.12  |        |      |      |                  |        |
|           |           | 500            | 435   | 6.67  | -2.64     | 2.70    |       |        |      |      |                  |        |
|           |           | 500            | 432   | 6.55  | -2.66     | 2.70    |       |        |      |      |                  |        |
| 6/22/95   | SS        | 3.9            | 1720  | 90.56 | 2.26      | 0.59    | 3.20  | Total= | 9154 | 9092 | R <sup>2</sup> = | 0.97   |
|           |           | 3.9            | 1708  | 89.85 | 2.18      | 0.59    |       |        | 9062 |      |                  |        |
|           |           | 3.9            | 1621  | 84.65 | 1.71      | 0.59    |       |        | 9062 |      | Slope b=         | -0.42  |
|           |           | 7.8            | 1614  | 84.25 | 1.68      | 0.89    | 9.58  |        |      |      |                  |        |
|           |           | 7.8            | 1347  | 68.21 | 0.76      | 0.89    |       | NSB=   | 209  | 209  | Y int=           | 1.49   |
|           |           | 7.8            | 1577  | 82.00 | 1.52      | 0.89    |       |        | 209  |      |                  |        |
|           |           | 15.6           | 1408  | 71.88 | 0.94      | 1.19    | 4.23  |        | 209  |      | EC 20=           | 118.59 |
|           |           | 15.6           | 1301  | 65.48 | 0.64      | 1.19    |       |        |      |      | EC 50=           | 30.57  |
|           |           | 15.6           | 1321  | 66.68 | 0.69      | 1.19    |       | 0 STD= | 1917 | 1877 | EC 80=           | 7.88   |
|           |           | 31.3           | 1093  | 52.97 | 0.12      | 1.50    | 19.77 |        | 1928 |      |                  |        |
|           |           | 31.3           | 1867* |       |           |         |       |        | 1786 |      | Bind Eff.        |        |
|           |           | 31.3           | 825   | 36.90 | -0.54     | 1.50    |       |        |      |      | Bo=              | 18.78  |

\* Outlier removed

**Hylebos Waterway Reproductive Toxicology in Flatfish - Reproductive Steroid RIAs**



**Table 1. Standard Curve Calibration for 17- $\beta$  Estradiol RIA.  
Hylebos Waterway Reproductive Toxicology in Flatfish**

| test date | tested by | Standard pg/ml | CPM   | B %   | logit B % | Log Std | RSD   |        |       |       |                  |        |
|-----------|-----------|----------------|-------|-------|-----------|---------|-------|--------|-------|-------|------------------|--------|
|           |           | 62.5           | 811   | 36.08 | -0.57     | 1.80    | 1.59  |        |       |       |                  |        |
|           |           | 62.5           | 835   | 37.53 | -0.51     | 1.80    |       |        |       |       |                  |        |
|           |           | 62.5           | 814   | 36.29 | -0.56     | 1.80    |       |        |       |       |                  |        |
|           |           | 125            | 577   | 22.06 | -1.26     | 2.10    | 16.89 |        |       |       |                  |        |
|           |           | 125            | 413   | 12.24 | -1.97     | 2.10    |       |        |       |       |                  |        |
|           |           | 125            | 477   | 16.05 | -1.65     | 2.10    |       |        |       |       |                  |        |
|           |           | 250            | 426   | 13.01 | -1.90     | 2.40    | 20.62 |        |       |       |                  |        |
|           |           | 250            | 318   | 6.51  | -2.66     | 2.40    |       |        |       |       |                  |        |
|           |           | 250            | 1021* |       |           |         |       |        |       |       |                  |        |
|           |           | 500            | 324   | 6.87  | -2.61     | 2.70    | 0.74  |        |       |       |                  |        |
|           |           | 500            | 326   | 7.04  | -2.58     | 2.70    |       |        |       |       |                  |        |
|           |           | 500            | 328   | 7.16  | -2.56     | 2.70    |       |        |       |       |                  |        |
| 6/27/95   | SS        | 3.9            | 2649  | 90.43 | 2.25      | 0.59    | 2.10  | Total= | 8992  | 9179  | R <sup>2</sup> = | 0.99   |
|           |           | 3.9            | 2728  | 93.49 | 2.66      | 0.59    |       |        | 9474  |       |                  |        |
|           |           | 3.9            | 2619  | 89.29 | 2.12      | 0.59    |       |        | 9072  |       | Slope b=         | -0.43  |
|           |           | 7.8            | 2264  | 75.51 | 1.13      | 0.89    | 5.77  |        |       |       |                  |        |
|           |           | 7.8            | 2437  | 82.22 | 1.53      | 0.89    |       | NSB=   | 323   | 318   | Y int=           | 1.57   |
|           |           | 7.8            | 2540  | 86.20 | 1.83      | 0.89    |       |        | 326   |       |                  |        |
|           |           | 15.6           | 2028  | 66.34 | 0.68      | 1.19    | 2.34  |        | 306   |       | EC 20=           | 144.62 |
|           |           | 15.6           | 2104  | 69.30 | 0.81      | 1.19    |       |        |       |       | EC 50=           | 36.76  |
|           |           | 15.6           | 2015  | 65.85 | 0.66      | 1.19    |       | 0 STD= | 2806  | 2895  | EC 80=           | 9.34   |
|           |           | 31.3           | 1626  | 50.73 | 0.03      | 1.50    | 6.19  |        | 2759  |       |                  |        |
|           |           | 31.3           | 1783  | 56.83 | 0.28      | 1.50    |       |        | 3121  |       | Bind Eff.        |        |
|           |           | 31.3           | 1833  | 58.76 | 0.35      | 1.50    |       |        |       |       | Bo=              | 29.08  |
|           |           | 62.5           | 1423  | 42.88 | -0.29     | 1.80    | 10.27 |        |       |       |                  |        |
|           |           | 62.5           | 1225  | 35.16 | -0.61     | 1.80    |       |        |       |       |                  |        |
|           |           | 62.5           | 1176  | 33.29 | -0.69     | 1.80    |       |        |       |       |                  |        |
|           |           | 125            | 902   | 22.66 | -1.23     | 2.10    | 3.55  |        |       |       |                  |        |
|           |           | 125            | 964   | 25.05 | -1.10     | 2.10    |       |        |       |       |                  |        |
|           |           | 125            | 913   | 23.09 | -1.20     | 2.10    |       |        |       |       |                  |        |
|           |           | 250            | 657   | 13.12 | -1.89     | 2.40    | 2.07  |        |       |       |                  |        |
|           |           | 250            | 630   | 12.09 | -1.98     | 2.40    |       |        |       |       |                  |        |
|           |           | 250            | 645   | 12.67 | -1.93     | 2.40    |       |        |       |       |                  |        |
|           |           | 500            | 525   | 8.00  | -2.44     | 2.70    | 5.50  |        |       |       |                  |        |
|           |           | 500            | 476   | 6.11  | -2.73     | 2.70    |       |        |       |       |                  |        |
|           |           | 500            | 480   | 6.26  | -2.71     | 2.70    |       |        |       |       |                  |        |
| 6/27/95   | SS ADD    | 3.9            | 3372  | 92.70 | 2.54      | 0.59    | 1.32  | Total= | 11107 | 11168 | R <sup>2</sup> = | 0.99   |
|           |           | 3.9            | 3389  | 93.22 | 2.62      | 0.59    |       |        | 11179 |       |                  |        |
|           |           | 3.9            | 3305  | 90.64 | 2.27      | 0.59    |       |        | 11219 |       | Slope b=         | -0.42  |
|           |           | 7.8            | 2874  | 77.40 | 1.23      | 0.89    | 3.79  |        |       |       |                  |        |
|           |           | 7.8            | 2990  | 80.97 | 1.45      | 0.89    |       | NSB=   | 369   | 358   | Y int=           | 1.56   |
|           |           | 7.8            | 3101  | 84.37 | 1.69      | 0.89    |       |        | 336   |       |                  |        |

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Hylebos Waterway Reproductive Toxicology in Flatfish - Reproductive Steroid RIAs

**Table 1. Standard Curve Calibration for 17- $\beta$  Estradiol RIA.  
Hylebos Waterway Reproductive Toxicology in Flatfish**

| test date | tested by | Standard pg/ml | CPM  | B %   | logit B % | Log Std | RSD  |        |       |       |                       |
|-----------|-----------|----------------|------|-------|-----------|---------|------|--------|-------|-------|-----------------------|
|           |           | 15.6           | 2616 | 69.44 | 0.82      | 1.19    | 2.27 |        | 369   |       | EC 20= 140.79         |
|           |           | 15.6           | 2575 | 68.18 | 0.76      | 1.19    |      |        |       |       | EC 50= 36.70          |
|           |           | 15.6           | 2501 | 65.91 | 0.66      | 1.19    |      | 0 STD= | 3460  | 3609  | EC 80= 9.57           |
|           |           | 31.3           | 2023 | 51.20 | 0.05      | 1.50    | 4.06 |        | 3514  |       |                       |
|           |           | 31.3           | 2165 | 55.58 | 0.22      | 1.50    |      |        | 3854  |       | Bind Eff.             |
|           |           | 31.3           | 2178 | 55.98 | 0.24      | 1.50    |      |        |       |       | Bo= 30.07             |
|           |           | 62.5           | 1643 | 39.53 | -0.43     | 1.80    | 6.67 |        |       |       |                       |
|           |           | 62.5           | 1543 | 36.45 | -0.56     | 1.80    |      |        |       |       |                       |
|           |           | 62.5           | 1438 | 33.20 | -0.70     | 1.80    |      |        |       |       |                       |
|           |           | 125            | 1124 | 23.56 | -1.18     | 2.10    | 0.80 |        |       |       |                       |
|           |           | 125            | 1124 | 23.57 | -1.18     | 2.10    |      |        |       |       |                       |
|           |           | 125            | 1140 | 24.04 | -1.15     | 2.10    |      |        |       |       |                       |
|           |           | 250            | 0*   |       |           |         | 2.69 |        |       |       |                       |
|           |           | 250            | 752  | 12.11 | -1.98     | 2.40    |      |        |       |       |                       |
|           |           | 250            | 781  | 13.00 | -1.90     | 2.40    |      |        |       |       |                       |
| 6/28/95   | SS        | 3.9            | 3222 | 91.39 | 2.36      | 0.59    | 1.14 | Total= | 10985 | 10930 | R <sup>2</sup> = 0.99 |
|           |           | 3.9            | 3151 | 89.10 | 2.10      | 0.59    |      |        | 10841 |       |                       |
|           |           | 3.9            | 3198 | 90.62 | 2.27      | 0.59    |      |        | 10965 |       | Slope b= -0.45        |
|           |           | 7.8            | 2770 | 76.91 | 1.20      | 0.89    | 3.97 |        |       |       |                       |
|           |           | 7.8            | 2996 | 84.13 | 1.67      | 0.89    |      | NSB=   | 377   | 368   | Y int= 1.57           |
|           |           | 7.8            | 2921 | 81.74 | 1.50      | 0.89    |      |        | 375   |       |                       |
|           |           | 15.6           | 2497 | 68.16 | 0.76      | 1.19    | 1.06 |        | 353   |       | EC 20= 157.40         |
|           |           | 15.6           | 2513 | 68.67 | 0.78      | 1.19    |      |        |       |       | EC 50= 37.16          |
|           |           | 15.6           | 2462 | 67.03 | 0.71      | 1.19    |      | 0 STD= | 3520  | 3491  | EC 80= 8.77           |
|           |           | 31.3           | 1928 | 49.95 | 0.00      | 1.50    | 4.40 |        | 3516  |       |                       |
|           |           | 31.3           | 1952 | 50.71 | 0.03      | 1.50    |      |        | 3438  |       | Bind Eff.             |
|           |           | 31.3           | 2091 | 55.15 | 0.21      | 1.50    |      |        |       |       | Bo= 29.57             |
|           |           | 62.5           | 1556 | 38.02 | -0.49     | 1.80    | 2.51 |        |       |       |                       |
|           |           | 62.5           | 1615 | 39.91 | -0.41     | 1.80    |      |        |       |       |                       |
|           |           | 62.5           | 1540 | 37.50 | -0.51     | 1.80    |      |        |       |       |                       |
|           |           | 125            | 1145 | 24.88 | -1.10     | 2.10    | 2.42 |        |       |       |                       |
|           |           | 125            | 1191 | 26.34 | -1.03     | 2.10    |      |        |       |       |                       |
|           |           | 125            | 1198 | 26.57 | -1.02     | 2.10    |      |        |       |       |                       |
|           |           | 250            | 867  | 15.97 | -1.66     | 2.40    | 6.99 |        |       |       |                       |
|           |           | 250            | 767  | 12.75 | -1.92     | 2.40    |      |        |       |       |                       |
|           |           | 250            | 774  | 12.99 | -1.90     | 2.40    |      |        |       |       |                       |
|           |           | 500            | 629  | 8.34  | -2.40     | 2.70    | 6.40 |        |       |       |                       |
|           |           | 500            | 625  | 8.20  | -2.41     | 2.70    |      |        |       |       |                       |
|           |           | 500            | 560  | 5.46  | -2.85     | 2.70    |      |        |       |       |                       |
| 7/6/95    | LC        | 3.9            | 3032 | 90.58 | 2.26      | 0.59    | 2.16 | Total= | 11401 | 11210 | R <sup>2</sup> = 0.97 |
|           |           | 3.9            | 2905 | 86.29 | 1.84      | 0.59    |      |        | 11271 |       |                       |
|           |           | 3.9            | 2988 | 89.07 | 2.10      | 0.59    |      |        | 10958 |       | Slope b= -0.41        |

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Hylebos Waterway Reproductive Toxicology in Flatfish - Reproductive Steroid RIAs

**Table 1. Standard Curve Calibration for 17-β Estradiol RIA.  
Hylebos Waterway Reproductive Toxicology in Flatfish**

| test date | tested by | Standard pg/ml | CPM    | B %   | logit B % | Log Std | RSD   |        |       |       |           |        |
|-----------|-----------|----------------|--------|-------|-----------|---------|-------|--------|-------|-------|-----------|--------|
|           |           | 7.8            | 2694   | 79.14 | 1.33      | 0.89    | 1.06  |        |       |       |           |        |
|           |           | 7.8            | 2696   | 79.21 | 1.34      | 0.89    |       | NSB=   | 356   | 357   | Y int=    | 1.50   |
|           |           | 7.8            | 2745   | 80.86 | 1.44      | 0.89    |       |        | 351   |       |           |        |
|           |           | 15.6           | 2336   | 66.99 | 0.71      | 1.19    | 2.09  |        | 366   |       | EC 20=    | 119.41 |
|           |           | 15.6           | 2338   | 67.07 | 0.71      | 1.19    |       |        |       |       | EC 50=    | 31.76  |
|           |           | 15.6           | 2253   | 64.21 | 0.58      | 1.19    |       | 0 STD= | 3191  | 3310  | EC 80=    | 8.45   |
|           |           | 31.3           | 1812   | 49.27 | -0.03     | 1.50    | 3.49  |        | 3369  |       |           |        |
|           |           | 31.3           | 1923   | 53.02 | 0.12      | 1.50    |       |        | 3371  |       | Bind Eff. |        |
|           |           | 31.3           | 1930   | 53.26 | 0.13      | 1.50    |       |        |       |       | Bo=       | 27.21  |
|           |           | 62.5           | 1511   | 39.05 | -0.45     | 1.80    | 3.01  |        |       |       |           |        |
|           |           | 62.5           | 1488   | 38.29 | -0.48     | 1.80    |       |        |       |       |           |        |
|           |           | 62.5           | 1425   | 36.15 | -0.57     | 1.80    |       |        |       |       |           |        |
|           |           | 125            | 1021   | 22.49 | -1.24     | 2.10    | 2.51  |        |       |       |           |        |
|           |           | 125            | 1073   | 24.23 | -1.14     | 2.10    |       |        |       |       |           |        |
|           |           | 125            | 1058   | 23.71 | -1.17     | 2.10    |       |        |       |       |           |        |
|           |           | 250            | 501    | 4.85  | -2.98     | 2.40    | 22.95 |        |       |       |           |        |
|           |           | 250            | 496    | 4.70  | -3.01     | 2.40    |       |        |       |       |           |        |
|           |           | 250            | 727    | 12.51 | -1.95     | 2.40    |       |        |       |       |           |        |
|           |           | 500            | 550    | 6.52  | -2.66     | 2.70    | 3.50  |        |       |       |           |        |
|           |           | 500            | 560    | 6.13  | -2.73     | 2.70    |       |        |       |       |           |        |
|           |           | 500            | 588.10 | 7.81  | -2.47     | 2.70    |       |        |       |       |           |        |
| 7/10/95   | LC        | 3.9            | 3223   | 90.12 | 2.21      | 0.59    | 9.30  | Total= | 15490 | 14705 | R^2=      | 0.95   |
|           |           | 3.9            | 3153   | 87.91 | 1.98      | 0.59    |       |        | 13682 |       |           |        |
|           |           | 3.9            | 2704   | 73.76 | 1.03      | 0.59    |       |        | 14943 |       | Slope b=  | -0.49  |
|           |           | 7.8            | 2356   | 62.78 | 0.52      | 0.89    | 11.08 |        |       |       |           |        |
|           |           | 7.8            | 2940   | 81.21 | 1.46      | 0.89    |       | NSB=   | 332   | 366   | Y int=    | 1.52   |
|           |           | 7.8            | 2621   | 71.15 | 0.90      | 0.89    |       |        | 399   |       |           |        |
|           |           | 15.6           | 2590   | 70.15 | 0.85      | 1.19    | 2.89  |        |       |       | EC 20=    | 158.05 |
|           |           | 15.6           | 2565   | 69.37 | 0.82      | 1.19    |       |        |       |       | EC 50=    | 33.34  |
|           |           | 15.6           | 2452   | 65.81 | 0.65      | 1.19    |       | 0 STD= | 3540  | 3536  | EC 80=    | 7.03   |
|           |           | 31.3           | 1957   | 50.20 | 0.01      | 1.50    | 5.50  |        | 3478  |       |           |        |
|           |           | 31.3           | 2144   | 56.10 | 0.25      | 1.50    |       |        | 3590  |       | Bind Eff. |        |
|           |           | 31.3           | 2166   | 56.79 | 0.27      | 1.50    |       |        |       |       | Bo=       | 22.11  |
|           |           | 62.5           | 1681   | 41.48 | -0.34     | 1.80    | 1.13  |        |       |       |           |        |
|           |           | 62.5           | 1672   | 41.22 | -0.35     | 1.80    |       |        |       |       |           |        |
|           |           | 62.5           | 1645   | 40.35 | -0.39     | 1.80    |       |        |       |       |           |        |
|           |           | 125            | 1221   | 26.99 | -1.00     | 2.10    | 1.92  |        |       |       |           |        |
|           |           | 125            | 1246   | 27.78 | -0.96     | 2.10    |       |        |       |       |           |        |
|           |           | 125            | 1269   | 28.50 | -0.92     | 2.10    |       |        |       |       |           |        |
|           |           | 250            | 893    | 16.62 | -1.61     | 2.40    | 6.84  |        |       |       |           |        |
|           |           | 250            | 797    | 13.61 | -1.85     | 2.40    |       |        |       |       |           |        |
|           |           | 250            | 792    | 13.46 | -1.86     | 2.40    |       |        |       |       |           |        |
|           |           | 500            | 556    | 6.01  | -2.75     | 2.70    | 5.26  |        |       |       |           |        |

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Hylebos Waterway Reproductive Toxicology in Flatfish - Reproductive Steroid RIAs

**Table 1. Standard Curve Calibration for 17- $\beta$  Estradiol RIA.  
Hylebos Waterway Reproductive Toxicology in Flatfish**

| test date | tested by | Standard<br>pg/ml | CPM  | B %   | logit<br>B % | Log<br>Std | RSD  |        |       |       |                       |
|-----------|-----------|-------------------|------|-------|--------------|------------|------|--------|-------|-------|-----------------------|
|           |           | 500               | 605  | 7.55  | -2.51        | 2.70       |      |        |       |       |                       |
|           |           | 500               | 614  | 7.83  | -2.47        | 2.70       |      |        |       |       |                       |
| 7/10/95   | SS        | 3.9               | 2973 | 94.45 | 2.83         | 0.59       | 5.03 | Total= | 14706 | 14631 | R <sup>2</sup> = 0.97 |
|           |           | 3.9               | 2747 | 86.56 | 1.86         | 0.59       |      |        | 14390 |       |                       |
|           |           | 3.9               | 3020 | 96.08 | 3.20         | 0.59       |      |        | 14797 |       | Slope b= -0.44        |
|           |           | 7.8               | 2774 | 87.53 | 1.95         | 0.89       | 3.28 |        |       |       |                       |
|           |           | 7.8               | 2740 | 86.34 | 1.84         | 0.89       |      | NSB=   | 252   | 257   | Y int= 1.71           |
|           |           | 7.8               | 2607 | 81.69 | 1.50         | 0.89       |      |        | 245   |       |                       |
|           |           | 15.6              | 2451 | 76.27 | 1.17         | 1.19       | 4.16 |        | 275   |       | EC 20= 210.23         |
|           |           | 15.6              | 2366 | 73.33 | 1.01         | 1.19       |      |        |       |       | EC 50= 50.86          |
|           |           | 15.6              | 2255 | 69.47 | 0.82         | 1.19       |      | 0 STD= | 3375  | 3133  | EC 80= 12.30          |
|           |           | 31.3              | 1836 | 54.91 | 0.20         | 1.50       | 3.67 |        | 2973  |       |                       |
|           |           | 31.3              | 1971 | 59.59 | -0.39        | 1.50       |      |        | 3051  |       | Bind Eff.             |
|           |           | 31.3              | 1939 | 58.49 | 0.34         | 1.50       |      |        |       |       | Bo= 20.01             |
|           |           | 62.5              | 1633 | 47.85 | -0.09        | 1.80       | 4.32 |        |       |       |                       |
|           |           | 62.5              | 1600 | 46.68 | -0.13        | 1.80       |      |        |       |       |                       |
|           |           | 62.5              | 1502 | 43.28 | -0.27        | 1.80       |      |        |       |       |                       |
|           |           | 125               | 1203 | 32.89 | -0.71        | 2.10       | 3.34 |        |       |       |                       |
|           |           | 125               | 1155 | 31.23 | -0.79        | 2.10       |      |        |       |       |                       |
|           |           | 125               | 1126 | 30.22 | -0.84        | 2.10       |      |        |       |       |                       |
|           |           | 250               | 808  | 19.15 | -1.44        | 2.40       | 4.44 |        |       |       |                       |
|           |           | 250               | 747  | 17.02 | -1.58        | 2.40       |      |        |       |       |                       |
|           |           | 250               | 751  | 17.18 | -1.57        | 2.40       |      |        |       |       |                       |
|           |           | 500               | 544  | 9.96  | -2.20        | 2.70       | 3.49 |        |       |       |                       |
|           |           | 500               | 583  | 11.32 | -2.06        | 2.70       |      |        |       |       |                       |
|           |           | 500               | 566  | 10.75 | -2.12        | 2.70       |      |        |       |       |                       |
| 7/14/95   | LC        | 3.9               | 2887 | 88.84 | 2.07         | 0.59       | 2.15 | Total= | 10734 | 10597 | R <sup>2</sup> = 0.99 |
|           |           | 3.9               | 2798 | 85.82 | 1.80         | 0.59       |      |        | 10530 |       |                       |
|           |           | 3.9               | 2771 | 84.91 | 1.73         | 0.59       |      |        | 10527 |       | Slope b= -0.45        |
|           |           | 7.8               | 2498 | 75.65 | 1.13         | 0.89       | 1.23 |        |       |       |                       |
|           |           | 7.8               | 2557 | 77.66 | 1.25         | 0.89       |      | NSB=   | 170   | 263   | Y int= 1.48           |
|           |           | 7.8               | 2511 | 76.11 | 1.16         | 0.89       |      |        | 171   |       |                       |
|           |           | 15.6              | 2090 | 61.84 | 0.48         | 1.19       | 3.29 |        | 186   |       | EC 20= 125.89         |
|           |           | 15.6              | 2139 | 63.51 | 0.55         | 1.19       |      |        |       |       | EC 50= 29.98          |
|           |           | 15.6              | 2230 | 66.57 | 0.69         | 1.19       |      | 0 STD= | 2959  | 3217  | EC 80= 7.14           |
|           |           | 31.3              | 1975 | 57.96 | 0.32         | 1.50       | 8.64 |        | 3309  |       |                       |
|           |           | 31.3              | 1661 | 47.32 | -0.11        | 1.50       |      |        | 3382  |       | Bind Eff.             |
|           |           | 31.3              | 1824 | 52.85 | 0.11         | 1.50       |      |        |       |       | Bo= 28.58             |
|           |           | 62.5              | 1293 | 34.85 | -0.63        | 1.80       | 6.45 |        |       |       |                       |
|           |           | 62.5              | 1410 | 38.81 | -0.46        | 1.80       |      |        |       |       |                       |
|           |           | 62.5              | 1245 | 33.23 | -0.70        | 1.80       |      |        |       |       |                       |
|           |           | 125               | 916  | 22.11 | -1.26        | 2.10       | 5.43 |        |       |       |                       |

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Hylebos Waterway Reproductive Toxicology in Flatfish - Reproductive Steroid RIAs

**Table 1. Standard Curve Calibration for 17- $\beta$  Estradiol RIA.  
Hylebos Waterway Reproductive Toxicology in Flatfish**

| test date | tested by | Standard pg/ml | CPM  | B%    | logit B% | Log Std | RSD  |        |       |       |                  |        |
|-----------|-----------|----------------|------|-------|----------|---------|------|--------|-------|-------|------------------|--------|
|           |           | 125            | 940  | 22.91 | -1.21    | 2.10    |      |        |       |       |                  |        |
|           |           | 125            | 1016 | 25.48 | -1.07    | 2.10    |      |        |       |       |                  |        |
|           |           | 250            | 657  | 13.32 | -1.87    | 2.40    | 5.87 |        |       |       |                  |        |
|           |           | 250            | 600  | 11.40 | -2.05    | 2.40    |      |        |       |       |                  |        |
|           |           | 250            | 590  | 11.04 | -2.09    | 2.40    |      |        |       |       |                  |        |
|           |           | 500            | 399  | 4.58  | -3.04    | 2.70    | 2.45 |        |       |       |                  |        |
|           |           | 500            | 418  | 5.25  | -2.89    | 2.70    |      |        |       |       |                  |        |
|           |           | 500            | 411  | 5.01  | -2.94    | 2.70    |      |        |       |       |                  |        |
| 7/14/95   | SS        | 3.9            | 3071 | 91.65 | 2.40     | 0.59    | 3.67 | Total= | 11803 | 11640 | R <sup>2</sup> = | 0.95   |
|           |           | 3.9            | 3146 | 94.10 | 2.77     | 0.59    |      |        | 11651 |       |                  |        |
|           |           | 3.9            | 3300 | 99.12 | 4.73     | 0.59    |      |        | 11466 |       | Slope b=         | -0.36  |
|           |           | 7.8            | 2724 | 80.30 | 1.41     | 0.89    | 3.45 |        |       |       |                  |        |
|           |           | 7.8            | 2918 | 86.65 | 1.87     | 0.89    |      | NSB=   | 265   | 265   | Y int=           | 1.64   |
|           |           | 7.8            | 2825 | 83.60 | 1.63     | 0.89    |      |        | 271   |       |                  |        |
|           |           | 15.6           | 2465 | 71.84 | 0.94     | 1.19    | 0.95 |        | 261   |       | EC 20=           | 136.67 |
|           |           | 15.6           | 2482 | 72.40 | 0.96     | 1.19    |      |        |       |       | EC 50=           | 43.72  |
|           |           | 15.6           | 2511 | 73.37 | 1.01     | 1.19    |      | 0 STD= | 3158  | 3327  | EC 80=           | 13.99  |
|           |           | 31.3           | 1882 | 52.79 | 0.11     | 1.50    | 3.85 |        | 3451  |       |                  |        |
|           |           | 31.3           | 2000 | 56.67 | 0.27     | 1.50    |      |        | 3371  |       | Bind Eff.        |        |
|           |           | 31.3           | 2023 | 57.40 | 0.30     | 1.50    |      |        |       |       | Bo=              | 26.91  |
|           |           | 62.5           | 1531 | 41.34 | -0.35    | 1.80    | 3.30 |        |       |       |                  |        |
|           |           | 62.5           | 1436 | 38.23 | -0.48    | 1.80    |      |        |       |       |                  |        |
|           |           | 62.5           | 1465 | 39.20 | -0.44    | 1.80    |      |        |       |       |                  |        |
|           |           | 125            | 929  | 21.67 | -1.28    | 2.10    | 7.27 |        |       |       |                  |        |
|           |           | 125            | 1075 | 26.44 | -1.02    | 2.10    |      |        |       |       |                  |        |
|           |           | 125            | 1007 | 24.21 | -1.14    | 2.10    |      |        |       |       |                  |        |
|           |           | 250            | 654  | 12.70 | -1.93    | 2.40    | 1.35 |        |       |       |                  |        |
|           |           | 250            | 672  | 13.28 | -1.88    | 2.40    |      |        |       |       |                  |        |
|           |           | 250            | 662  | 12.95 | -1.91    | 2.40    |      |        |       |       |                  |        |
|           |           | 500            | 432  | 5.44  | -2.85    | 2.70    | 3.35 |        |       |       |                  |        |
|           |           | 500            | 461  | 6.39  | -2.69    | 2.70    |      |        |       |       |                  |        |
|           |           | 500            | 454  | 6.16  | -2.72    | 2.70    |      |        |       |       |                  |        |
| 7/18/95   | LC        | 3.9            | 2996 | 81.15 | 1.46     | 0.59    | 0.93 | Total= | 11210 | 11168 | R <sup>2</sup> = | 0.98   |
|           |           | 3.9            | 2980 | 80.70 | 1.43     | 0.59    |      |        | 11142 |       |                  |        |
|           |           | 3.9            | 2942 | 79.59 | 1.36     | 0.59    |      |        | 11151 |       | Slope b=         | -0.57  |
|           |           | 7.8            | 2518 | 67.30 | 0.72     | 0.89    | 1.87 |        |       |       |                  |        |
|           |           | 7.8            | 2452 | 65.39 | 0.64     | 0.89    |      | NSB=   | 201   | 195   | Y int=           | 1.45   |
|           |           | 7.8            | 2543 | 68.03 | 0.76     | 0.89    |      |        | 190   |       |                  |        |
|           |           | 15.6           | 2581 | 69.12 | 0.81     | 1.19    | 4.54 |        | 196   |       | EC 20=           | 173.85 |
|           |           | 15.6           | 2540 | 67.94 | 0.75     | 1.19    |      |        |       |       | EC 50=           | 28.15  |
|           |           | 15.6           | 2367 | 62.94 | 0.53     | 1.19    |      | 0 STD= | 3699  | 3646  | EC 80=           | 4.56   |
|           |           | 31.3           | 1948 | 50.80 | 0.03     | 1.50    | 3.12 |        | 3714  |       |                  |        |

\* Outlier removed

Hylebos Waterway Reproductive Toxicology in Flatfish - Reproductive Steroid RIAs

**Table 1. Standard Curve Calibration for 17- $\beta$  Estradiol RIA.  
Hylebos Waterway Reproductive Toxicology in Flatfish**

| test date | tested by | Standard pg/ml | CPM   | B %   | logit B % | Log Std | RSD   |        |       |           |                       |
|-----------|-----------|----------------|-------|-------|-----------|---------|-------|--------|-------|-----------|-----------------------|
|           |           | 31.3           | 2074  | 54.43 | 0.18      | 1.50    |       |        | 3527  | Bind Eff. |                       |
|           |           | 31.3           | 2015  | 52.74 | 0.11      | 1.50    |       |        |       | Bo= 31.45 |                       |
|           |           | 62.5           | 1249  | 30.52 | -0.82     | 1.80    | 8.04  |        |       |           |                       |
|           |           | 62.5           | 1446  | 36.23 | -0.57     | 1.80    |       |        |       |           |                       |
|           |           | 62.5           | 1435  | 35.91 | -0.58     | 1.80    |       |        |       |           |                       |
|           |           | 125            | 1025  | 24.04 | -1.15     | 2.10    | 2.41  |        |       |           |                       |
|           |           | 125            | 1069  | 25.32 | -1.08     | 2.10    |       |        |       |           |                       |
|           |           | 125            | 1026  | 24.07 | -1.15     | 2.10    |       |        |       |           |                       |
|           |           | 250            | 732   | 15.54 | -1.69     | 2.40    | 5.58  |        |       |           |                       |
|           |           | 250            | 764   | 16.48 | -1.62     | 2.40    |       |        |       |           |                       |
|           |           | 250            | 683   | 14.14 | -1.80     | 2.40    |       |        |       |           |                       |
|           |           | 500            | 489   | 8.51  | -2.38     | 2.70    | 12.44 |        |       |           |                       |
|           |           | 500            | 532   | 9.75  | -2.23     | 2.70    |       |        |       |           |                       |
|           |           | 500            | 622   | 12.37 | -1.96     | 2.70    |       |        |       |           |                       |
| 7/18/95   | SS        | 3.9            | 607*  |       |           |         |       | Total= | 11671 | 11382     | R <sup>2</sup> = 0.96 |
|           |           | 3.9            | 613*  |       |           |         |       |        | 11439 |           |                       |
|           |           | 3.9            | 954*  |       |           |         |       |        | 11037 |           | Slope b= -0.49        |
|           |           | 7.8            | 2434  | 72.50 | 0.97      | 0.89    | 2.20  |        |       |           |                       |
|           |           | 7.8            | 1611* |       |           |         |       | NSB=   | 207   | 212       | Y int= 1.52           |
|           |           | 7.8            | 2366  | 70.28 | 0.86      | 0.89    |       |        | 225   |           |                       |
|           |           | 15.6           | 1953  | 56.79 | 0.27      | 1.19    | 12.15 |        | 205   |           | EC 20= 157.82         |
|           |           | 15.6           | 2413  | 71.78 | 0.93      | 1.19    |       |        |       |           | EC 50= 32.71          |
|           |           | 15.6           | 2092  | 61.33 | 0.46      | 1.19    |       | 0 STD= | 3133  | 3277      | EC 80= 6.78           |
|           |           | 31.3           | 1799  | 51.78 | 0.07      | 1.50    | 4.32  |        | 3430  |           |                       |
|           |           | 31.3           | 1779  | 51.11 | 0.04      | 1.50    |       |        | 3270  |           | Bind Eff.             |
|           |           | 31.3           | 1909  | 55.35 | 0.21      | 1.50    |       |        |       |           | Bo= 27.44             |
|           |           | 62.5           | 1639  | 46.53 | -0.14     | 1.80    | 9.79  |        |       |           |                       |
|           |           | 62.5           | 1426  | 39.61 | -0.42     | 1.80    |       |        |       |           |                       |
|           |           | 62.5           | 1417  | 39.29 | -0.44     | 1.80    |       |        |       |           |                       |
|           |           | 125            | 1068  | 27.92 | -0.95     | 2.10    | 4.39  |        |       |           |                       |
|           |           | 125            | 1010  | 26.03 | -1.04     | 2.10    |       |        |       |           |                       |
|           |           | 125            | 1079  | 28.27 | -0.93     | 2.10    |       |        |       |           |                       |
|           |           | 250            | 623   | 13.39 | -1.87     | 2.40    | 3.23  |        |       |           |                       |
|           |           | 250            | 630   | 13.62 | -1.85     | 2.40    |       |        |       |           |                       |
|           |           | 250            | 604   | 12.79 | -1.92     | 2.40    |       |        |       |           |                       |
|           |           | 500            | 445   | 7.58  | -2.50     | 2.70    | 13.84 |        |       |           |                       |
|           |           | 500            | 474   | 8.54  | -2.37     | 2.70    |       |        |       |           |                       |
|           |           | 500            | 410   | 6.46  | -2.67     | 2.70    |       |        |       |           |                       |
| 3/13/96   | BB        | 3.9            | 1964  | 67.23 | 0.72      | 0.59    | 7.12  | Total= | 8464  | 8206      | R <sup>2</sup> = 0.94 |
|           |           | 3.9            | 1985  | 68.08 | 0.76      | 0.59    |       |        | 8049  |           |                       |
|           |           | 3.9            | 1777  | 59.68 | 0.39      | 0.59    |       |        | 8107  |           | Slope b= -0.52        |
|           |           | 7.8            | 1747  | 58.47 | 0.34      | 0.89    | 3.06  |        |       |           |                       |

\* Outlier removed

Hylebos Waterway Reproductive Toxicology in Flatfish - Reproductive Steroid RIAs

**Table 1. Standard Curve Calibration for 17- $\beta$  Estradiol RIA.  
Hylebos Waterway Reproductive Toxicology in Flatfish**

| test date | tested by | Standard<br>pg/ml | CPM  | B %   | logit<br>B % | Log<br>Std | RSD   |        |      |      |                  |       |
|-----------|-----------|-------------------|------|-------|--------------|------------|-------|--------|------|------|------------------|-------|
|           |           | 7.8               | 1737 | 58.08 | 0.33         | 0.89       |       | NSB=   | 286  | 300  | Y int=           | 1.20  |
|           |           | 7.8               | 1820 | 61.40 | 0.46         | 0.89       |       |        | 298  |      |                  |       |
|           |           | 15.6              | 1906 | 64.89 | 0.61         | 1.19       | 6.24  |        | 316  |      | EC 20=           | 83.65 |
|           |           | 15.6              | 1738 | 58.10 | 0.33         | 1.19       |       |        |      |      | EC 50=           | 15.86 |
|           |           | 15.6              | 1751 | 58.62 | 0.35         | 1.19       |       | 0 STD= | 2738 | 2775 | EC 80=           | 3.01  |
|           |           | 31.3              | 1439 | 46.03 | -0.16        | 1.50       | 6.47  |        | 2717 |      |                  |       |
|           |           | 31.3              | 1437 | 45.93 | -0.16        | 1.50       |       |        | 2871 |      | Bind Eff.        |       |
|           |           | 31.3              | 1315 | 41.01 | -0.36        | 1.50       |       |        |      |      | Bo=              | 31.31 |
|           |           | 62.5              | 941  | 25.91 | -1.05        | 1.80       | 8.25  |        |      |      |                  |       |
|           |           | 62.5              | 1000 | 28.27 | -0.93        | 1.80       |       |        |      |      |                  |       |
|           |           | 62.5              | 1057 | 30.57 | -0.82        | 1.80       |       |        |      |      |                  |       |
|           |           | 125               | 652  | 14.22 | -1.80        | 2.10       | 10.22 |        |      |      |                  |       |
|           |           | 125               | 592  | 11.79 | -2.01        | 2.10       |       |        |      |      |                  |       |
|           |           | 125               | 648  | 14.08 | -1.81        | 2.10       |       |        |      |      |                  |       |
|           |           | 250               | 533  | 9.44  | -2.26        | 2.40       | 5.95  |        |      |      |                  |       |
|           |           | 250               | 508  | 8.41  | -2.39        | 2.40       |       |        |      |      |                  |       |
|           |           | 250               | 516  | 8.74  | -2.35        | 2.40       |       |        |      |      |                  |       |
|           |           | 500               | 425  | 5.04  | -2.94        | 2.70       | 16.75 |        |      |      |                  |       |
|           |           | 500               | 451  | 6.13  | -2.73        | 2.70       |       |        |      |      |                  |       |
|           |           | 500               | 409  | 4.41  | -3.08        | 2.70       |       |        |      |      |                  |       |
| 3/13/96   | SS        | 3.9               | 2192 | 64.90 | 0.61         | 0.59       | 2.02  | Total= | 8320 | 8075 | R <sup>2</sup> = | 0.95  |
|           |           | 3.9               | 2116 | 62.33 | 0.50         | 0.59       |       |        | 7966 |      |                  |       |
|           |           | 3.9               | 2118 | 62.42 | 0.51         | 0.59       |       |        | 7939 |      | Slope b=         | -0.57 |
|           |           | 7.8               | 1725 | 49.11 | -0.04        | 0.89       | 0.39  |        |      |      |                  |       |
|           |           | 7.8               | 1716 | 48.81 | -0.05        | 0.89       |       | NSB=   | 256  | 271  | Y int=           | 1.05  |
|           |           | 7.8               | 1712 | 48.67 | -0.05        | 0.89       |       |        | 276  |      |                  |       |
|           |           | 15.6              | 1906 | 55.24 | 0.21         | 1.19       | 8.24  |        | 283  |      | EC 20=           | 69.06 |
|           |           | 15.6              | 1624 | 45.70 | -0.17        | 1.19       |       |        |      |      | EC 50=           | 11.30 |
|           |           | 15.6              | 1839 | 52.99 | 0.12         | 1.19       |       | 0 STD= | 3399 | 3230 | EC 80=           | 1.85  |
|           |           | 31.3              | 1443 | 39.60 | -0.42        | 1.50       | 3.84  |        | 3180 |      |                  |       |
|           |           | 31.3              | 1531 | 42.57 | -0.30        | 1.50       |       |        | 3112 |      | Bind Eff.        |       |
|           |           | 31.3              | 1426 | 39.01 | -0.45        | 1.50       |       |        |      |      | Bo=              | 37.92 |
|           |           | 62.5              | 960  | 23.26 | -1.19        | 1.80       | 12.05 |        |      |      |                  |       |
|           |           | 62.5              | 1032 | 25.72 | -1.06        | 1.80       |       |        |      |      |                  |       |
|           |           | 62.5              | 811  | 18.25 | -1.50        | 1.80       |       |        |      |      |                  |       |
|           |           | 125               | 669  | 13.43 | -1.86        | 2.10       | 6.65  |        |      |      |                  |       |
|           |           | 125               | 585  | 10.61 | -2.13        | 2.10       |       |        |      |      |                  |       |
|           |           | 125               | 633  | 12.23 | -1.97        | 2.10       |       |        |      |      |                  |       |
|           |           | 250               | 509  | 8.02  | -2.44        | 2.40       | 2.75  |        |      |      |                  |       |
|           |           | 250               | 489  | 7.36  | -2.53        | 2.40       |       |        |      |      |                  |       |
|           |           | 250               | 483  | 7.13  | -2.57        | 2.40       |       |        |      |      |                  |       |
|           |           | 500               | 455  | 6.21  | -2.71        | 2.70       | 2.52  |        |      |      |                  |       |
|           |           | 500               | 445  | 5.85  | -2.78        | 2.70       |       |        |      |      |                  |       |

\* Outlier removed

Hylebos Waterway Reproductive Toxicology in Flatfish - Reproductive Steroid RIAs

**Table 1. Standard Curve Calibration for 17- $\beta$  Estradiol RIA.  
Hylebos Waterway Reproductive Toxicology in Flatfish**

| test date | tested by | Standard<br>pg/ml | CPM | B%   | logit<br>B% | Log<br>Std | RSD |
|-----------|-----------|-------------------|-----|------|-------------|------------|-----|
|           |           | 500               | 433 | 5.46 | -2.85       | 2.70       |     |

\* Outlier removed

**Hylebos Waterway Reproductive Toxicology in Flatfish - Reproductive Steroid RIAs**



Table 2. Plasma 17β estradiol concentrations (pg/ml Plasma) of quality control. Hylebos Waterway Reproductive Toxicology in Flatfish .

| test date | sample tested by | volume (ul) | B%    |       |       | E2 (pg/ml) |      |      | RSD following adjustment | E2 (pg/ml) | RSD   | comment |       |
|-----------|------------------|-------------|-------|-------|-------|------------|------|------|--------------------------|------------|-------|---------|-------|
|           |                  |             | rep1  | rep2  | rep3  | avg        | rep1 | rep2 |                          |            |       |         | rep3  |
| 6/20/95   | S.S.             | 10          | 54.48 | 48.44 | 49.39 | 50.77      | 2708 | 3544 | 3398                     | 3217       | 13.88 | 3217    |       |
| 6/21/95   | S.S.             | 10          | 51.61 | 48.89 | 49.28 | 49.92      | 3283 | 3697 | 3634                     | 3538       | 6.30  | 3538    |       |
| 6/22/95   | B.B.             | 10          | 52.07 | 50.3  | 50.83 | 51.07      | 2963 | 3188 | 3119                     | 3090       | 3.73  | 3090    |       |
| 6/22/95   | S.S.             | 10          | 45.8  | 43.2  | 38.6  | 42.53      | 3602 | 3992 | 4808                     | 4134       | 14.88 | 4134    |       |
| 6/27/95   | S.S.             | 10          | 50.93 | 48.97 | 56.69 | 52.2       | 3541 | 3820 | 2829                     | 3396       | 15.04 | 3393    |       |
| 6/28/95   | S.S.             | 10          | 59.04 | 49.65 | 52.71 | 53.8       | 2542 | 3770 | 3320                     | 3211       | 19.35 | 3211    |       |
| 7/6/95    | LC               | 10          | 41.23 | 46.65 | 45.2  | 44.36      | 4452 | 3609 | 3816                     | 3959       | 11.10 | 3959    |       |
| 7/10/95   | LC               | 10          | 58.61 | 57.96 | 56.28 | 57.62      | 2258 | 2327 | 2513                     | 2366       | 5.57  | 2366    |       |
| 7/10/95   | S.S.             | 10          | 59.5  | 61.33 | 62.8  | 61.21      | 3434 | 3176 | 2980                     | 3197       | 7.12  | 3197    |       |
| 7/14/95   | LC               | 10          | 34.41 | 43.21 | 45.08 | 40.9       | 5834 | 3975 | 3676                     | 4495       | 26.01 | 3826    |       |
| 7/14/95   | S.S.             | 10          | 48.31 | 48.11 | 49.53 | 48.65      | 4621 | 4652 | 4440                     | 4571       | 2.51  | 4571    |       |
| 7/18/95   | LC               | 10          | 49.44 | 51.53 | 45.71 | 48.9       | 2899 | 2598 | 3526                     | 3008       | 15.74 | 3008    |       |
| 7/18/95   | S.S.             | 10          | 50.42 | 46.97 | 47.01 | 48.13      | 3210 | 3753 | 3746                     | 3569       | 8.72  | 3569    |       |
| 7/18/95   | S.S.             | 10          | 49.55 | 47.92 | 45.92 | 47.8       | 3338 | 3595 | 3936                     | 3623       | 8.29  | 3623    |       |
| 3/13/96   | B.B.             | 10          | 41.85 | 39.23 | 44.99 | 42.02      | 2351 | 2677 | 2017                     | 2349       | 14.05 | 2349    |       |
| 3/13/96   | S.S.             | 10          | 35.23 | 39.34 | 35.14 | 36.57      | 2468 | 1987 | 2511                     | 2332       | 12.82 | 2332    |       |
|           |                  |             |       |       |       |            |      |      |                          | RSD=       | 20.49 | RSD=    | 19.13 |

a=outlier taken out.

Table 3. Plasma 17- $\beta$  estradiol concentrations (pg/ml Plasma).  
Hylebos Waterway Reproductive Toxicology in Flatfish.

| specimen # | re- runs | sample test date | volume ( $\mu$ l) | B %   |       |       | E2 (pg/ml) |          |         | E2 rep3 (pg/ml) | E2 avg (pg/ml) | E2 (pg/ml) adjusted RSD | com- ment | E2 (pg/ml) assay |     | final comment |      |   |
|------------|----------|------------------|-------------------|-------|-------|-------|------------|----------|---------|-----------------|----------------|-------------------------|-----------|------------------|-----|---------------|------|---|
|            |          |                  |                   | rep1  | rep2  | rep3  | rep1       | rep2     | rep3    |                 |                |                         |           | RSD              | RSD |               |      |   |
| 3271       |          | 6/22/95          | 10                | 37.91 | 39.08 | 42.65 | 39.88      | 4945.22  | 4713.84 | 4080.44         | 4579.83        | 9.78                    | 4579.83   |                  |     |               |      |   |
| 3273       |          | 6/22/95          | 50                | 42.06 | 47.90 | 46.77 | 45.58      | 835.47   | 663.53  | 693.45          | 730.81         | 12.57                   | 730.81    |                  |     |               |      |   |
| 3276       | R        | 6/22/95          | 50                | 41.2  | 40.59 | 30.24 | 37.34      | 864.93   | 886.54  | 1381.61         | 1044.36        | 27.99                   | 875.73    | 1.74             | a   | 875.73        | 1.74 | a |
| 3276       | R        | 3/13/96          | 50                | 0.78  | 20.39 | 8.82  | 10.00      | 104759.1 | 1617.52 | 5189.26         | 37188.63       | 157.43                  |           | B                |     |               |      |   |
| 3281       |          | 6/22/95          | 10                | 63.56 | 68.56 | 64.42 | 65.51      | 1776.54  | 1428.83 | 1712.79         | 1639.39        | 11.29                   | 1639.39   |                  |     |               |      |   |
| 3283       |          | 6/22/95          | 50                | 42.01 | 36.61 | 35.65 | 38.09      | 837.07   | 1044.48 | 1087.7          | 989.75         | 13.54                   | 989.75    |                  |     |               |      |   |
| 3284       |          | 7/10/95          | 100               | 67.65 | 69.28 | 71.55 | 69.50      | 145.99   | 134.13  | 118.71          | 132.94         | 10.29                   | 132.94    |                  |     |               |      |   |
| 3287       |          | 6/22/95          | 50                | 27.24 | 26.34 | 28.76 | 27.45      | 1593.79  | 1666.69 | 1480.57         | 1580.35        | 5.93                    | 1580.35   |                  |     |               |      |   |
| 3288       |          | 7/10/95          | 100               | 34.62 | 41.98 | 35.52 | 37.38      | 679.26   | 478.86  | 649.8           | 602.64         | 17.95                   | 602.64    |                  |     |               |      |   |
| 3289       |          | 6/22/95          | 10                | 39.17 | 38.70 | 39.89 | 39.26      | 4695.36  | 4786.3  | 4559.52         | 4680.39        | 2.44                    | 4680.39   |                  |     |               |      |   |
| 3290       |          | 7/10/95          | 100               | 68.78 | 65.43 | 61.63 | 65.28      | 137.72   | 163.24  | 196.15          | 165.7          | 17.68                   | 165.7     |                  |     |               |      |   |
| 3291       |          | 7/10/95          | 100               | 49.67 | 46.00 | 46.43 | 47.37      | 338.39   | 398.9   | 391.38          | 376.23         | 8.77                    | 376.23    |                  |     |               |      |   |
| 3292       |          | 7/10/95          | 100               | 47.82 | 44.29 | 42.43 | 44.85      | 367.62   | 431.08  | 469.12          | 422.61         | 12.13                   | 422.61    |                  |     |               |      |   |
| 3293       |          | 6/22/95          | 10                | 64.22 | 60.14 | 62.71 | 62.36      | 1727.69  | 2046.24 | 1841.07         | 1871.67        | 8.63                    | 1871.67   |                  |     |               |      |   |
| 3294       |          | 7/10/95          | 100               | 25.82 | 23.06 | 22.16 | 23.68      | 1086.51  | 1284.31 | 1361.18         | 1244           | 11.39                   | 1244      |                  |     |               |      |   |
| 3295       |          | 7/10/95          | 100               | 48.75 | 48.52 | 55.30 | 50.86      | 352.54   | 356.25  | 262.78          | 323.86         | 16.34                   | 323.86    |                  |     |               |      |   |
| 3296       |          | 7/10/95          | 100               | 52.31 | 52.43 | 52.76 | 52.50      | 300.59   | 298.98  | 294.61          | 298.06         | 1.04                    | 298.06    |                  |     |               |      |   |
| 3298       |          | 7/10/95          | 100               | 92.02 | 92.70 | 82.65 | 89.12      | 41.9     | 38      | 103.35          | 61.08          | 60.02                   | 60.15     | A                |     | 60.15         |      | A |
| 3299       | R        | 7/10/95          | 100               | 65.49 | 55.25 | 49.39 | 56.71      | 264.43   | 410.07  | 521.42          | 398.64         | 32.33                   |           |                  |     |               |      |   |
| 3299       | R        | 7/18/95          | 100               | 36.73 | 38.05 | 42.32 | 39.03      | 605.56   | 567.97  | 464.48          | 546            | 13.38                   | 546       |                  |     |               |      |   |
| 3300       |          | 7/10/95          | 100               | 72.76 | 75.59 | 69.61 | 72.65      | 186.55   | 160.41  | 218.18          | 188.38         | 15.35                   | 188.38    |                  |     |               |      |   |
| 3301       |          | 7/14/95          | 100               | 27.24 | 25.34 | 20.98 | 24.52      | 826.55   | 914.42  | 1178.27         | 973.08         | 18.81                   | 973.08    |                  |     |               |      |   |

A=below effective concentrations of the assay ; B=above effective concentrations of the assay; C=average of 2 or more runs; D=Delete; R=run to run RSD of outside limit; O=non-target species; a=outlier taken out; s=within run RSD slightly above limit; B%=B% slightly above limit; CNC=could not calculate; NDB=not included in database.

Hylebos Waterway Reproductive Toxicology in Flatfish - Reproductive Steroid RIAs

Table 3. Plasma 17- $\beta$  estradiol concentrations(pg/ml Plasma).  
Hylebos Waterway Reproductive Toxicology in Flatfish.

| specimen # | re- runs | test date | sample volume ( $\mu$ l) | B %   |       |       | E2 (pg/ml) |          |         | E2 avg (pg/ml) | RSD      | E2 (pg/ml) adjusted | RSD     | comment | E2 (pg/ml) assay |        | final comment |   |
|------------|----------|-----------|--------------------------|-------|-------|-------|------------|----------|---------|----------------|----------|---------------------|---------|---------|------------------|--------|---------------|---|
|            |          |           |                          | rep1  | rep2  | rep3  | rep1       | rep2     | rep3    |                |          |                     |         |         | final            | RSD    |               |   |
| 3271       |          | 6/22/95   | 10                       | 37.91 | 39.08 | 42.65 | 39.88      | 4945.22  | 4713.84 | 4080.44        | 4579.83  | 9.78                | 4579.83 |         |                  |        |               |   |
| 3273       |          | 6/22/95   | 50                       | 42.06 | 47.90 | 46.77 | 45.58      | 835.47   | 663.53  | 693.45         | 730.81   | 12.57               | 730.81  |         |                  |        |               |   |
| 3276       | R        | 6/22/95   | 50                       | 41.2  | 40.59 | 30.24 | 37.34      | 864.93   | 886.54  | 1381.61        | 1044.36  | 27.99               | 875.73  | 1.74    | a                | 875.73 | 1.74          | a |
| 3276       | R        | 3/13/96   | 50                       | 0.78  | 20.39 | 8.82  | 10.00      | 104759.1 | 1617.52 | 5189.26        | 37188.63 | 157.43              |         | B       |                  |        |               |   |
| 3281       |          | 6/22/95   | 10                       | 63.56 | 68.56 | 64.42 | 65.51      | 1776.54  | 1428.83 | 1712.79        | 1639.39  | 11.29               | 1639.39 |         |                  |        |               |   |
| 3283       |          | 6/22/95   | 50                       | 42.01 | 36.61 | 35.65 | 38.09      | 837.07   | 1044.48 | 1087.7         | 989.75   | 13.54               | 989.75  |         |                  |        |               |   |
| 3284       |          | 7/10/95   | 100                      | 67.65 | 69.28 | 71.55 | 69.50      | 145.99   | 134.13  | 118.71         | 132.94   | 10.29               | 132.94  |         |                  |        |               |   |
| 3287       |          | 6/22/95   | 50                       | 27.24 | 26.34 | 28.76 | 27.45      | 1593.79  | 1666.69 | 1480.57        | 1580.35  | 5.93                | 1580.35 |         |                  |        |               |   |
| 3288       |          | 7/10/95   | 100                      | 34.62 | 41.98 | 35.52 | 37.38      | 679.26   | 478.86  | 649.8          | 602.64   | 17.95               | 602.64  |         |                  |        |               |   |
| 3289       |          | 6/22/95   | 10                       | 39.17 | 38.70 | 39.89 | 39.26      | 4695.36  | 4786.3  | 4559.52        | 4680.39  | 2.44                | 4680.39 |         |                  |        |               |   |
| 3290       |          | 7/10/95   | 100                      | 68.78 | 65.43 | 61.63 | 65.28      | 137.72   | 163.24  | 196.15         | 165.7    | 17.68               | 165.7   |         |                  |        |               |   |
| 3291       |          | 7/10/95   | 100                      | 49.67 | 46.00 | 46.43 | 47.37      | 338.39   | 398.9   | 391.38         | 376.23   | 8.77                | 376.23  |         |                  |        |               |   |
| 3292       |          | 7/10/95   | 100                      | 47.82 | 44.29 | 42.43 | 44.85      | 367.62   | 431.08  | 469.12         | 422.61   | 12.13               | 422.61  |         |                  |        |               |   |
| 3293       |          | 6/22/95   | 10                       | 64.22 | 60.14 | 62.71 | 62.36      | 1727.69  | 2046.24 | 1841.07        | 1871.67  | 8.63                | 1871.67 |         |                  |        |               |   |
| 3294       |          | 7/10/95   | 100                      | 25.82 | 23.06 | 22.16 | 23.68      | 1086.51  | 1284.31 | 1361.18        | 1244     | 11.39               | 1244    |         |                  |        |               |   |
| 3295*      |          | 7/10/95   | 100                      | 48.75 | 48.52 | 55.30 | 50.86      | 352.54   | 356.25  | 262.78         | 323.86   | 16.34               | 323.86  |         |                  |        |               |   |
| 3296       |          | 7/10/95   | 100                      | 52.31 | 52.43 | 52.76 | 52.50      | 300.59   | 298.98  | 294.61         | 298.06   | 1.04                | 298.06  |         |                  |        |               |   |
| 3298       |          | 7/10/95   | 100                      | 92.02 | 92.70 | 82.65 | 89.12      | 41.9     | 38      | 103.35         | 61.08    | 60.02               | 60.15   | A       |                  | 60.15  | A             |   |
| 3299       | R        | 7/10/95   | 100                      | 65.49 | 55.25 | 49.39 | 56.71      | 264.43   | 410.07  | 521.42         | 398.64   | 32.33               |         |         |                  |        |               |   |
| 3299       | R        | 7/18/95   | 100                      | 36.73 | 38.05 | 42.32 | 39.03      | 605.56   | 567.97  | 464.48         | 546      | 13.38               | 546     |         |                  |        |               |   |
| 3300       |          | 7/10/95   | 100                      | 72.76 | 75.59 | 69.61 | 72.65      | 186.55   | 160.41  | 218.18         | 188.38   | 15.35               | 188.38  |         |                  |        |               |   |
| 3301       |          | 7/14/95   | 100                      | 27.24 | 25.34 | 20.98 | 24.52      | 826.55   | 914.42  | 1178.27        | 973.08   | 18.81               | 973.08  |         |                  |        |               |   |

A=below effective concentrations of the assay ; B=above effective concentrations of the assay; C=average of 2 or more runs; D=Delete; R=run to run RSD of outside limit; a=outlier taken out; s=within run RSD slightly above limit; B%=B% slightly above limit; CNC=could not calculate.

Hylebos Waterway Reproductive Toxicology in Flatfish - Reproductive Steroid RIAs

Table 3. Plasma 17- $\beta$  estradiol concentrations (pg/ml Plasma).  
Hylebos Waterway Reproductive Toxicology in Flatfish.

| specimen # | re- runs | test date | sample volume ( $\mu$ l) | B %   |       |       | E2 (pg/ml) |         |         | E2 (pg/ml) avg | E2 (pg/ml) rep3 | E2 (pg/ml) rep2 | E2 (pg/ml) rep1 | RSD adjusted | E2 (pg/ml) com- ment | E2 final | intra assay RSD | inter assay RSD | final comment |
|------------|----------|-----------|--------------------------|-------|-------|-------|------------|---------|---------|----------------|-----------------|-----------------|-----------------|--------------|----------------------|----------|-----------------|-----------------|---------------|
|            |          |           |                          | rep1  | rep2  | rep3  | rep1       | rep2    | rep3    |                |                 |                 |                 |              |                      |          |                 |                 |               |
| 3302       |          | 6/22/95   | 10                       | 50.55 | 51.75 | 49.88 | 50.73      | 2991.63 | 2854.82 | 3071.04        | 2972.5          | 3.68            | 2972.5          |              | 2972.5               |          |                 |                 |               |
| 3303       | R        | 7/10/95   | 100                      | 78.88 | 62.06 | 64.75 | 68.56      | 132.49  | 307.72  | 273.34         | 237.85          | 39.04           |                 |              |                      |          |                 |                 |               |
| 3303       | R        | 7/18/95   | 100                      | 48.68 | 47.90 | 45.80 | 47.46      | 347.24  | 359.79  | 395.75         | 367.6           | 6.85            | 367.6           |              | 367.6                |          |                 |                 |               |
| 3304       |          | 7/10/95   | 100                      | 44.61 | 45.11 | 41.49 | 43.74      | 634.3   | 621.33  | 722.42         | 659.35          | 8.34            | 659.35          |              | 659.35               |          |                 |                 |               |
| 3305       |          | 7/14/95   | 100                      | 35.62 | 35.85 | 35.05 | 35.50      | 552.38  | 546.69  | 566.76         | 555.27          | 1.86            | 555.27          |              | 555.27               |          |                 |                 |               |
| 3306       |          | 7/14/95   | 100                      | 42.38 | 40.73 | 42.15 | 41.75      | 411.75  | 441.58  | 415.78         | 423.04          | 3.83            | 423.04          |              | 423.04               |          |                 |                 |               |
| 3307       |          | 7/6/95    | 100                      | 65.32 | 59.03 | 58.99 | 61.11      | 173.72  | 224.26  | 224.62         | 207.53          | 14.11           | 207.53          |              | 207.53               |          |                 |                 |               |
| 3308       |          | 7/14/95   | 100                      | 54.92 | 51.58 | 50.22 | 52.24      | 244.49  | 280.88  | 297.04         | 274.14          | 9.82            | 274.14          |              | 274.14               |          |                 |                 |               |
| 3309       |          | 7/10/95   | 100                      | 71.58 | 73.20 | 73.30 | 72.69      | 198.04  | 182.37  | 181.38         | 187.26          | 4.99            | 187.26          |              | 187.26               |          |                 |                 |               |
| 3310       |          | 7/10/95   | 100                      | 49.24 | 48.35 | 49.36 | 48.98      | 524.69  | 544.12  | 522.01         | 530.27          | 2.28            | 530.27          |              | 530.27               |          |                 |                 |               |
| 3311       |          | 7/14/95   | 100                      | 40.09 | 33.93 | 37.47 | 37.16      | 453.91  | 596.44  | 508.73         | 519.69          | 13.83           | 519.69          |              | 519.69               |          |                 |                 |               |
| 3312       | R        | 7/10/95   | 100                      | 30.81 | 26.66 | 23.07 | 26.85      | 1161.8  | 1429.4  | 1738.88        | 1443.36         | 20.01           |                 |              |                      |          |                 |                 |               |
| 3312       | R        | 3/13/96   | 50                       | 22.07 | 23.23 | 23.28 | 22.86      | 1168.86 | 1072.08 | 1067.86        | 1102.93         | 5.18            | 1102.93         |              | 1102.93              |          |                 |                 |               |
| 3313       |          | 7/6/95    | 100                      | 70.03 | 73.91 | 71.63 | 71.85      | 141.47  | 117.75  | 131.42         | 130.21          | 9.14            | 130.21          |              | 130.21               |          |                 |                 |               |
| 3314       |          | 7/10/95   | 100                      | 39.18 | 38.35 | 46.28 | 41.27      | 796.78  | 825.65  | 592.17         | 738.2           | 17.24           | 738.2           |              | 738.2                |          |                 |                 |               |
| 3315       | R        | 7/6/95    | 100                      | 13.03 | 20.66 | 27.32 | 20.34      | 1938.26 | 1144.84 | 806.7          | 1296.6          | 44.80           |                 |              |                      |          |                 |                 |               |
| 3315       | R        | 7/18/95   | 100                      | 21.28 | 19.36 | 20.30 | 20.31      | 1561.68 | 1823.98 | 1688.21        | 1691.29         | 7.76            | 1691.29         |              | 1691.29              |          |                 |                 |               |
| 3316       |          | 7/14/95   | 100                      | 36.07 | 32.20 | 29.92 | 32.73      | 541.24  | 646.47  | 721.67         | 636.46          | 14.24           | 636.46          |              | 636.46               |          |                 |                 |               |
| 3317       |          | 7/6/95    | 100                      | 63.17 | 66.00 | 63.76 | 64.31      | 189.95  | 168.81  | 185.36         | 181.38          | 6.13            | 181.38          |              | 181.38               |          |                 |                 |               |
| 3318       |          | 7/14/95   | 100                      | 35.41 | 37.78 | 35.79 | 36.33      | 557.46  | 501.66  | 548.19         | 535.77          | 5.58            | 535.77          |              | 535.77               |          |                 |                 |               |
| 3319       |          | 7/14/95   | 100                      | 29.29 | 29.62 | 29.87 | 29.59      | 744.79  | 732.61  | 723.6          | 733.67          | 1.45            | 733.67          |              | 733.67               |          |                 |                 |               |
| 3320       |          | 7/14/95   | 100                      | 49.7  | 45.25 | 44.58 | 46.51      | 303.59  | 365.03  | 375.38         | 348             | 11.15           | 348             |              | 348                  |          |                 |                 |               |

A=below effective concentrations of the assay ; B=above effective concentrations of the assay; C=average of 2 or more runs; D=Delete; R=run to run RSD of outside limit; a=outlier taken out; s=within run RSD slightly above limit; B%=B% slightly above limit; CNC=could not calculate.

Hylebos Waterway Reproductive Toxicology in Flatfish - Reproductive Steroid RIAs

Table 3. Plasma 17- $\beta$  estradiol concentrations(pg/ml Plasma).  
Hylebos Waterway Reproductive Toxicology in Flatfish.

| specimen # | re-<br>runs | test<br>date | sample<br>volume<br>( $\mu$ l) | E2 (pg/ml) |        |       | E2<br>rep3<br>(pg/ml) | E2<br>avg<br>(pg/ml) | E2<br>RSD<br>(%) | E2<br>adjusted<br>(pg/ml) | RSD     | comment | E2<br>final<br>(pg/ml) | Intra<br>assay<br>RSD | Inter<br>assay<br>RSD | final<br>comment |
|------------|-------------|--------------|--------------------------------|------------|--------|-------|-----------------------|----------------------|------------------|---------------------------|---------|---------|------------------------|-----------------------|-----------------------|------------------|
|            |             |              |                                | rep1       | rep2   | rep3  |                       |                      |                  |                           |         |         |                        |                       |                       |                  |
| 3321       |             | 7/6/95       | 100                            | 80.19      | 80.71  | 23.66 | 61.52                 | 83.81                | 81.2             | 969.41                    | 378.14  | 135.41  | 82.51                  | 2.23                  | a                     | a                |
| 3322       |             | 7/6/95       | 100                            | 55.42      | 62.90  | 59.33 | 59.22                 | 258.05               | 192.07           | 221.58                    | 223.9   | 14.76   | 223.9                  |                       |                       |                  |
| 3323       |             | 7/6/95       | 100                            | 68.63      | 70.96  | 71.94 | 70.51                 | 150.65               | 135.54           | 129.5                     | 138.56  | 7.86    | 138.56                 |                       |                       |                  |
| 3332       |             | 6/22/95      | 50                             | 77.92      | 71.29  | 72.61 | 73.94                 | 178.65               | 251.72           | 236.17                    | 222.18  | 17.33   | 222.18                 |                       |                       |                  |
| 3333       | R           | 7/6/95       | 10                             | 77.91      | 68.21  | 74.89 | 73.67                 | 955.81               | 1534.63          | 1121.04                   | 1203.83 | 24.77   |                        |                       |                       |                  |
| 3333       | R           | 7/18/95      | 10                             | 49.06      | 46.06  | 48.91 | 48.01                 | 3413.4               | 3910.69          | 3435.67                   | 3586.59 | 7.83    | 3586.59                |                       |                       |                  |
| 3335       | R           | 7/6/95       | 100                            | 64.11      | 87.65  | 72.69 | 74.82                 | 182.73               | 49.07            | 124.95                    | 118.92  | 56.37   |                        |                       |                       |                  |
| 3335       | R           | 7/18/95      | 100                            | 54.54      | 57.15  | 55.50 | 55.73                 | 221.79               | 193.04           | 210.78                    | 208.54  | 6.96    | 208.54                 |                       |                       |                  |
| 3340       | R           | 7/10/95      | 50                             | 84.67      | 92.86  | 96.88 | 91.47                 | 98.43                | 37.7             | 14.23                     | 50.12   | 86.70   |                        |                       | A                     |                  |
| 3340       | R           | 7/18/95      | 100                            | 47.18      | 107.70 | 87.16 | 80.68                 | 371.71               | CNC              | 37.41                     | CNC     |         | 33.9                   |                       | A                     | A                |
| 3341       | R           | 7/10/95      | 50                             | 84.28      | 86.26  | 88.10 | 86.21                 | 101.78               | 85.28            | 70.94                     | 86      | 17.94   |                        |                       | A                     |                  |
| 3341       | R           | 7/18/95      | 100                            | 80.98      | 86.69  | 82.32 | 83.33                 | 63.41                | 39.18            | 57.34                     | 53.31   | 23.65   | 33.9                   |                       | A                     | A                |
| 3347       | R           | 7/6/95       | 100                            | 50.72      | 68.42  | 69.78 | 62.97                 | 309.02               | 152.05           | 143.09                    | 201.38  | 46.34   |                        |                       |                       |                  |
| 3347       | R           | 3/13/96      | 100                            | 46.3       | 49.76  | 46.85 | 47.64                 | 189.39               | 160.39           | 184.46                    | 178.08  | 8.71    | 178.08                 |                       |                       |                  |
| 3348       |             | 7/10/95      | 100                            | 80.03      | 83.50  | 77.60 | 80.38                 | 70.5                 | 54.29            | 82.94                     | 69.24   | 20.74   | 35.15                  |                       | A                     | A                |
| 3350       |             | 7/10/95      | 100                            | 79.66      | 86.15  | 80.62 | 82.14                 | 72.31                | 43.1             | 67.6                      | 61      | 25.71   | 35.15                  |                       | A                     | A                |
| 3352       |             | 6/22/95      | 100                            | 74.77      | 70.57  | 70.40 | 71.92                 | 105.93               | 130.22           | 131.25                    | 122.46  | 11.70   | 122.46                 |                       |                       |                  |
| 3355       |             | 6/22/95      | 100                            | 81         | 98.40  | 88.86 | 89.42                 | 74.28                | 5.49             | 40.33                     | 40.03   | 85.90   | 39.4                   |                       | A                     | A                |
| 3358       | R           | 6/22/95      | 100                            | 67.35      | 49.49  | 71.94 | 62.93                 | 150.85               | 311.74           | 122.01                    | 194.86  | 52.47   | 136.43                 |                       | a                     | 27.00            |
| 3358       | R           | 7/18/95      | 100                            | 57.63      | 60.70  | 30.57 | 49.63                 | 230.94               | 199.95           | 827.86                    | 419.58  | 84.35   | 215.44                 |                       | a                     |                  |
| 3358       | R           | 3/13/96      | 50                             | 86.02      |        |       | 86.02                 | 21.23                |                  |                           | 21.23   |         |                        |                       | A                     |                  |
| 3359       | R           | 6/28/95      | 50                             | 77.19      | 81.44  | 82.60 | 80.41                 | 209.63               | 160.05           | 147.5                     | 172.39  | 19.06   |                        |                       |                       |                  |

A=below effective concentrations of the assay ; B=above effective concentrations of the assay; C=average of 2 or more runs; D=Delete; R=run to run RSD of outside limit;  
a=outlier taken out; s=within run RSD slightly above limit; B%=B% slightly above limit; CNC=could not calculate.

Hylebos Waterway Reproductive Toxicology in Flatfish - Reproductive Steroid RIAs



Table 3. Plasma 17- $\beta$  estradiol concentrations (pg/ml Plasma).  
Hylebos Waterway Reproductive Toxicology in Flatfish.

| specimen # | re-runs | sample test date | volume (ul) | B %   |       |        | E2 (pg/ml) |         |         | E2 (pg/ml) avg | RSD     | E2 (pg/ml) adjusted | RSD | com-ment | E2 (pg/ml) assay |       |         | inter-assay RSD | final comment |
|------------|---------|------------------|-------------|-------|-------|--------|------------|---------|---------|----------------|---------|---------------------|-----|----------|------------------|-------|---------|-----------------|---------------|
|            |         |                  |             | rep1  | rep2  | rep3   | rep1       | rep2    | rep3    |                |         |                     |     |          | final            | RSD   | comment |                 |               |
| 3359       | R       | 7/14/95          | 100         | 79.46 | 79.46 | 144.18 | 144.07     | 144.07  | 144.07  |                | 144.07  |                     |     |          |                  |       |         |                 |               |
| 3359       | R       | 3/13/96          | 25          | 67.25 | 67.25 | 177.16 | 177.16     | 177.16  | 177.16  |                | 177.16  |                     |     |          |                  | 15.00 |         |                 | C             |
| 3360       |         | 6/22/95          | 100         | 89.82 | 93.47 | 86.58  | 36.56      | 22.78   | 36.31   | 36.93          | 39.4    |                     | A   |          |                  |       |         |                 | A             |
| 3361       |         | 6/28/95          | 100         | 85.47 | 84.69 | 85.58  | 59.03      | 62.9    | 60.14   | 3.99           | 43.85   |                     | A   |          |                  |       |         |                 | A             |
| 3362       |         | 6/28/95          | 100         | 90.83 | 95.15 | 101.82 | 34.34      | 16.89   | CNC     | CNC            | 43.85   |                     | A   |          |                  |       |         |                 | A             |
| 3363       | R       | 6/28/95          | 100         | 70.15 | 81.81 | 78.79  | 153        | 77.99   | 108.71  | 36.15          |         |                     |     |          |                  |       |         |                 | D             |
| 3363       | R       | 7/14/95          | 100         | 76.43 | 76.43 | 166.64 | 166.75     | 166.75  | 166.75  |                |         |                     |     |          |                  |       |         |                 | D             |
| 3363       | R       | 3/13/96          | 100         | 53.13 | 58.31 | 49.94  | 96.04      | 73.03   | 113.38  | 21.50          |         |                     |     |          |                  |       |         |                 | D             |
| 3366       |         | 6/28/95          | 50          | 34    | 35.15 | 33.09  | 34.08      | 1480.06 | 1403.77 | 4.76           | 1475.98 |                     |     |          |                  |       |         |                 | 1475.98       |
| 3368       |         | 6/28/95          | 50          | 68.83 | 64.13 | 62.39  | 326.43     | 406.61  | 439.4   | 14.87          | 390.81  |                     | A   |          |                  |       |         |                 | 390.81        |
| 3369       | R       | 6/28/95          | 10          | 86.5  | 84.32 | 87.00  | 539.83     | 647.79  | 516.31  | 12.34          |         |                     |     |          |                  |       |         |                 |               |
| 3369       | R       | 7/14/95          | 50          | 63.93 | 61.65 | 66.97  | 546.89     | 592.6   | 489.82  | 9.48           | 543.1   |                     |     |          |                  |       |         |                 | 543.1         |
| 3370       | R       | 6/28/95          | 100         | 65.04 | 56.68 | 55.30  | 195.02     | 281.13  | 297.93  | 21.40          | 289.53  |                     | a   |          |                  |       |         |                 | D,R           |
| 3370       | R       | 3/13/96          | 100         | 48.63 | 45.85 | 48.17  | 121.4      | 140.37  | 124.34  | 7.93           | 128.7   |                     |     |          |                  |       |         |                 | D,R           |
| 3372       | R       | 6/28/95          | 50          | 29.26 | 22.96 | 21.66  | 1859.49    | 2612.92 | 2824.64 | 20.86          |         |                     |     |          |                  |       |         |                 |               |
| 3372       | R       | 7/14/95          | 10          | 54.36 | 55.75 | 57.02  | 3786.22    | 3618.28 | 3467.79 | 4.42           | 3624.76 |                     |     |          |                  |       |         |                 | 3624.76       |
| 3374       | R       | 6/20/95          | 100         | 79.13 | 77.34 | 76.79  | 75.06      | 84.35   | 87.33   | 7.79           | 82.25   |                     |     |          |                  |       |         |                 | 82.25         |
| 3374       | R       | 7/14/95          | 50          | 90.05 | 92.06 | 87.34  | 143.62     | 117.26  | 179.53  | 21.29          |         |                     | A   |          |                  |       |         |                 |               |
| 3374       | R       | 3/13/96          | 100         | 22.38 | 33.54 | 53.88  | 570.61     | 275.32  | 92.34   | 77.16          |         |                     |     |          |                  |       |         |                 |               |
| 3376       | R       | 6/20/95          | 100         | 86.55 | 84.72 | 88.85  | 86.70      | 41.68   | 32.86   | 19.80          | 35.2    |                     | A   |          |                  |       |         |                 | A             |
| 3376       | R       | 6/22/95          | 50          | 49.83 | 1.19  | 44.30  | 615.36     | CNC     | 764.21  | 689.78         |         |                     |     |          |                  |       |         |                 |               |
| 3377       |         | 6/22/95          | 100         | 44.37 | 47.42 | 45.89  | 381.02     | 338.04  | 359     | 5.98           | 359.36  |                     |     |          |                  |       |         |                 | 359.36        |

A=below effective concentrations of the assay; B=above effective concentrations of the assay; C=average of 2 or more runs; D=Delete; R=run to run RSD of outside limit; a=outlier taken out; s=within run RSD slightly above limit; B%=B% slightly above limit; CNC=could not calculate.

Hylebos Waterway Reproductive Toxicology in Flatfish - Reproductive Steroid RIAs

Table 3. Plasma 17- $\beta$  estradiol concentrations(pg/ml Plasma).  
Hylebos Waterway Reproductive Toxicology In Flatfish.

| specimen # | re- runs | test date | sample volume (ul) | B %   |       |       | E2 (pg/ml) |         |         | E2 avg  | E2 (pg/ml) rep3 | E2 (pg/ml) rep2 | E2 (pg/ml) rep1 | RSD     | E2 adjusted (pg/ml) | RSD | comment | E2 (pg/ml) assay |     | final comment |  |
|------------|----------|-----------|--------------------|-------|-------|-------|------------|---------|---------|---------|-----------------|-----------------|-----------------|---------|---------------------|-----|---------|------------------|-----|---------------|--|
|            |          |           |                    | rep1  | rep2  | rep3  | rep1       | rep2    | rep3    |         |                 |                 |                 |         |                     |     |         | final            | RSD |               |  |
| 3378       |          | 6/28/95   | 50                 | 32.47 | 33.92 | 32.49 | 32.96      | 1589.99 | 1485.34 | 1588.54 | 1554.62         | 1554.62         | 3.86            | 1554.62 |                     |     |         |                  |     | 1554.62       |  |
| 3380       | R        | 6/22/95   | 100                | 16.03 | 14.66 | 14.82 | 15.17      | 1537.19 | 1703.42 | 1682.93 | 1641.18         | 1641.18         | 5.52            |         |                     |     | B       |                  |     |               |  |
| 3380       | R        | 3/13/96   | 5                  | 78.37 | 79.45 | 75.17 | 77.67      | 679.76  | 628.85  | 842.74  | 717.12          | 717.12          | 15.58           | 717.12  |                     |     |         |                  |     | 717.12        |  |
| 3381       | R        | 6/28/95   | 100                | 67.45 | 76.15 | 65.56 | 69.72      | 174.38  | 111.28  | 190.5   | 158.72          | 158.72          | 26.38           |         |                     |     |         |                  |     |               |  |
| 3381       | R        | 7/14/95   | 100                | 75.57 | 76.27 | 77.15 | 76.33      | 173.23  | 167.83  | 161.22  | 167.43          | 167.43          | 3.59            | 167.43  |                     |     |         |                  |     | 167.43        |  |
| 3383       |          | 6/22/95   | 50                 | 21.45 | 19.78 | 21.63 | 20.95      | 2168.31 | 2394.85 | 2145.92 | 2236.36         | 2236.36         | 6.16            | 2236.36 |                     |     |         |                  |     | 2236.36       |  |
| 3384       |          | 6/28/95   | 100                | 32.11 | 28.83 | 30.91 | 30.62      | 808.9   | 949.76  | 856.89  | 871.85          | 871.85          | 8.21            | 871.85  |                     |     |         |                  |     | 871.85        |  |
| 3385       | R        | 6/20/95   | 50                 | 29    | 28.36 | 27.69 | 28.35      | 1790.87 | 1854.01 | 1924.42 | 1856.43         | 1856.43         | 3.60            | 1856.43 |                     |     |         |                  |     | 1856.43       |  |
| 3385       | R        | 3/13/96   | 10                 | 52.29 | 34.60 | 45.59 | 44.16      | 1421.01 | 3397.04 | 1959.15 | 2259.07         | 2259.07         | 45.22           |         |                     |     |         |                  |     |               |  |
| 3386       |          | 6/28/95   | 100                | 45.98 | 42.38 | 40.97 | 43.11      | 439.27  | 511.34  | 543.06  | 497.89          | 497.89          | 10.68           | 497.89  |                     |     |         |                  |     | 497.89        |  |
| 3387       |          | 6/28/95   | 100                | 24.56 | 22.27 | 20.96 | 22.59      | 1191.85 | 1361.2  | 1475.31 | 1342.79         | 1342.79         | 10.62           | 1342.79 |                     |     |         |                  |     | 1342.79       |  |
| 3389       |          | 6/22/95   | 10                 | 67.04 | 66.12 | 64.52 | 65.89      | 1529.43 | 1591.77 | 1705.81 | 1609            | 1609            | 5.46            | 1609    |                     |     |         |                  |     | 1609          |  |
| 3391       |          | 6/22/95   | 50                 | 30.55 | 29.28 | 31.07 | 30.30      | 1361.89 | 1444.86 | 1330.14 | 1378.96         | 1378.96         | 4.30            | 1378.96 |                     |     |         |                  |     | 1378.96       |  |
| 3392       |          | 6/28/95   | 50                 | 37.04 | 31.36 | 34.62 | 34.34      | 1289.66 | 1676.45 | 1438.3  | 1468.14         | 1468.14         | 13.29           | 1468.14 |                     |     |         |                  |     | 1468.14       |  |
| 3393       | R        | 6/28/95   | 100                | 37.11 | 27.92 | 25.94 | 30.32      | 642.63  | 995.27  | 1104.88 | 914.26          | 914.26          | 26.42           |         |                     |     |         |                  |     |               |  |
| 3393       | R        | 7/14/95   | 50                 | 49.22 | 47.66 | 37.94 | 44.94      | 897.15  | 944.14  | 1308.99 | 1050.09         | 1050.09         | 21.47           |         |                     |     |         |                  |     |               |  |
| 3393       | R        | 3/13/96   | 50                 | 31.66 | 36.01 | 31.91 | 33.19      | 615.75  | 477.9   | 606.57  | 566.74          | 566.74          | 13.60           | 566.74  |                     |     |         |                  |     | 566.74        |  |
| 3398       |          | 6/22/95   | 100                | 30.48 | 30.92 | 31.10 | 30.83      | 683.2   | 669.45  | 663.98  | 672.21          | 672.21          | 1.47            | 672.21  |                     |     |         |                  |     | 672.21        |  |
| 3400       |          | 6/22/95   | 10                 | 50.48 | 49.41 | 47.98 | 49.29      | 3000.04 | 3127.59 | 3306.78 | 3144.8          | 3144.8          | 4.90            | 3144.8  |                     |     |         |                  |     | 3144.8        |  |
| 3401       |          | 6/27/95   | 100                | 36.41 | 32.35 | 34.99 | 34.58      | 636.86  | 760.52  | 676.9   | 691.43          | 691.43          | 9.13            | 691.43  |                     |     |         |                  |     | 691.43        |  |
| 3402       |          | 6/27/95   | 50                 | 36.61 | 37.25 | 38.84 | 37.57      | 1262.83 | 1228.88 | 1150.17 | 1213.96         | 1213.96         | 4.76            | 1213.96 |                     |     |         |                  |     | 1213.96       |  |
| 3403       |          | 6/22/95   | 50                 | 65.36 | 63.80 | 66.44 | 65.20      | 329.11  | 351.73  | 314.05  | 331.63          | 331.63          | 5.72            | 331.63  |                     |     |         |                  |     | 331.63        |  |

A=below effective concentrations of the assay ; B=above effective concentrations of the assay; C=average of 2 or more runs; D=Delete; R=run to run RSD of outside limit; a=outlier taken out; s=within run RSD slightly above limit; B%=B% slightly above limit; CNC=could not calculate.

Hylebos Waterway Reproductive Toxicology in Flatfish - Reproductive Steroid RIAs

Table 3. Plasma 17- $\beta$  estradiol concentrations (pg/ml Plasma).  
Hylebos Waterway Reproductive Toxicology in Flatfish.

| specimen # | re- test runs | date    | sample volume ( $\mu$ l) | B %   |       |       | E2 (pg/ml) |         |         | E2 (pg/ml) rep3 | E2 (pg/ml) avg | E2 (pg/ml) RSD adjusted | com- ment | E2 (pg/ml) final |       | inter assay RSD | final comment |
|------------|---------------|---------|--------------------------|-------|-------|-------|------------|---------|---------|-----------------|----------------|-------------------------|-----------|------------------|-------|-----------------|---------------|
|            |               |         |                          | rep1  | rep2  | rep3  | rep1       | rep2    | rep3    |                 |                |                         |           | final            | RSD   |                 |               |
| 3404       |               | 6/27/95 | 50                       | 27.53 | 24.42 | 23.40 | 25.12      | 1908.26 | 2237.67 | 2365.35         | 2170.43        | 10.87                   |           | 2170.43          |       |                 |               |
| 3405       | R             | 6/27/95 | 50                       | 55.09 | 68.09 | 59.76 | 60.98      | 601.15  | 348.27  | 497.89          | 482.44         | 26.35                   |           |                  |       |                 |               |
| 3405       | R             | 7/18/95 | 50                       | 72.08 | 49.05 | 44.93 | 55.35      | 223.54  | 682.88  | 823.76          | 576.73         | 54.42                   | a         | 753.32           | 13.22 |                 | D,R           |
| 3405       | R             | 3/13/96 | 50                       | 35.71 | 30.03 | 36.32 | 34.02      | 486.2   | 680.14  | 469.6           | 545.31         | 21.47                   | a         | 477.9            | 2.46  |                 | D,R           |
| 3407       | R             | 6/22/95 | 10                       | 84.49 | 85.38 | 81.99 | 83.95      | 584.81  | 546.55  | 696.99          | 609.45         | 12.83                   | A         |                  |       |                 |               |
| 3407       | R             | 7/14/95 | 50                       | 62.89 | 50.38 | 61.63 | 58.30      | 567.34  | 863.57  | 592.87          | 674.59         | 24.33                   |           |                  |       |                 |               |
| 3407       | R             | 7/18/95 | 50                       | 53.31 | 51.43 | 51.38 | 52.04      | 473.41  | 522.31  | 523.74          | 506.49         | 5.66                    |           | 506.49           |       |                 |               |
| 3408       | R             | 6/22/95 | 10                       | 80.64 | 76.28 | 71.86 | 76.26      | 759.77  | 978.11  | 1225.04         | 987.64         | 23.57                   |           |                  |       |                 |               |
| 3408       | R             | 7/14/95 | 100                      | 32.41 | 33.83 | 31.28 | 32.51      | 798.84  | 757.81  | 833.69          | 796.78         | 4.77                    |           | 796.78           |       |                 |               |
| 3410       |               | 6/27/95 | 100                      | 75.94 | 76.20 | 77.14 | 76.43      | 118.42  | 116.74  | 110.9           | 115.35         | 3.42                    |           | 115.35           |       |                 |               |
| 3411       |               | 6/27/95 | 100                      | 37.43 | 39.78 | 38.83 | 38.68      | 603.33  | 548.14  | 569.61          | 573.69         | 4.85                    |           | 573.69           |       |                 |               |
| 3412       | R             | 6/22/95 | 50                       | 35.56 | 27.29 | 27.91 | 30.25      | 1091.6  | 1590.03 | 1542.37         | 1408           | 19.53                   |           | 1408             |       |                 | D,R           |
| 3412       | R             | 3/13/96 | 10                       | 66.06 | 67.29 | 62.36 | 65.24      | 475.06  | 441.84  | 585.85          | 500.92         | 15.05                   |           | 500.92           |       |                 |               |
| 3413       |               | 6/27/95 | 100                      | 26.53 | 27.03 | 24.04 | 25.87      | 983.17  | 959     | 1116.67         | 1019.62        | 8.33                    |           | 1019.62          |       |                 |               |
| 3414       |               | 6/27/95 | 10                       | 71.37 | 72.75 | 70.69 | 71.60      | 1494.28 | 1396.57 | 1543.96         | 1478.27        | 5.07                    |           | 1478.27          |       |                 |               |
| 3415       |               | 6/27/95 | 10                       | 66.15 | 66.26 | 68.44 | 66.95      | 1899.65 | 1889.93 | 1714.52         | 1834.7         | 5.68                    |           | 1834.7           |       |                 |               |
| 3466       |               | 6/22/95 | 10                       | 59.41 | 58.88 | 64.69 | 60.99      | 2177.14 | 2226.36 | 1726.08         | 2043.19        | 13.50                   |           | 2043.19          |       |                 |               |
| 3467       |               | 6/22/95 | 10                       | 80.11 | 75.90 | 74.44 | 76.82      | 763.9   | 985.25  | 1068.44         | 939.2          | 16.76                   |           | 939.2            |       |                 |               |
| 3468       |               | 6/22/95 | 10                       | 64.95 | 63.63 | 61.44 | 63.34      | 1674.64 | 1771.16 | 1940.62         | 1795.47        | 7.50                    |           | 1795.47          |       |                 |               |
| 3469       | R             | 6/22/95 | 100                      | 55.44 | 36.73 | 38.69 | 43.62      | 257.55  | 566.7   | 519.82          | 448.02         | 37.19                   |           |                  |       |                 |               |
| 3469       | R             | 7/14/95 | 100                      | 61.2  | 59.69 | 60.15 | 60.34      | 300.96  | 316.94  | 311.95          | 309.95         | 2.64                    |           | 309.95           |       |                 |               |
| 3493       |               | 6/22/95 | 50                       | 41.51 | 36.43 | 35.65 | 37.86      | 920.39  | 1148.53 | 1189.41         | 1086.11        | 13.35                   |           | 1086.11          |       |                 |               |

A=below effective concentrations of the assay ; B=above effective concentrations of the assay; C=average of 2 or more runs; D=Delete; R=run to run RSD of outside limit; a=outlier taken out; s=within run RSD slightly above limit; B%=B% slightly above limit; CNC=could not calculate.

Hylebos Waterway Reproductive Toxicology in Flatfish - Reproductive Steroid RIAs



Table 3. Plasma 17- $\beta$  estradiol concentrations (pg/ml Plasma).  
Hylebos Waterway Reproductive Toxicology in Flatfish.

| specimen # | re-runs | test date | sample volume ( $\mu$ l) | B%     |       |       | E2 (pg/ml) |         |         | E2 (pg/ml) avg | E2 (pg/ml) RSD | com-ment | E2 (pg/ml) assay |      |     | final comment |
|------------|---------|-----------|--------------------------|--------|-------|-------|------------|---------|---------|----------------|----------------|----------|------------------|------|-----|---------------|
|            |         |           |                          | rep1   | rep2  | rep3  | rep1       | rep2    | rep3    |                |                |          | final            | RSD  | RSD |               |
| 3494       |         | 6/22/95   | 50                       | 29.81  | 31.52 | 29.16 | 30.16      | 1565.86 | 1440.74 | 1617.4         | 1541.34        | 5.89     | 1541.34          |      |     |               |
| 3496       |         | 6/22/95   | 50                       | 15.59  | 17.58 | 17.01 | 16.73      | 3703.6  | 3192.44 | 3326.91        | 3407.65        | 7.78     |                  | B    |     |               |
| 3497       | R       | 7/10/95   | 100                      | 49.68  | 37.03 | 36.14 | 40.95      | 338.2   | 604.03  | 630.53         | 524.25         | 30.84    |                  |      |     |               |
| 3497       | R       | 7/18/95   | 50                       | 39.7   | 46.32 | 46.91 | 44.31      | 973.24  | 682.95  | 662.03         | 772.74         | 22.51    |                  |      |     |               |
| 3497       | R       | 3/13/96   | 50                       | 26.61  | 30.57 | 31.28 | 29.49      | 847.15  | 657.84  | 629.99         | 711.66         | 16.60    | 711.66           |      |     |               |
| 3498       | R       | 7/10/95   | 100                      | 19.71  | 20.19 | 16.77 | 18.89      | 1605.81 | 1552.74 | 2004.06        | 1720.87        | 14.33    |                  | B    |     |               |
| 3498       | R       | 3/13/96   | 50                       | 20.3   | 26.22 | 18.60 | 21.71      | 1629.14 | 1093.52 | 1854.62        | 1525.76        | 25.62    | 1741.88          | 9.15 | a   | a             |
| 3500       | R       | 6/22/95   | 10                       | 42.2   | 41.25 | 39.79 | 41.08      | 4470.3  | 4654.29 | 4954.07        | 4692.88        | 5.20     | 4692.88          |      |     | D,R           |
| 3500       | R       | 7/10/95   | 10                       | 86.33  | 86.77 | 93.50 | 88.87      | 423.63  | 406.01  | 168.48         | 332.71         | 42.83    |                  | A    |     | D,R           |
| 3500       | R       | 7/18/95   | 50                       | 72.38  | 75.11 | 72.12 | 73.20      | 219.71  | 187.37  | 222.97         | 210.01         | 9.37     | 210.01           |      |     | D,R           |
| 3501       | R       | 6/22/95   | 100                      | 124.73 | 60.79 | 33.28 | 72.93      | CNC     | 205.11  | 662.92         | CNC            |          |                  |      |     | D,R           |
| 3501       | R       | 7/14/95   | 100                      | 53.2   |       |       | 53.20      | 393.55  |         |                | 393.55         |          |                  |      |     | D,R           |
| 3503       | R       | 7/10/95   | 100                      | 40.7   | 52.10 | 48.08 | 46.96      | 508.03  | 303.5   | 363.38         | 391.64         | 26.85    |                  |      |     |               |
| 3503       | R       | 7/18/95   | 100                      | 41.72  | 36.29 | 14.95 | 30.99      | 477.62  | 618.63  | 2341.25        | 1145.83        | 90.56    |                  |      |     |               |
| 3503       | R       | 3/13/96   | 100                      | 26.8   | 29.36 | 29.41 | 28.52      | 418.09  | 354.56  | 353.51         | 375.39         | 9.85     | 375.39           |      |     |               |
| 3504*      | R       | 6/22/95   | 100                      | 40.58  | 40.06 | 51.12 | 43.92      | 478.9   | 489.77  | 308.16         | 425.61         | 23.93    |                  |      |     |               |
| 3504       | R       | 3/13/96   | 100                      | 24.65  | 25.75 | 23.01 | 24.47      | 484.29  | 448.8   | 544.6          | 492.56         | 9.83     | 492.56           |      |     |               |
| 3514       |         | 6/20/95   | 50                       | 81.92  | 83.38 | 81.74 | 82.34      | 123.14  | 109.94  | 124.79         | 119.29         | 6.82     |                  |      |     |               |
| 3515       |         | 6/20/95   | 10                       | 56.51  | 59.08 | 61.86 | 59.15      | 2470.6  | 2197.15 | 1930.33        | 2199.36        | 12.28    | 2199.36          |      | A   | 2199.36       |
| 3516       |         | 6/20/95   | 50                       | 39.71  | 39.40 | 40.92 | 40.01      | 1052.34 | 1067.66 | 994.99         | 1038.33        | 3.69     | 1038.33          |      |     | 1038.33       |
| 3518       |         | 6/21/95   | 10                       | 37.85  | 33.50 | 34.17 | 35.18      | 6047.86 | 7435.87 | 7197.34        | 6893.69        | 10.77    | 6893.69          |      |     | 6893.69       |
| 3520       | R       | 6/21/95   | 50                       | 86.09  | 88.11 | 86.37 | 86.86      | 96.51   | 79.29   | 94.07          | 89.96          | 10.36    |                  | A    |     |               |

A=below effective concentrations of the assay ; B=above effective concentrations of the assay; C=average of 2 or more runs; D=Delete; R=run to run RSD of outside limit; a=outlier taken out; s=within run RSD slightly above limit; B%=B% slightly above limit; CNC=could not calculate.

Hylebos Waterway Reproductive Toxicology in Flatfish - Reproductive Steroid RIAs

Table 3. Plasma 17-β estradiol concentrations(pg/ml Plasma).  
Hylebos Waterway Reproductive Toxicology in Flatfish.

| specimen # | re- runs | test date | sample volume (ul) | B %   |       |       | E2 (pg/ml) |         |         | E2 (pg/ml) avg | RSD     | E2 (pg/ml) adjusted | RSD     | comment | E2 (pg/ml) final |     | comment |
|------------|----------|-----------|--------------------|-------|-------|-------|------------|---------|---------|----------------|---------|---------------------|---------|---------|------------------|-----|---------|
|            |          |           |                    | rep1  | rep2  | rep3  | rep1       | rep2    | rep3    |                |         |                     |         |         | assay            | RSD |         |
| 3520       | R        | 7/18/95   | 100                | 86.22 | 82.60 | 85.45 | 84.76      | 41      | 56.09   | 44.05          | 47.05   | 16.96               | 33.9    | A       | 33.9             | A   |         |
| 3521       |          | 6/27/95   | 10                 | 57.11 | 57.18 | 56.93 | 57.07      | 2771.7  | 2764.78 | 2791.66        | 2776.05 | 0.50                | 2776.05 |         | 2776.05          |     |         |
| 3523       |          | 6/21/95   | 50                 | 48.43 | 48.60 | 43.98 | 47.00      | 754.34  | 748.62  | 917.03         | 806.66  | 11.85               | 806.66  |         | 806.66           |     |         |
| 3524       |          | 7/14/95   | 100                | 41.05 | 37.10 | 40.70 | 39.62      | 435.49  | 517.01  | 442.22         | 464.91  | 9.73                | 464.91  |         | 464.91           |     |         |
| 3525       |          | 7/10/95   | 100                | 62.68 | 61.42 | 61.55 | 61.89      | 299.54  | 316.35  | 314.55         | 310.15  | 2.97                | 310.15  |         | 310.15           |     |         |
| 3526       | R        | 6/20/95   | 100                | 86.66 | 84.36 | 85.73 | 85.59      | 41.21   | 50.69   | 44.97          | 45.62   | 10.45               |         | A       |                  |     |         |
| 3526       | R        | 3/13/96   | 100                | 63.05 | 58.09 | 56.74 | 59.29      | 83.7    | 107.29  | 114.62         | 101.87  | 15.86               | 101.87  |         | 101.87           |     |         |
| 3527       |          | 7/14/95   | 10                 | 31.38 | 36.40 | 36.93 | 34.90      | 6724.03 | 5334.38 | 5209.02        | 5755.81 | 14.61               | 5755.81 |         | 5755.81          |     |         |
| 3529       | R        | 7/6/95    | 50                 | 24.5  | 41.58 | 81.34 | 49.14      | 1856.43 | 878.21  | 156.19         | 963.61  | 88.56               |         |         |                  |     |         |
| 3529       | R        | 7/14/95   | 100                | 22.59 | 24.78 |       | 23.69      | 1200.34 | 1086.51 |                | 1143.42 | 7.04                | 1143.42 | a       | 1143.42          | a   |         |
| 3529       | R        | 3/13/96   | 100                | 12.92 | 11.24 | 10.99 | 11.71      | 1355.57 | 1666.75 | 1722.62        | 1581.65 | 12.50               |         | B       |                  |     |         |
| 3530       |          | 7/6/95    | 100                | 45.83 | 49.08 | 55.05 | 49.99      | 372.37  | 328.94  | 261.78         | 321.03  | 17.36               | 321.03  |         | 321.03           |     |         |
| 3531       |          | 7/6/95    | 100                | 19.63 | 21.78 | 19.71 | 20.37      | 1216.35 | 1073.76 | 1210.9         | 1167    | 6.92                | 1167    |         | 1167             |     |         |
| 3532       | R        | 7/6/95    | 100                | 71.71 | 77.15 | 66.87 | 71.91      | 130.88  | 99.63   | 162.63         | 131.04  | 24.04               | 146.75  | a       | 146.75           | a   |         |
| 3532       | R        | 3/13/96   | 100                | 68.16 | 50.03 | 81.68 | 66.62      | 63.8    | 158.36  | 26.53          | 82.9    | 81.98               |         |         |                  |     |         |
| 3533       |          | 7/6/95    | 10                 | 55.65 | 53.24 | 54.09 | 54.33      | 2557.68 | 2806.72 | 2716.41        | 2693.6  | 4.68                | 2693.6  |         | 2693.6           |     |         |
| 3535       |          | 7/6/95    | 100                | 80.5  | 76.68 | 73.79 | 76.99      | 82.25   | 102.16  | 118.42         | 100.94  | 17.95               | 100.94  |         | 100.94           |     |         |
| 3536       | R        | 7/6/95    | 100                | 22.1  | 31.38 | 18.28 | 23.92      | 1054.91 | 669.34  | 1323.01        | 1015.75 | 32.35               |         |         |                  |     |         |
| 3536       | R        | 7/18/95   | 50                 | 35.48 | 32.70 | 33.57 | 33.92      | 1231.94 | 1449.4  | 1376.32        | 1352.55 | 8.18                | 1352.55 |         | 1352.55          |     |         |
| 3537       |          | 6/21/95   | 10                 | 49.35 | 48.04 | 49.40 | 48.93      | 3622.1  | 3835.4  | 3614.4         | 3690.63 | 3.40                | 3690.63 |         | 3690.63          |     |         |
| 3538       | R        | 7/6/95    | 50                 | 17.64 | 17.80 | 18.62 | 18.02      | 2758.47 | 2729.31 | 2588.76        | 2692.18 | 3.37                |         | B       |                  |     |         |
| 3538       | R        | 3/13/96   | 10                 | 44.9  | 45.37 | 28.56 | 39.61      | 2026.01 | 1980.65 | 4748.54        | 2918.4  | 54.31               | 2003.33 | a       | 2003.33          | a   |         |

A=below effective concentrations of the assay ; B=above effective concentrations of the assay; C=average of 2 or more runs; D=Delete; R=run to run RSD of outside limit; a=outlier taken out; s=within run RSD slightly above limit; B%=B% slightly above limit; CNC=could not calculate.

Hylebos Waterway Reproductive Toxicology in Flatfish - Reproductive Steroid RIAs

Table 3. Plasma 17- $\beta$  estradiol concentrations(pg/ml Plasma).  
Hylebos Waterway Reproductive Toxicology in Flatfish.

| specimen # | re-runs | test date | sample volume ( $\mu$ l) | B %   |       |       | E2 (pg/ml) |         |         | E2 (pg/ml) avg | E2 (pg/ml) RSD | comment | E2 (pg/ml) assay |      |     | final comment |      |   |
|------------|---------|-----------|--------------------------|-------|-------|-------|------------|---------|---------|----------------|----------------|---------|------------------|------|-----|---------------|------|---|
|            |         |           |                          | rep1  | rep2  | rep3  | rep1       | rep2    | rep3    |                |                |         | final            | RSD  | RSD |               |      |   |
| 3539       |         | 6/20/95   | 50                       | 72.87 | 78.80 | 74.49 | 75.39      | 220.3   | 153.51  | 200.7          | 191.5          | 17.93   | 191.5            |      |     |               |      |   |
| 3540       | R       | 7/6/95    | 10                       | 45.41 | 48.69 | 36.05 | 43.39      | 3784.29 | 3338.18 | 5482.79        | 4201.75        | 26.93   |                  |      |     |               |      |   |
| 3540       | R       | 7/18/95   | 10                       | 47.52 | 49.11 | 44.94 | 47.19      | 3659.19 | 3405.33 | 4116.32        | 3726.95        | 9.67    | 3726.95          |      |     |               |      |   |
| 3541       |         | 7/6/95    | 100                      | 53.74 | 57.00 | 62.07 | 57.61      | 275.26  | 242.76  | 198.63         | 238.88         | 16.10   | 238.88           |      |     |               |      |   |
| 3542       |         | 6/21/95   | 100                      | 69.56 | 71.33 | 68.18 | 69.69      | 142.99  | 130.32  | 153.4          | 142.24         | 8.12    | 142.24           |      |     |               |      |   |
| 3543       |         | 7/10/95   | 100                      | 74.04 | 73.56 | 74.97 | 74.19      | 174.48  | 178.96  | 165.95         | 173.13         | 3.81    | 173.13           |      |     |               |      |   |
| 3544       |         | 7/14/95   | 100                      | 27.35 | 26.16 | 26.29 | 26.60      | 821.9   | 875.16  | 868.86         | 855.31         | 3.40    | 855.31           |      |     |               |      |   |
| 3546       |         | 6/20/95   | 10                       | 49.19 | 46.79 | 42.09 | 46.02      | 3428.39 | 3814.28 | 4714.62        | 3985.77        | 16.56   | 3985.77          |      |     |               |      |   |
| 3548       |         | 7/10/95   | 50                       | 29.01 | 29.73 | 34.24 | 30.99      | 1816.16 | 1746.43 | 1384.7         | 1649.1         | 14.04   | 1649.1           |      |     |               |      |   |
| 3550       | R       | 7/10/95   | 50                       | 33.66 | 23.77 | 20.46 | 25.96      | 1425.41 | 2458.74 | 3049.11        | 2311.09        | 35.56   | 2753.93          | 7.58 | a   | 2753.93       | 7.60 | a |
| 3550       | R       | 7/18/95   | 50                       | 16.86 | 15.42 | 17.65 | 16.64      | 3984.4  | 4493.07 | 3740.63        | 4072.7         | 9.43    |                  |      | B   |               |      |   |
| 3550       | R       | 3/13/96   | 50                       | 9.37  | 33.31 | 12.83 | 18.50      | 4792.47 | 727.61  | 3138.46        | 2886.18        | 70.83   |                  |      | B   |               |      |   |
| 3552       |         | 6/20/95   | 10                       | 63.03 | 60.30 | 61.75 | 61.69      | 1826.52 | 2076.67 | 1940.96        | 1948.05        | 6.43    | 1948.05          |      |     |               |      |   |
| 3554       | R       | 6/20/95   | 50                       | 85.76 | 82.90 | 86.84 | 85.17      | 89.68   | 114.23  | 81.01          | 94.97          | 18.14   |                  |      | A   |               |      |   |
| 3554       | R       | 3/13/96   | 50                       | 57.29 | 69.78 | 63.18 | 63.42      | 223.17  | 116.52  | 166.22         | 168.64         | 31.65   |                  |      |     |               |      |   |
| 3558       |         | 7/10/95   | 10                       | 55.45 | 49.79 | 52.79 | 52.68      | 2609.09 | 3365.8  | 2942.76        | 2972.55        | 12.76   | 2972.55          |      |     |               |      |   |
| 3560       |         | 7/6/95    | 50                       | 27.27 | 25.74 | 22.58 | 25.20      | 1617.61 | 1742.84 | 2054.81        | 1805.09        | 12.47   | 1805.09          |      |     |               |      |   |
| 3562       |         | 7/6/95    | 100                      | 71    | 65.10 | 66.18 | 67.43      | 135.33  | 175.3   | 167.51         | 159.38         | 13.30   | 159.38           |      |     |               |      |   |
| 3564       | R       | 7/6/95    | 50                       | 16.94 | 17.73 | 22.16 | 18.94      | 2889.76 | 2741.4  | 2102.71        | 2577.96        | 16.22   |                  |      | B   |               |      |   |
| 3564       | R       | 3/13/96   | 10                       | 37.05 | 59.77 | 38.05 | 44.96      | 2990.61 | 987.41  | 2841.58        | 2273.2         | 49.09   | 2916.1           | 3.60 | a   | 2916.1        | 3.60 | a |
| 3565       |         | 7/6/95    | 10                       | 38.41 | 39.66 | 37.47 | 38.51      | 4980.26 | 4737.82 | 5172.26        | 4963.45        | 4.39    | 4963.45          |      |     |               |      |   |
| 3566       | R       | 7/6/95    | 50                       | 24.45 | 23.34 | 43.08 | 30.29      | 1860.98 | 1972.53 | 828.15         | 1553.88        | 40.61   |                  |      |     |               |      |   |

A=below effective concentrations of the assay ; B=above effective concentrations of the assay; C=average of 2 or more runs; D=Delete; R=run to run RSD of outside limit; a=outlier taken out; s=within run RSD slightly above limit; B%=B% slightly above limit; CNC=could not calculate.

Hylebos Waterway Reproductive Toxicology in Flatfish - Reproductive Steroid RIAs

Table 3. Plasma 17- $\beta$  estradiol concentrations (pg/ml Plasma).  
Hylebos Waterway Reproductive Toxicology in Flatfish.

| specimen # | re-runs | test date | sample volume ( $\mu$ l) | B %   |        |       | E2 (pg/ml) |         |         | E2 avg  | E2 (pg/ml) rep3 | E2 (pg/ml) rep2 | E2 (pg/ml) rep1 | E2 avg | RSD     | E2 adjusted (pg/ml) | RSD   | comment | E2 (pg/ml) final | Intra assay RSD | Inter assay RSD | final comment |
|------------|---------|-----------|--------------------------|-------|--------|-------|------------|---------|---------|---------|-----------------|-----------------|-----------------|--------|---------|---------------------|-------|---------|------------------|-----------------|-----------------|---------------|
|            |         |           |                          | rep1  | rep2   | rep3  | rep1       | rep2    | rep3    |         |                 |                 |                 |        |         |                     |       |         |                  |                 |                 |               |
| 3566       | R       | 7/18/95   | 10                       | 72.8  | 70.19  | 70.47 | 71.15      | 1072.74 | 1240.24 | 1221.93 | 1178.3          | 7.80            | 1178.3          | 7.80   | 1178.3  |                     |       | 1178.3  |                  |                 |                 |               |
| 3567       | R       | 7/6/95    | 10                       | 45.79 | 31.28  | 31.26 | 36.11      | 3729.55 | 6723.6  | 6727.64 | 5726.93         | 30.20           |                 |        |         |                     |       |         |                  |                 |                 |               |
| 3567       | R       | 7/18/95   | 10                       | 46.87 | 46.33  | 47.12 | 46.77      | 3317.22 | 3413.71 | 3275.04 | 3335.33         | 2.13            | 3335.33         | 2.13   | 3335.33 |                     |       | 3086.28 | 11.00            |                 |                 | C             |
| 3567       | R       | 3/13/96   | 10                       | 36.6  | 37.95  | 39.84 | 38.13      | 3059.73 | 2855.61 | 2596.35 | 2837.23         | 8.19            | 2837.23         | 8.19   | 2837.23 |                     |       |         |                  |                 |                 |               |
| 3568       | R       | 7/10/95   | 50                       | 30.23 | 33.48  | 31.86 | CNC        | CNC     | 1701.19 | 1438.11 | 1569.65         | 11.85           | 1569.65         | 11.85  | 1569.65 |                     |       | 1569.65 | 11.85            |                 |                 | a             |
| 3568       | R       | 7/18/95   | 100                      | 15.1  | 16.42  | 17.09 | 16.20      | 2311.64 | 2064.58 | 1954.96 | 2110.39         | 8.66            |                 |        |         |                     |       |         |                  |                 |                 |               |
| 3568       | R       | 3/13/96   | 50                       | 17.64 | 19.34  | 18.97 | 18.65      | 2004.15 | 1750.38 | 1801.35 | 1851.96         | 7.25            |                 |        |         |                     |       |         |                  |                 |                 |               |
| 3569       | R       | 7/10/95   | 10                       | 47.52 | 49.57  | 50.19 | 49.09      | 3725.4  | 3399.24 | 3306.42 | 3477.02         | 6.33            | 3477.02         | 6.33   | 3477.02 |                     |       | 3477.02 |                  |                 |                 |               |
| 3572       | R       | 6/20/95   | 100                      | 92.41 | 100.64 | 95.18 | 96.08      | 20.49   | CNC     | 11.95   | CNC             |                 |                 |        |         |                     |       |         |                  |                 |                 |               |
| 3572       | R       | 3/13/96   | 100                      | 90.74 | 83.15  | 82.22 | 85.37      | 10.34   | 23.5    | 25.39   | 19.74           | 41.54           | 15.05           |        |         |                     | 15.05 |         |                  |                 |                 | A             |
| 3574       | R       | 6/20/95   | 10                       | 50.61 | 48.11  | 48.58 | 49.10      | 3217.11 | 3597.28 | 3522.14 | 3445.51         | 5.84            | 3445.51         | 5.84   | 3445.51 |                     |       | 3445.51 |                  |                 |                 |               |
| 3575       | R       | 6/27/95   | 10                       | 46.9  | 44.31  | 47.61 | 46.27      | 4154.18 | 4604.86 | 4038.55 | 4265.86         | 7.01            | 4265.86         | 7.01   | 4265.86 |                     |       | 4265.86 |                  |                 |                 |               |
| 3576       | R       | 6/20/95   | 10                       | 53.44 | 57.01  | 54.88 | 55.11      | 2836.05 | 2415.54 | 2659.17 | 2636.92         | 8.01            | 2636.92         | 8.01   | 2636.92 |                     |       | 2636.92 |                  |                 |                 |               |
| 3577       | R       | 6/27/95   | 10                       | 40.12 | 34.71  | 34.56 | 36.46      | 5454.96 | 6851.58 | 6895.7  | 6400.75         | 12.80           | 6400.75         | 12.80  | 6400.75 |                     |       | 6400.75 |                  |                 |                 |               |
| 3578       | R       | 6/27/95   | 10                       | 82.81 | 90.97  | 88.68 | 87.49      | 780.73  | 377.17  | 483.28  | 547.06          | 38.24           |                 |        |         |                     |       |         |                  |                 |                 | D,R           |
| 3578       | R       | 7/18/95   | 50                       | 80.83 | 82.68  | 79.62 | 81.05      | 128.22  | 111.44  | 139.82  | 126.49          | 11.28           |                 |        |         |                     |       |         |                  |                 |                 | D,R           |
| 3581       | R       | 6/20/95   | 10                       | 45.57 | 44.94  | 47.70 | 46.07      | 4029.71 | 4143.74 | 3662.64 | 3945.36         | 6.37            | 3945.36         | 6.37   | 3945.36 |                     |       | 3945.36 |                  |                 |                 |               |
| 3586       | R       | 6/20/95   | 10                       | 77.75 | 75.03  | 72.32 | 75.03      | 822.06  | 972.28  | 1135.98 | 976.77          | 16.07           | 976.77          | 16.07  | 976.77  |                     |       | 976.77  |                  |                 |                 |               |
| 3587       | R       | 6/20/95   | 50                       | 91.33 | 94.63  | 93.82 | 93.26      | 48.15   | 27.15   | 32.04   | 35.78           | 30.71           |                 |        |         |                     |       |         |                  |                 |                 |               |
| 3587       | R       | 3/13/96   | 100                      | 27.34 | 32.27  | 73.14 | 44.25      | 510.55  | 384.98  | 47.83   | 314.45          | 76.10           | 447.77          | 19.80  | 447.77  |                     |       | 447.77  | 19.80            |                 |                 | a             |
| 3588       | R       | 6/27/95   | 10                       | 75.17 | 77.73  | 70.96 | 74.62      | 1233.73 | 1072.71 | 1524.09 | 1276.84         | 17.92           | 1276.84         | 17.92  | 1276.84 |                     |       | 1276.84 |                  |                 |                 |               |
| 3589       | R       | 6/27/95   | 50                       | 76.07 | 79.83  | 74.71 | 76.87      | 244.59  | 189.51  | 252.85  | 228.99          | 15.04           | 228.99          | 15.04  | 228.99  |                     |       | 228.99  |                  |                 |                 |               |

A=below effective concentrations of the assay ; B=above effective concentrations of the assay; C=average of 2 or more runs; D=Delete; R=run to run RSD of outside limit; a=outlier taken out; s=within run RSD slightly above limit; B%=B% slightly above limit; CNC=could not calculate.

Hylebos Waterway Reproductive Toxicology in Flatfish - Reproductive Steroid RIAs

Table 3. Plasma 17- $\beta$  estradiol concentrations (pg/ml Plasma).  
Hylebos Waterway Reproductive Toxicology in Flatfish.

| specimen # | re- runs | test date | sample volume ( $\mu$ l) | B%     |       |        | E2 (pg/ml) |          |          | E2 (pg/ml) rep3 | E2 (pg/ml) avg | RSD adjusted | RSD   | comment | E2 (pg/ml) assay |       | inter assay RSD | final comment |
|------------|----------|-----------|--------------------------|--------|-------|--------|------------|----------|----------|-----------------|----------------|--------------|-------|---------|------------------|-------|-----------------|---------------|
|            |          |           |                          | rep1   | rep2  | rep3   | rep1       | rep2     | rep3     |                 |                |              |       |         | final            | RSD   |                 |               |
| 3590       |          | 6/20/95   | 10                       | 51.61  | 52.92 | 53.17  | 52.57      | 3077.79  | 2902.78  | 2870.73         | 2950.43        | 3.78         |       |         | 2950.43          |       |                 |               |
| 3591       | R        | 6/27/95   | 50                       | 18.08  | 17.22 | 17.93  | 17.74      | 3257.71  | 3453.66  | 3291.64         | 3334.34        | 3.14         |       | B       |                  |       |                 |               |
| 3591       | R        | 3/13/96   | 10                       | 36.29  | 33.07 | 40.67  | 36.68      | 3108.64  | 3685.92  | 2492.06         | 3095.54        | 19.29        |       |         | 3095.54          |       |                 |               |
| 3592       |          | 6/20/95   | 10                       | 57.03  | 52.84 | 52.22  | 54.03      | 2412.99  | 2913.88  | 2995.79         | 2774.22        | 11.37        |       |         | 2774.22          |       |                 |               |
| 3594       | R        | 6/27/95   | 50                       | 97.53  | 94.60 | 105.68 | 99.27      | 19.63    | 43.77    | CNC             | 21.13          | 53.85        |       | A       |                  |       |                 |               |
| 3594       | R        | 3/13/96   | 100                      | 72.01  | 64.14 | 60.47  | 65.54      | 33.04    | 53.01    | 65.01           | 50.35          | 32.07        | 14.37 | a       | 59               | 14.37 |                 | a             |
| 3595       |          | 6/21/95   | 10                       | 20.53  | 22.14 | 23.30  | 21.99      | 15408.44 | 13876.74 | 12908.46        | 14064.55       | 8.96         |       |         | 14064.55         |       |                 |               |
| 3596       |          | 6/20/95   | 10                       | 58.34  | 53.42 | 57.61  | 56.45      | 2273.65  | 2839.01  | 2350.73         | 2487.8         | 12.32        |       |         | 2487.8           |       |                 |               |
| 3598       |          | 6/21/95   | 100                      | 92.65  | 90.15 | 88.90  | 90.57      | 22.21    | 31.5     | 36.43           | 30.05          | 24.03        |       | A       | 35.2             |       |                 | A             |
| 3600       |          | 7/6/95    | 100                      | 90.4   | 88.35 | 72.32  | 83.69      | 37.48    | 46.07    | 127.16          | 70.24          | 70.45        |       | A       | 43.85            |       |                 | A             |
| 3602       |          | 6/21/95   | 10                       | 60.99  | 60.47 | 57.54  | 59.67      | 2162.97  | 2215.32  | 2528.09         | 2302.13        | 8.58         |       |         | 2302.13          |       |                 |               |
| 3603       |          | 7/10/95   | 100                      | 92.42  | 96.22 | 98.14  | 95.59      | 20.27    | 8.89     | 3.94            | 11.03          | 75.90        |       | A       | 35.15            |       |                 | A             |
| 3604       | R        | 6/21/95   | 10                       | 88.42  | 94.63 | 91.47  | 91.51      | 383.83   | 154.1    | 284.89          | 267.61         | 42.93        |       | A       |                  |       |                 |               |
| 3604       | R        | 7/18/95   | 50                       | 64.03  | 82.64 | 82.44  | 76.37      | 340.46   | 111.84   | 113.57          | 188.62         | 69.71        |       |         |                  |       |                 |               |
| 3604       | R        | 3/13/96   | 50                       | 72.5   | 60.89 | 73.22  | 68.87      | 64       | 127.03   | 61.05           | 84.02          | 44.36        | 3.26  | a       | 62.53            | 3.26  |                 | a             |
| 3605       |          | 7/10/95   | 100                      | 101.36 | 95.89 | 90.61  | 95.95      | CNC      | 9.82     | 26.37           | CNC            |              |       | A       | 35.15            |       |                 | A             |
| 3607       | R        | 6/21/95   | 10                       | 19.96  | 19.89 | 17.17  | 19.01      | 16007.57 | 16084.03 | 19590.79        | 17227.46       | 11.88        |       | B       |                  |       |                 |               |
| 3607       | R        | 3/13/96   | 5                        | 34.87  | 20.65 | 32.38  | 29.30      | 6996.31  | 15871.37 | 7652.13         | 10073.27       | 50.07        | 9.42  | a       | 7174.22          | 9.42  |                 | a             |
| 3615       |          | 7/10/95   | 10                       | 27.88  | 28.09 | 29.22  | 28.40      | 9659.42  | 9548.37  | 8978.84         | 9395.54        | 3.89         |       |         | 9395.54          |       |                 |               |
| 3616       |          | 7/10/95   | 10                       | 27.04  | 25.46 | 28.15  | 26.88      | 10129.43 | 11101.06 | 9518.39         | 10249.63       | 7.79         |       |         | 10249.63         |       |                 |               |
| 3617       |          | 7/10/95   | 10                       | 39.98  | 38.34 | 37.06  | 38.46      | 5254.68  | 5675.75  | 6033.03         | 5654.48        | 6.89         |       |         | 5654.48          |       |                 |               |
| 3618       |          | 6/20/95   | 100                      | 69.37  | 73.08 | 65.90  | 69.45      | 133.13   | 108.85   | 158.85          | 133.61         | 18.71        |       |         | 133.61           |       |                 |               |

A=below effective concentrations of the assay ; B=above effective concentrations of the assay; C=average of 2 or more runs; D=Delete; R=run to run RSD of outside limit; a=outlier taken out; s=within run RSD slightly above limit; B%=B% slightly above limit; CNC=could not calculate.

Hylebos Waterway Reproductive Toxicology in Flatfish - Reproductive Steroid RIAs



Table 3. Plasma 17- $\beta$  estradiol concentrations(pg/ml Plasma).  
Hylebos Waterway Reproductive Toxicology in Flatfish.

| specimen # | re- runs | test date | sample volume ( $\mu$ l) | B%    |       |       | E2 (pg/ml) |          |          | E2 avg   | RSD      | E2 (pg/ml) adjusted | RSD | com- ment | E2 (pg/ml) final assay |     |         | inter assay RSD | final comment |
|------------|----------|-----------|--------------------------|-------|-------|-------|------------|----------|----------|----------|----------|---------------------|-----|-----------|------------------------|-----|---------|-----------------|---------------|
|            |          |           |                          | rep1  | rep2  | rep3  | rep1       | rep2     | rep3     |          |          |                     |     |           | final                  | RSD | comment |                 |               |
| 3628       | R        | 6/20/95   | 10                       | 8.38  | 9.14  | 8.13  | 8.55       | 47349.4  | 42540.31 | 49111.08 | 46333.6  | 7.34                | B   |           |                        |     |         |                 |               |
| 3628       | R        | 3/13/96   | 5                        | 27.12 | 21.93 | 24.04 | 24.36      | 10351.42 | 14488.63 | 12563.15 | 12467.73 | 16.60               |     | 12467.73  |                        |     |         |                 | 12467.73      |
| 3629       |          | 6/20/95   | 10                       | 58.85 | 63.86 | 59.76 | 60.82      | 2220.82  | 1755.12  | 2129.7   | 2035.21  | 12.13               |     | 2035.21   |                        |     |         |                 | 2035.21       |
| 3630       |          | 6/21/95   | 10                       | 20.23 | 20.41 | 20.92 | 20.52      | 15714.69 | 15531.28 | 15007.3  | 15417.76 | 2.38                |     | 15417.76  |                        |     |         |                 | 15417.76      |
| 3631       | R        | 6/27/95   | 100                      | 64.59 | 45.33 | 43.54 | 51.16      | 205.22   | 439.87   | 471.83   | 372.3    | 39.10               |     |           |                        |     |         |                 |               |
| 3631       | R        | 7/18/95   | 100                      | 46.96 | 47.10 | 45.47 | 46.51      | 375.48   | 373.04   | 401.81   | 383.44   | 4.16                |     | 383.44    |                        |     |         |                 | 383.44        |
| 3632       |          | 6/22/95   | 50                       | 72.58 | 75.84 | 79.32 | 75.91      | 235.94   | 197.77   | 160.75   | 198.15   | 18.97               |     | 198.15    |                        |     |         |                 | 198.15        |
| 3633       |          | 6/21/95   | 50                       | 48.95 | 44.08 | 47.33 | 46.78      | 737.32   | 913.08   | 791.54   | 813.98   | 11.06               |     | 813.98    |                        |     |         |                 | 813.98        |
| 3634       | R        | 6/27/95   | 100                      | 71.17 | 79.39 | 77.33 | 75.96      | 153.17   | 99.61    | 112.05   | 121.61   | 23.19               |     | 116.83    | 5.78                   | a   |         |                 | D,R           |
| 3634       | R        | 3/13/96   | 100                      | 68.74 | 78.96 | 75.36 | 74.35      | 61.77    | 32.59    | 41.64    | 45.33    | 32.96               |     | 36.17     | 8.70                   | a   |         |                 | D,R           |
| 3635       |          | 6/27/95   | 100                      | 88.31 | 82.32 | 87.13 | 85.92      | 51.93    | 82.9     | 57.74    | 64.19    | 25.64               |     | 47.8      |                        | A   |         |                 | A             |
| 3636       |          | 6/21/95   | 10                       | 32.9  | 32.97 | 31.17 | 32.35      | 7658.37  | 7632.05  | 8354.24  | 7881.55  | 5.20                |     | 7881.55   |                        |     |         |                 | 7881.55       |
| 3637       |          | 6/22/95   | 100                      | 87.63 | 85.03 | 86.76 | 86.48      | 42.59    | 53.54    | 46.16    | 47.43    | 11.77               |     | 38.35     |                        | A   |         |                 | A             |
| 3639       |          | 6/27/95   | 100                      | 93.12 | 91.02 | 87.74 | 90.63      | 29.54    | 39.09    | 54.7     | 41.11    | 30.89               |     | 47.8      |                        | A   |         |                 | A             |
| 3644       |          | 6/27/95   | 100                      | 89.4  | 94.13 | 91.59 | 91.71      | 46.69    | 25.05    | 36.46    | 36.07    | 30.01               |     | 47.8      |                        | A   |         |                 | A             |
| 3645       | R        | 6/20/95   | 10                       | 27.45 | 24.54 | 24.61 | 25.53      | 9747.85  | 11537.89 | 11489.58 | 10925.1  | 9.33                |     | 10925.1   |                        |     |         |                 | D,R           |
| 3645       | R        | 3/13/96   | 5                        | 36.94 | 33.53 | 32.98 | 34.49      | 5972.71  | 7191.23  | 7407.35  | 6870.71  | 10.92               |     | 6870.71   |                        |     |         |                 | D,R           |
| 3647       |          | 6/20/95   | 10                       | 42.44 | 43.33 | 48.07 | 44.62      | 4640.08  | 4457.15  | 3602.26  | 4233.17  | 13.09               |     | 4233.17   |                        |     |         |                 | 4233.17       |
| 3648       |          | 7/14/95   | 100                      | 47.36 | 51.20 | 49.66 | 49.41      | 334.37   | 285.32   | 304.02   | 307.9    | 8.04                |     | 307.9     |                        |     |         |                 | 307.9         |
| 3649       |          | 6/20/95   | 100                      | 89.68 | 88.34 | 93.82 | 90.61      | 29.84    | 34.74    | 32.04    | 32.2     | 7.62                |     | 35.2      |                        | A   |         |                 | A             |
| 3650       |          | 6/22/95   | 10                       | 36.4  | 37.77 | 36.26 | 36.81      | 5750.91  | 5411.03  | 5786.14  | 5649.36  | 3.67                |     | 5649.36   |                        |     |         |                 | 5649.36       |
| 3651       | R        | 7/10/95   | 100                      | 76.16 | 73.74 | 62.81 | 70.91      | 155.33   | 177.26   | 297.82   | 210.14   | 36.51               |     |           |                        |     |         |                 |               |

A=below effective concentrations of the assay ; B=above effective concentrations of the assay; C=average of 2 or more runs; D=Delete; R=run to run RSD of outside limit; a=outlier taken out; s=within run RSD slightly above limit; B%=B% slightly above limit; CNC=could not calculate.

**Hylebos Waterway Reproductive Toxicology in Flatfish - Reproductive Steroid RIAs**

Table 3. Plasma 17- $\beta$  estradiol concentrations (pg/ml Plasma).  
Hylebos Waterway Reproductive Toxicology in Flatfish.

| specimen # | re-runs | test date | sample volume ( $\mu$ l) | B%    |       |       | E2 (pg/ml) |          |          | E2 (pg/ml) avg | RSD      | E2 (pg/ml) adjusted | RSD      | comment | E2 (pg/ml) intra assay |     |          | final comment |
|------------|---------|-----------|--------------------------|-------|-------|-------|------------|----------|----------|----------------|----------|---------------------|----------|---------|------------------------|-----|----------|---------------|
|            |         |           |                          | rep1  | rep2  | rep3  | rep1       | rep2     | rep3     |                |          |                     |          |         | RSD                    | RSD | RSD      |               |
| 3651       | R       | 7/18/95   | 100                      | 74.92 | 75.07 | 74.92 | 74.97      | 94.76    | 93.91    | 94.76          | 94.48    | 0.52                | 94.48    |         |                        |     | 94.48    |               |
| 3652       |         | 6/22/95   | 10                       | 49.6  | 46.72 | 43.20 | 46.51      | 3282.54  | 3698.12  | 4284.45        | 3755.04  | 13.41               | 3755.04  | A       |                        |     | 3755.04  |               |
| 3653       |         | 6/27/95   | 10                       | 22.92 | 23.01 | 22.99 | 22.97      | 12146.13 | 12083.29 | 12098.95       | 12109.46 | 0.27                | 12109.46 |         |                        |     | 12109.46 |               |
| 3654       |         | 6/28/95   | 50                       | 32.59 | 30.79 | 30.54 | 31.31      | 1580.86  | 1723.43  | 1745.13        | 1683.14  | 5.30                | 1683.14  |         |                        |     | 1683.14  |               |
| 3655       |         | 6/27/95   | 10                       | 25.88 | 25.88 | 27.20 | 26.32      | 10365.5  | 10369.64 | 9696.74        | 10143.96 | 3.82                | 10143.96 |         |                        |     | 10143.96 |               |
| 3656       |         | 6/28/95   | 10                       | 26.21 | 24.26 | 23.98 | 24.82      | 10886.06 | 12122.16 | 12313.05       | 11773.76 | 6.58                | 11773.76 |         |                        |     | 11773.76 |               |
| 3657       | R       | 6/28/95   | 10                       | 95.46 | 94.69 | 95.30 | 95.15      | 157.07   | 186.6    | 163.29         | 168.98   | 9.21                |          | A       |                        |     |          |               |
| 3657       | R       | 7/14/95   | 100                      | 67.79 | 68.83 | 65.90 | 67.51      | 237.5    | 228.39   | 254.75         | 240.22   | 5.57                | 240.22   |         |                        |     | 240.22   |               |
| 3658       |         | 6/27/95   | 10                       | 23.13 | 24.43 | 21.32 | 22.96      | 12005.43 | 11183.7  | 13309.78       | 12166.3  | 8.81                | 12166.3  |         |                        |     | 12166.3  |               |
| 3659       |         | 6/28/95   | 10                       | 29.4  | 30.36 | 29.84 | 29.87      | 9229.09  | 8799.95  | 9028.16        | 9019.07  | 2.38                | 9019.07  |         |                        |     | 9019.07  |               |
| 3660       |         | 6/27/95   | 10                       | 61.27 | 64.97 | 58.98 | 61.74      | 2338.8   | 1999.34  | 2570.45        | 2302.86  | 12.47               | 2302.86  |         |                        |     | 2302.86  |               |
| 3661       | R       | 6/21/95   | 50                       | 80.87 | 88.75 | 83.38 | 84.33      | 146.28   | 74.1     | 121.3          | 113.9    | 32.19               |          | A       |                        |     |          |               |
| 3661       | R       | 7/18/95   | 100                      | 68.98 | 69.74 | 69.89 | 69.54      | 132.34   | 127.07   | 126.05         | 128.49   | 2.63                | 128.49   |         |                        |     | 128.49   |               |
| 3662       |         | 6/22/95   | 10                       | 51.17 | 45.08 | 44.79 | 47.02      | 3075.44  | 3959.46  | 4007.63        | 3680.85  | 14.26               | 3680.85  |         |                        |     | 3680.85  |               |
| 3663       |         | 6/22/95   | 10                       | 52.06 | 54.47 | 52.96 | 53.16      | 2963.91  | 2681.37  | 2856.26        | 2833.84  | 5.03                | 2833.84  |         |                        |     | 2833.84  |               |
| 3664       |         | 6/21/95   | 10                       | 46.25 | 40.44 | 40.80 | 42.50      | 4148.32  | 5370.58  | 5284.92        | 4934.61  | 13.83               | 4934.61  |         |                        |     | 4934.61  |               |
| 3666       |         | 6/22/95   | 100                      | 76.18 | 79.11 | 76.28 | 77.19      | 97.01    | 81.44    | 96.43          | 91.63    | 9.63                | 91.63    |         |                        |     | 91.63    |               |
| 3667       | R       | 6/21/95   | 50                       | 19.15 | 19.96 | 16.96 | 18.69      | 3386.15  | 3201.51  | 3981.83        | 3523.16  | 11.57               |          | B       |                        |     |          |               |
| 3669       |         | 7/10/95   | 10                       | 35.56 | 37.71 | 37.00 | 36.76      | 9330.08  | 8491.09  | 8755.34        | 8858.84  | 4.84                | 8858.84  |         |                        |     | 8858.84  |               |
| 3670       |         | 6/21/95   | 10                       | 25.18 | 25.74 | 25.44 | 25.45      | 11547.44 | 11178.71 | 11376.85       | 11367.67 | 1.62                | 11367.67 |         |                        |     | 11367.67 |               |
| 3671       |         | 6/21/95   | 50                       | 82.82 | 80.46 | 84.69 | 82.66      | 126.74   | 150.48   | 109.1          | 128.77   | 16.12               |          | A       |                        |     |          |               |
| 3672       | R       | 6/21/95   | 100                      | 61.77 | 70.90 | 64.01 | 65.56      | 208.66   | 133.39   | 188            | 176.69   | 22.01               |          |         |                        |     |          | D             |

A=below effective concentrations of the assay ; B=above effective concentrations of the assay; C=average of 2 or more runs; D=Delete; R=run to run RSD of outside limit; a=outlier taken out; s=within run RSD slightly above limit; B%=B% slightly above limit; CNC=could not calculate.

Hylebos Waterway Reproductive Toxicology in Flatfish - Reproductive Steroid RIAs

Table 3. Plasma 17- $\beta$  estradiol concentrations(pg/ml Plasma).  
Hylebos Waterway Reproductive Toxicology in Flatfish.

| specimen # | re- runs | test date | sample volume ( $\mu$ l) | B%    |       |       | E2 (pg/ml) |          |          | E2 avg   | E2 RSD   | E2 (pg/ml) adjusted RSD | comment  | E2 (pg/ml) assay |     | final comment |      |   |
|------------|----------|-----------|--------------------------|-------|-------|-------|------------|----------|----------|----------|----------|-------------------------|----------|------------------|-----|---------------|------|---|
|            |          |           |                          | rep1  | rep2  | rep3  | rep1       | rep2     | rep3     |          |          |                         |          | final            | RSD |               |      |   |
| 3672       | R        | 3/13/96   | 100                      | 54.01 | 56.74 | 62.49 | 57.75      | 91.69    | 79.43    | 58.17    | 76.43    | 22.19                   |          |                  |     | D             |      |   |
| 3681       | R        | 6/22/95   | 10                       | 19.57 | 16.49 | 17.95 | 18.01      | 13927.19 | 17281.3  | 15549.86 | 15586.12 | 10.76                   | B        |                  |     |               |      |   |
| 3681       | R        | 3/13/96   | 5                        | 43.97 | 20.47 | 42.98 | 35.81      | 4238.61  | 16088.88 | 4446.97  | 8258.15  | 82.13                   | 4342.79  | 3.39             | a   | 4342.79       | 3.39 | a |
| 3682       |          | 6/22/95   | 10                       | 20.95 | 20.82 | 19.32 | 20.36      | 12747.56 | 12857.03 | 14158.14 | 13254.24 | 5.92                    | 13254.24 |                  |     | 13254.24      |      |   |
| 3683       |          | 7/14/95   | 10                       | 29.46 | 33.55 | 29.60 | 30.87      | 7382.94  | 6071.67  | 7331.05  | 6928.55  | 10.72                   | 6928.55  |                  |     | 6928.55       |      |   |
| 3684       | R        | 6/21/95   | 10                       | 18.47 | 17.65 | 19.20 | 18.44      | 17777.31 | 18888.72 | 16880.96 | 17849    | 5.64                    |          | B                |     | 7984.79       |      |   |
| 3684       | R        | 2/13/96   | 5                        | 31.62 | 33.81 | 29.71 | 31.71      | 7981.96  | 7086.18  | 8886.22  | 7984.79  | 11.27                   | 7984.79  |                  |     | 7984.79       |      |   |
| 3685       |          | 6/22/95   | 100                      | 72.26 | 72.50 | 71.91 | 72.22      | 119.9    | 118.41   | 122.1    | 120.14   | 1.54                    | 120.14   |                  |     | 120.14        |      |   |
| 3687       | R        | 7/10/95   | 10                       | 18.95 | 17.58 | 19.46 | 18.66      | 22424.73 | 24628.19 | 21687.99 | 22913.64 | 6.68                    |          | B                |     |               |      |   |
| 3687       | R        | 3/13/96   | 5                        | 40.49 | 33.10 | 32.63 | 35.40      | 5028.27  | 7362.18  | 7551.66  | 6647.37  | 21.14                   | 7456.92  | 1.80             | a   | 7456.92       | 1.80 | a |
| 3688       |          | 6/22/95   | 10                       | 33.83 | 34.49 | 34.36 | 34.23      | 6463.23  | 6268     | 6305.03  | 6345.42  | 1.63                    | 6345.42  |                  |     | 6345.42       |      |   |
| 3689       |          | 7/10/95   | 100                      | 78.83 | 81.68 | 77.19 | 79.23      | 132.88   | 110.56   | 146.46   | 129.97   | 13.95                   | 129.97   |                  |     | 129.97        |      |   |
| 3690       |          | 6/22/95   | 50                       | 69.86 | 68.16 | 69.95 | 69.32      | 270.61   | 293.85   | 269.44   | 277.97   | 4.95                    | 277.97   |                  |     | 277.97        |      |   |
| 3716       |          | 6/21/95   | 100                      | 65.54 | 64.99 | 72.21 | 67.58      | 174.71   | 179.4    | 124.3    | 159.47   | 19.16                   | 159.47   |                  |     | 159.47        |      |   |
| 3717       |          | 6/28/95   | 10                       | 24.39 | 24.79 | 25.02 | 24.73      | 12030.54 | 11765.99 | 11620.45 | 11805.66 | 1.76                    | 11805.66 |                  |     | 11805.66      |      |   |
| 3719       | R        | 6/28/95   | 50                       | 82.83 | 75.91 | 82.29 | 80.34      | 145.05   | 225.68   | 150.79   | 173.84   | 25.88                   |          | A                |     |               |      |   |
| 3719       | R        | 7/14/95   | 100                      | 78.78 | 70.20 | 74.03 | 74.34      | 149.17   | 216.53   | 185.2    | 183.63   | 18.35                   | 183.63   |                  |     | 183.63        |      |   |
| 3720       |          | 6/21/95   | 100                      | 75.83 | 76.59 | 73.10 | 75.17      | 101.21   | 96.69    | 118.4    | 105.43   | 10.87                   | 105.43   |                  |     | 105.43        |      |   |
| 3722       |          | 6/21/95   | 10                       | 28.97 | 28.11 | 29.21 | 28.77      | 9362.46  | 9801.79  | 9245.86  | 9470.04  | 3.10                    | 9470.04  |                  |     | 9470.04       |      |   |
| 3724       |          | 6/28/95   | 100                      | 92.54 | 83.60 | 92.64 | 89.60      | 27.18    | 68.46    | 26.79    | 40.81    | 58.68                   | 43.85    | A                |     | 43.85         |      | A |
| 3725       | R        | 6/21/95   | 50                       | 91.49 | 85.55 | 79.47 | 85.50      | 52.82    | 101.33   | 160.97   | 105.04   | 51.57                   |          | A                |     |               |      | A |
| 3725       | R        | 7/18/95   | 100                      | 81.34 | 78.84 | 81.72 | 80.63      | 61.74    | 73.75    | 60.04    | 65.18    | 11.46                   | 33.9     | A                |     | 33.9          |      | A |

A=below effective concentrations of the assay ; B=above effective concentrations of the assay; C=average of 2 or more runs; D=Delete; R=run to run RSD of outside limit; a=outlier taken out; s=within run RSD slightly above limit; B%=B% slightly above limit; CNC=could not calculate.

Hylebos Waterway Reproductive Toxicology in Flatfish - Reproductive Steroid RIAs



Table 3. Plasma 17- $\beta$  estradiol concentrations(pg/ml Plasma).  
Hylebos Waterway Reproductive Toxicology in Flatfish.

| specimen # | re- runs | test date | sample volume ( $\mu$ l) | B% rep1 | B% rep2 | B% rep3 | B% avg | E2 (pg/ml) |          |          | E2 (pg/ml) rep3 | E2 (pg/ml) avg | RSD      | E2 (pg/ml) adjusted | RSD | comment | E2 (pg/ml) final |       |       | inter assay RSD | comment |   |
|------------|----------|-----------|--------------------------|---------|---------|---------|--------|------------|----------|----------|-----------------|----------------|----------|---------------------|-----|---------|------------------|-------|-------|-----------------|---------|---|
|            |          |           |                          |         |         |         |        | rep1       | rep2     | rep3     |                 |                |          |                     |     |         | final            | final | final |                 |         |   |
| 3726       | R        | 6/28/95   | 50                       | 84.26   | 89.74   | 83.19   | 85.73  | 130.14     | 78.19    | 141.27   | 116.54          | 28.89          |          |                     | A   |         |                  |       |       |                 |         |   |
| 3726       | R        | 7/14/95   | 50                       | 92.01   | 43.40   | 91.64   | 75.68  | 117.95     | 1087.02  | 122.76   | 442.58          | 126.10         |          |                     | A   |         |                  |       |       |                 |         |   |
| 3726       | R        | 7/18/95   | 50                       | 80.39   | 83.48   | 84.95   | 82.94  | 88.71      | 67.49    | 58.34    | 71.51           | 21.78          |          |                     | A   |         |                  |       |       |                 |         |   |
| 3726       | R        | 3/13/96   | 100                      | 53.11   | 47.47   | 53.93   | 51.50  | 96.11      | 128.99   | 92.08    | 105.73          | 19.15          | 105.73   |                     |     |         | 105.73           |       |       |                 |         |   |
| 3732       |          | 6/21/95   | 100                      | 36.97   | 37.83   | 37.30   | 37.37  | 630.05     | 605.37   | 620.47   | 618.63          | 2.01           | 618.63   |                     |     |         | 618.63           |       |       |                 |         |   |
| 3737       | R        | 6/22/95   | 50                       | 74.91   | 86.46   | 74.30   | 78.56  | 208.31     | 94.87    | 215.28   | 172.82          | 39.11          |          |                     |     |         |                  |       |       |                 |         |   |
| 3737       | R        | 7/14/95   | 100                      | 70.2    | 62.60   | 69.85   | 67.55  | 216.53     | 286.61   | 219.53   | 240.89          | 16.45          | 240.89   |                     |     |         | 240.89           |       |       |                 |         |   |
| 3738       |          | 6/22/95   | 100                      | 84.61   | 83.47   | 80.16   | 82.75  | 55.37      | 60.46    | 76.15    | 63.99           | 16.93          | 38.35    |                     |     | A       | 38.35            |       |       |                 | A       |   |
| 3739       | R        | 6/28/95   | 10                       | 14.79   | 15.86   | 16.59   | 15.75  | 22895.55   | 21027.64 | 19886.53 | 21269.91        | 7.14           |          |                     | B   |         | 12164.01         |       |       |                 |         |   |
| 3739       | R        | 3/13/96   | 5                        | 21.95   | 26.36   | 25.86   | 24.72  | 14464.12   | 10842.89 | 11185.02 | 12164.01        | 16.44          | 12164.01 |                     |     |         | 12164.01         |       |       |                 |         |   |
| 3740       | R        | 6/21/95   | 50                       | 71.49   | 79.54   | 77.61   | 76.21  | 258.51     | 160.3    | 181.59   | 200.13          | 25.82          |          |                     |     |         |                  |       |       |                 |         |   |
| 3740       | R        | 7/18/95   | 100                      | 70.94   | 74.28   | 71.00   | 72.08  | 119.05     | 98.41    | 118.67   | 112.04          | 10.54          | 112.04   |                     |     |         | 112.04           |       |       |                 |         |   |
| 3741       |          | 6/22/95   | 100                      | 95.8    | 89.75   | 92.73   | 92.76  | 12.7       | 34.2     | 23.2     | 23.37           | 46.02          | 38.35    |                     |     | A       | 38.35            |       |       |                 | A       |   |
| 3742       | R        | 6/22/95   | 100                      | 60.31   | 85.90   | 74.42   | 73.55  | 209.37     | 49.77    | 106.98   | 122.04          | 66.25          |          |                     |     |         |                  |       |       |                 |         |   |
| 3742       | R        | 7/14/95   | 100                      | 64.61   | 67.65   | 75.53   | 69.26  | 266.89     | 238.79   | 173.58   | 226.4           | 21.16          | 252.84   |                     |     | a       | 252.84           |       |       |                 | a       |   |
| 3743       |          | 6/21/95   | 100                      | 41.34   | 44.57   | 42.22   | 42.71  | 515.7      | 446.66   | 495.78   | 486.05          | 7.31           | 486.05   |                     |     |         | 486.05           |       |       |                 |         |   |
| 3744       |          | 6/28/95   | 10                       | 30.36   | 28.76   | 26.57   | 28.56  | 8799.95    | 9534.66  | 10882.98 | 9672.53         | 9.81           | 9672.53  |                     |     |         | 9672.53          |       |       |                 |         |   |
| 3745       |          | 6/22/95   | 100                      | 84.21   | 82.46   | 84.92   | 83.86  | 57.14      | 65.11    | 54       | 58.75           | 9.75           | 38.35    |                     |     | A       | 38.35            |       |       |                 | A       |   |
| 3756       | R        | 7/14/95   | 100                      | 62.25   | 83.53   | 82.27   | 76.02  | 290.1      | 115.51   | 124.25   | 176.62          | 55.70          |          |                     |     |         |                  |       |       |                 |         |   |
| 3756       | R        | 7/18/95   | 100                      | 83.95   | 83.01   | 86.32   | 84.43  | 50.26      | 54.26    | 40.65    | 48.39           | 14.46          | 33.9     |                     |     | A       | 33.9             |       |       |                 | A       |   |
| 3761       | R        | 7/10/95   | 50                       | 89.93   | 98.82   | 92.66   | 93.80  | 108.73     | 11.11    | 76.4     | 65.41           | 76.02          |          |                     | A   |         |                  |       |       |                 |         |   |
| 3761       | R        | 7/18/95   | 100                      | 74.75   | 103.18  | 73.42   | 83.78  | 95.73      | CNC      | 103.51   | CNC             |                | 99.62    |                     | a   |         | 99.62            |       |       |                 | 5.52    | a |

A=below effective concentrations of the assay ; B=above effective concentrations of the assay; C=average of 2 or more runs; D=Delete; R=run to run RSD of outside limit;  
a=outlier taken out; s=within run RSD slightly above limit; B%=B% slightly above limit; CNC=could not calculate.

Hylebos Waterway Reproductive Toxicology in Flatfish - Reproductive Steroid RIAs

Table 3. Plasma 17-β estradiol concentrations (pg/ml Plasma).  
Hylebos Waterway Reproductive Toxicology in Flatfish.

| specimen # | re- runs | test date | sample volume (μl) | B %    |        |        | E2 (pg/ml) |        |        | E2 (pg/ml) rep3 | E2 (pg/ml) rep2 | E2 (pg/ml) rep1 | E2 avg | RSD    | E2 adjusted (pg/ml) | RSD | com- ment | E2 final (pg/ml) | intra assay RSD | inter assay RSD | final comment |
|------------|----------|-----------|--------------------|--------|--------|--------|------------|--------|--------|-----------------|-----------------|-----------------|--------|--------|---------------------|-----|-----------|------------------|-----------------|-----------------|---------------|
|            |          |           |                    | rep1   | rep2   | rep3   | rep1       | rep2   | rep3   |                 |                 |                 |        |        |                     |     |           |                  |                 |                 |               |
| 3762       |          | 7/10/95   | 100                | 55.3   | 53.68  | 52.57  | 53.85      | 409.25 | 437.49 | 457.91          | 434.88          | 434.88          | 5.62   | 434.88 |                     |     | 434.88    |                  |                 |                 |               |
| 3764       | R        | 7/14/95   | 100                | 82.32  | 78.97  | 82.96  | 81.42      | 123.89 | 147.74 | 119.44          | 130.36          | 130.36          | 11.68  |        |                     | A   |           |                  |                 |                 |               |
| 3764       | R        | 3/13/96   | 100                | 73.95  | 77.73  | 79.53  | 77.07      | 29.07  | 22.2   | 19.31           | 23.53           | 23.53           | 21.29  | 20.76  | 9.84                | a   | 20.76     | 9.80             |                 |                 | a             |
| 3766       | R        | 7/14/95   | 100                | 89.03  | 75.01  | 72.88  | 78.98      | 34.52  | 96.37  | 108.05          | 79.65           | 79.65           | 49.61  |        |                     |     |           |                  |                 |                 |               |
| 3766       | R        | 7/18/95   | 100                | 82.57  | 81.62  | 86.52  | 83.57      | 36.7   | 39.97  | 24.67           | 33.78           | 33.78           | 23.86  | 22.8   |                     | A   | 22.8      |                  |                 |                 | A             |
| 3768       |          | 7/10/95   | 100                | 72.51  | 77.64  | 77.90  | 76.02      | 188.97 | 142.71 | 140.5           | 157.39          | 157.39          | 17.39  | 157.39 |                     |     | 157.39    |                  |                 |                 |               |
| 3769       |          | 7/10/95   | 100                | 75.25  | 77.57  | 73.01  | 75.28      | 163.45 | 143.29 | 184.09          | 163.61          | 163.61          | 12.47  | 163.61 |                     |     | 163.61    |                  |                 |                 |               |
| 3771       |          | 7/14/95   | 100                | 92.73  | 90.18  | 84.95  | 89.29      | 54.24  | 70.95  | 105.78          | 76.99           | 76.99           | 34.15  | 69.95  |                     | A   | 69.95     |                  |                 |                 | A             |
| 3775       |          | 7/14/95   | 100                | 99.54  | 81.31  | 84.66  | 88.50      | 5.32   | 131    | 107.73          | 81.35           | 81.35           | 82.20  | 69.95  |                     | A   | 69.95     |                  |                 |                 | A             |
| 3780       | R        | 7/10/95   | 50                 | 105.05 | 103.20 | 101.32 | 103.19     | CNC    | CNC    | CNC             | CNC             | CNC             |        |        |                     | A   |           |                  |                 |                 | A             |
| 3780       | R        | 7/18/95   | 100                | 87.12  | 94.54  | 106.51 | 96.05      | 37.56  | 12.96  | CNC             | CNC             | CNC             |        |        |                     | A   |           |                  |                 |                 | A             |
| 3781       | R        | 7/10/95   | 10                 | 107.17 | 107.23 | 113.61 | 109.34     | CNC    | CNC    | CNC             | CNC             | CNC             |        |        |                     | A   |           |                  |                 |                 | A             |
| 3781       | R        | 7/18/95   | 100                | 86.18  | 87.25  | 86.63  | 86.69      | 41.18  | 37.07  | 39.41           | 39.22           | 39.22           | 5.26   | 33.9   |                     | A   | 33.9      |                  |                 |                 | A             |
| 3783       |          | 7/10/95   | 100                | 106.91 | 102.66 | 103.93 | 104.50     | CNC    | CNC    | CNC             | CNC             | CNC             |        |        |                     | A   | 60.15     |                  |                 |                 | A             |
| 3793       | R        | 7/14/95   | 50                 | 61.96  | 51.35  | 50.91  | 54.74      | 362.39 | 567.13 | 577.54          | 502.35          | 502.35          | 24.15  |        |                     |     |           |                  |                 |                 |               |
| 3793       | R        | 3/13/96   | 50                 | 47.68  | 54.64  | 51.15  | 51.16      | 255.08 | 177.43 | 212.92          | 215.14          | 215.14          | 18.07  | 215.14 |                     |     | 215.14    |                  |                 |                 |               |
| 3793       |          | 7/10/95   | 100                | 76.14  | 73.24  | 74.76  | 74.71      | 155.57 | 181.97 | 167.84          | 168.46          | 168.46          | 7.84   | 168.46 |                     |     | 168.46    |                  |                 |                 |               |
| 3795       |          | 7/10/95   | 100                | 98.54  | 102.42 | 103.54 | 101.50     | 6.88   | CNC    | CNC             | CNC             | CNC             |        |        |                     | A   | 60.15     |                  |                 |                 | A             |
| 3803       |          | 7/14/95   | 100                | 79.45  | 89.08  | 75.12  | 81.22      | 144.27 | 78.22  | 176.66          | 133.05          | 133.05          | 37.71  | 69.95  |                     | A   | 69.95     |                  |                 |                 | A             |
| 3805       |          | 7/14/95   | 100                | 93.03  | 93.42  | 74.67  | 87.04      | 52.22  | 49.67  | 180.17          | 94.02           | 94.02           | 79.37  | 69.95  |                     | A   | 69.95     |                  |                 |                 | A             |
| 3806       | R        | 7/14/95   | 100                | 76.44  | 67.84  | 90.91  | 78.40      | 88.98  | 138.76 | 27.81           | 85.18           | 85.18           | 65.24  |        |                     |     |           |                  |                 |                 |               |
| 3806       | R        | 7/18/95   | 100                | 74.92  | 71.79  | 79.87  | 75.52      | 67.17  | 82.85  | 46.3            | 65.44           | 65.44           | 28.02  |        |                     |     |           |                  |                 |                 |               |

A=below effective concentrations of the assay ; B=above effective concentrations of the assay; C=average of 2 or more runs; D=Delete; R=run to run RSD of outside limit; a=outlier taken out; s=within run RSD slightly above limit; B%=B% slightly above limit; CNC=could not calculate.

**Hylebos Waterway Reproductive Toxicology in Flatfish - Reproductive Steroid RIAs**

Table 3. Plasma 17- $\beta$  estradiol concentrations (pg/ml Plasma).  
Hylebos Waterway Reproductive Toxicology in Flatfish.

| specimen # | re- test runs | date    | sample volume ( $\mu$ l) | B %   |       |       | E2 (pg/ml) |        |        | E2 (pg/ml) avg | RSD    | E2 (pg/ml) adjusted | RSD    | comment | E2 (pg/ml) final | intra assay RSD | inter assay RSD | final comment |
|------------|---------------|---------|--------------------------|-------|-------|-------|------------|--------|--------|----------------|--------|---------------------|--------|---------|------------------|-----------------|-----------------|---------------|
|            |               |         |                          | rep1  | rep2  | rep3  | rep1       | rep2   | rep3   |                |        |                     |        |         |                  |                 |                 |               |
| 3806       | R             | 3/13/96 | 100                      | 64.31 | 63.35 | 71.06 | 66.24      | 52.53  | 55.45  | 35.1           | 47.69  | 23.07               | 53.99  | 3.83    | a                | 53.99           | 3.80            | a             |
| 3829       |               | 7/14/95 | 100                      | 89.42 | 89.15 | 80.82 | 86.46      | 33.09  | 34.1   | 67.93          | 45.04  | 44.02               | 35.7   |         | A                | 35.7            |                 | A             |
| 3830       |               | 7/14/95 | 100                      | 67.49 | 73.92 | 73.82 | 71.75      | 141.04 | 102.25 | 102.81         | 115.37 | 19.27               | 115.37 |         |                  | 115.37          |                 |               |
| 3831       |               | 7/14/95 | 100                      | 66.52 | 62.09 | 70.94 | 66.52      | 147.56 | 180.12 | 119.33         | 149.01 | 20.41               | 149.01 |         | s                | 149.01          |                 | s             |
| 3832       | R             | 7/14/95 | 100                      | 47.26 | 56.41 | 60.18 | 54.62      | 335.69 | 229.72 | 195.76         | 253.72 | 28.77               |        |         |                  |                 |                 |               |
| 3832       | R             | 7/18/95 | 100                      | 55.8  | 54.33 | 54.02 | 54.72      | 251.17 | 268.7  | 272.49         | 264.12 | 4.31                | 264.12 |         |                  | 264.12          |                 |               |
| 3834       |               | 7/14/95 | 100                      | 63.71 | 65.90 | 64.89 | 64.83      | 167.67 | 151.87 | 159.02         | 159.52 | 4.96                | 159.52 |         |                  | 159.52          |                 |               |
| 3835       |               | 7/14/95 | 100                      | 48.06 | 56.89 | 54.90 | 53.28      | 465.93 | 348.3  | 372.17         | 395.46 | 15.72               | 395.46 |         |                  | 395.46          |                 |               |
| 3836       |               | 7/14/95 | 100                      | 99.87 | 76.91 | 84.23 | 87.00      | 1.92   | 162.99 | 110.67         | 91.86  | 89.45               | 69.95  |         | A                | 69.95           |                 | A             |
| 3838       | R             | 7/14/95 | 100                      | 81.17 | 77.20 | 84.57 | 80.98      | 131.97 | 160.88 | 108.36         | 133.73 | 19.67               | 69.95  |         | A                | 69.95           |                 | D,R           |
| 3838       | R             | 3/13/96 | 100                      | 81.45 | 81.44 | 79.07 | 80.65      | 16.47  | 16.49  | 20.03          | 17.66  | 11.61               | 9.25   |         | A                | 9.25            |                 | D,R           |
| 3839       |               | 7/14/95 | 100                      | 72.84 | 80.14 | 75.59 | 76.19      | 108.28 | 71.02  | 93.34          | 90.88  | 20.63               | 90.88  |         | s                | 90.88           |                 | s             |
| 3840       | R             | 7/14/95 | 50                       | 67.82 | 76.29 | 56.28 | 66.80      | 277.7  | 179.47 | 461.92         | 306.37 | 46.80               |        |         |                  |                 |                 |               |
| 3840       | R             | 7/18/95 | 50                       | 66.03 | 63.42 | 63.33 | 64.26      | 235.83 | 273.81 | 275.25         | 261.63 | 8.54                | 261.63 |         |                  | 261.63          |                 |               |
| 3841       | R             | 7/10/95 | 100                      | 81.89 | 80.82 | 77.40 | 80.04      | 108.99 | 117.08 | 144.76         | 123.61 | 15.17               | 123.61 |         | B%               | 123.61          |                 | B%            |
| 3841       | R             | 3/13/96 | 100                      | 50.82 | 59.37 | 54.77 | 54.99      | 108.31 | 69.01  | 88.12          | 88.48  | 22.21               |        |         |                  |                 |                 |               |
| 3842       |               | 7/10/95 | 100                      | 70.62 | 69.29 | 65.93 | 68.61      | 207.78 | 221.56 | 259.23         | 229.53 | 11.60               | 229.53 |         |                  | 229.53          |                 |               |
| 3843       |               | 7/10/95 | 100                      | 91.08 | 89.52 | 88.00 | 89.54      | 47.43  | 56.9   | 66.51          | 56.94  | 16.75               | 60.15  |         | A                | 60.15           |                 | A             |
| 3844       |               | 7/14/95 | 100                      | 48.57 | 56.76 | 52.23 | 52.52      | 318.02 | 226.41 | 273.36         | 272.59 | 16.81               | 272.59 |         |                  | 272.59          |                 |               |
| 3845       |               | 7/14/95 | 100                      | 55.15 | 53.25 | 50.37 | 52.92      | 369.12 | 392.96 | 431.97         | 398.02 | 7.97                | 398.02 |         |                  | 398.02          |                 |               |
| 3846       |               | 7/14/95 | 100                      | 55.38 | 62.92 | 62.57 | 60.29      | 366.25 | 283.41 | 286.87         | 312.18 | 15.01               | 312.18 |         |                  | 312.18          |                 |               |
| 3847       | R             | 7/14/95 | 100                      | 56.76 | 70.75 | 64.21 | 63.90      | 349.82 | 211.95 | 270.79         | 277.52 | 24.93               |        |         |                  |                 |                 |               |

A=below effective concentrations of the assay ; B=above effective concentrations of the assay; C=average of 2 or more runs; D=Delete; R=run to run RSD of outside limit;  
a=outlier taken out; s=within run RSD slightly above limit; B%=B% slightly above limit; CNC=could not calculate.

Hylebos Waterway Reproductive Toxicology in Flatfish - Reproductive Steroid RIAs

Table 3. Plasma 17- $\beta$  estradiol concentrations(pg/ml Plasma).  
Hylebos Waterway Reproductive Toxicology in Flatfish.

| specimen # | re- runs | test date | sample volume ( $\mu$ l) | B %   |       |       | E2 (pg/ml) |          |        | E2 (pg/ml) adjusted | RSD     | comment | E2 (pg/ml) assay |       | inter assay RSD | final comment |       |     |
|------------|----------|-----------|--------------------------|-------|-------|-------|------------|----------|--------|---------------------|---------|---------|------------------|-------|-----------------|---------------|-------|-----|
|            |          |           |                          | rep1  | rep2  | rep3  | avg        | rep1     | rep2   |                     |         |         | rep3             | avg   |                 |               | final | RSD |
| 3847       | R        | 3/13/96   | 100                      | 46.73 | 48.87 | 34.36 | 43.32      | 134.05   | 119.87 | 262.55              | 172.16  | 45.66   | 126.96           | 7.90  | a               | 126.96        | 7.90  | a   |
| 3848       | R        | 7/14/95   | 10                       | 19.65 | 91.29 | 96.58 | 69.17      | 12829.14 | 265.02 | 95.44               | 4396.54 | 166.12  |                  |       |                 |               |       |     |
| 3848       | R        | 7/18/95   | 50                       | 66.38 | 71.91 | 72.46 | 70.25      | 231      | 164.41 | 158.61              | 184.67  | 21.78   | 161.51           | 2.54  | a               | 157.59        | 3.50  | C   |
| 3848       | R        | 3/13/96   | 100                      | 42.5  | 45.93 | 30.69 | 39.71      | 167.56   | 139.77 | 326.48              | 211.27  | 47.68   | 153.66           | 12.79 | a               |               |       |     |
| 3855       | R        | 7/14/95   | 100                      | 62.12 | 57.39 | 28.45 | 49.32      | 179.91   | 220.48 | 776.64              | 392.34  | 84.98   | 200.19           | 10.13 | a               | 215.39        | 10.00 | C   |
| 3855       | R        | 7/18/95   | 100                      | 54.02 | 53.59 | 61.71 | 56.44      | 227.98   | 233.19 | 150.69              | 203.96  | 22.65   | 230.59           | 1.60  | a               |               |       |     |

A=below effective concentrations of the assay ; B=above effective concentrations of the assay; C=average of 2 or more runs; D=Delete; R=run to run RSD of outside limit;  
a=outlier taken out; s=within run RSD slightly above limit; B%=B% slightly above limit; CNC=could not calculate.  
Hylebos Waterway Reproductive Toxicology in Flatfish - Reproductive Steroid RIAs