

Appendix for Section 20

Fish Bile

| <i>Naphthalene Meta (ref)</i> | <i>Naphthalene Meta In NAS)</i> | <i>Naphthalene Meta (Out NAS)</i> | <i>Naphthalene Meta In SWM)</i> | <i>Naphthalene Meta (Out SWM)</i> | <i>Benzo[a]pyren e Meta (ug/mg protein)</i> | <i>Benzo[a]pyren e Meta In NAS</i> | | | | | | | |
|-----------------------------------|-------------------------------------|---|-------------------------------------|---|---|--|--------|----------------------------|--------|----------------------------|-------|----------------------------|-------|
| Mean | 79.00 | Mean | 74.50 | Mean | 84.20 | Mean | 68.9 | Mean | 74.00 | Mean | 2.07 | Mean | 2.92 |
| Standard Error | 8.67 | Standard Error | 14.46 | Standard Error | 7.84 | Standard Error | 3.54 | Standard Error | 8.06 | Standard Error | 0.39 | Standard Error | 0.51 |
| Median | 72.00 | Median | 70.50 | Median | 81.00 | Median | 65.50 | Median | 65.00 | Median | 1.85 | Median | 2.55 |
| Mode | 72.00 | Mode | #N/A | Mode | 64.00 | Mode | #N/A | Mode | #N/A | Mode | #N/A | Mode | 2.30 |
| Standard Deviation | 27.41 | Standard Deviation | 45.74 | Standard Deviation | 24.80 | Standard Deviation | 11.18 | Standard Deviation | 25.50 | Standard Deviation | 1.25 | Standard Deviation | 1.63 |
| Variance | 751.11 | Variance | 2092.28 | Variance | 614.84 | Variance | 124.99 | Variance | 650.22 | Variance | 1.56 | Variance | 2.65 |
| Kurtosis | 5.71 | Kurtosis | -0.01 | Kurtosis | 6.73 | Kurtosis | 3.82 | Kurtosis | 1.32 | Kurtosis | 0.28 | Kurtosis | 0.34 |
| Skewness | 2.24 | Skewness | 0.85 | Skewness | 2.39 | Skewness | 1.64 | Skewness | 1.27 | Skewness | 0.88 | Skewness | 0.63 |
| Range | 92.00 | Range | 134.00 | Range | 86.00 | Range | 41.00 | Range | 81.00 | Range | 3.90 | Range | 5.50 |
| Minimum | 58.00 | Minimum | 26.00 | Minimum | 64.00 | Minimum | 55.00 | Minimum | 49.00 | Minimum | 0.70 | Minimum | 0.50 |
| Maximum | 150.00 | Maximum | 160.00 | Maximum | 150.00 | Maximum | 96.00 | Maximum | 130.00 | Maximum | 4.60 | Maximum | 6.00 |
| Sum | 790.00 | Sum | 745.00 | Sum | 842.00 | Sum | 689.00 | Sum | 740.00 | Sum | 20.70 | Sum | 29.20 |
| Count | 10.00 | Count | 10.00 | Count | 10.00 | Count | 10.00 | Count | 10.00 | Count | 10.00 | Count | 10.00 |
| Confidence Level(95.0%) | 19.61 | Confidence Level(95.0%) | 32.72 | Confidence Level(95.0%) | 17.74 | Confidence Level(95.0%) | 8.00 | Confidence Level(95.0%) | 18.24 | Confidence Level(95.0%) | 0.89 | Confidence Level(95.0%) | 1.16 |

| <i>Benzo[a]pyrene Meta Out NAS</i> | <i>Benzo[a]pyrene Meta In SWM</i> | <i>Benzo[a]pyrene Meta Out SWM</i> | <i>Phenanthrene Meta ref</i> | <i>Phenanthrene Meta In NAS</i> | <i>Phenanthrene Meta Out NAS</i> | <i>Phenanthrene Meta In SWM</i> | | | | | | | |
|------------------------------------|-----------------------------------|------------------------------------|------------------------------|---------------------------------|----------------------------------|---------------------------------|--------|-------------------------|--------|-------------------------|--------|-------------------------|--------|
| Mean | 5.32 | Mean | 1.67 | Mean | 5.95 | Mean | 12.75 | Mean | 13.55 | Mean | 26.70 | Mean | 13.90 |
| Standard Error | 0.65 | Standard Error | 0.27 | Standard Error | 0.49 | Standard Error | 1.50 | Standard Error | 2.35 | Standard Error | 2.46 | Standard Error | 0.59 |
| Median | 4.85 | Median | 1.60 | Median | 6.15 | Median | 12.00 | Median | 13.00 | Median | 25.50 | Median | 14.00 |
| Mode | #N/A | Mode | 1.90 | Mode | #N/A | Mode | 11.00 | Mode | #N/A | Mode | 20.00 | Mode | 14.00 |
| Standard Deviation | 2.06 | Standard Deviation | 0.87 | Standard Deviation | 1.55 | Standard Deviation | 4.74 | Standard Deviation | 7.44 | Standard Deviation | 7.79 | Standard Deviation | 1.85 |
| Sample Variance | 4.25 | Sample Variance | 0.75 | Sample Variance | 2.42 | Sample Variance | 22.46 | Sample Variance | 55.40 | Sample Variance | 60.68 | Sample Variance | 3.43 |
| Kurtosis | 1.46 | Kurtosis | 2.78 | Kurtosis | 1.16 | Kurtosis | 5.91 | Kurtosis | 0.08 | Kurtosis | 4.29 | Kurtosis | 2.48 |
| Skewness | 1.03 | Skewness | 1.44 | Skewness | -0.50 | Skewness | 2.10 | Skewness | 0.83 | Skewness | 1.88 | Skewness | 0.84 |
| Range | 7.10 | Range | 3.00 | Range | 5.70 | Range | 17.90 | Range | 22.30 | Range | 26.00 | Range | 7.00 |
| Minimum | 2.70 | Minimum | 0.70 | Minimum | 2.80 | Minimum | 7.10 | Minimum | 5.70 | Minimum | 20.00 | Minimum | 11.00 |
| Maximum | 9.80 | Maximum | 3.70 | Maximum | 8.50 | Maximum | 25.00 | Maximum | 28.00 | Maximum | 46.00 | Maximum | 18.00 |
| Sum | 53.20 | Sum | 16.70 | Sum | 59.50 | Sum | 127.50 | Sum | 135.50 | Sum | 267.00 | Sum | 139.00 |
| Count | 10.00 | Count | 10.00 | Count | 10.00 | Count | 10.00 | Count | 10.00 | Count | 10.00 | Count | 10.00 |
| Confidence Level(95.0%) | 1.47 | Confidence Level(95.0%) | 0.62 | Confidence Level(95.0%) | 1.11 | Confidence Level(95.0%) | 3.39 | Confidence Level(95.0%) | 5.32 | Confidence Level(95.0%) | 5.57 | Confidence Level(95.0%) | 1.33 |

Phenanthrene
Meta Out
SWM

| | |
|-------------------------|--------|
| Mean | 18.90 |
| Standard Error | 0.98 |
| Median | 18.50 |
| Mode | 17.00 |
| Standard Deviation | 3.11 |
| Sample Variance | 9.66 |
| Kurtosis | 0.61 |
| Skewness | 0.59 |
| Range | 11.00 |
| Minimum | 14.00 |
| Maximum | 25.00 |
| Sum | 189.00 |
| Count | 10.00 |
| Confidence Level(95.0%) | 2.22 |

| Reference | | | Inside NASSCO | | | Outside NASSCO | | | Inside SWM | | | Outside SWM | | |
|----------------------------------|-------------------------------------|-----------------------------------|----------------------------------|-------------------------------------|-----------------------------------|----------------------------------|-------------------------------------|-----------------------------------|----------------------------------|-------------------------------------|-----------------------------------|----------------------------------|-------------------------------------|-----------------------------------|
| Naphthalene Meta (ug/mg protein) | Benzo[a]pyrene Meta (ug/mg protein) | Phenanthrene Meta (ug/mg protein) | Naphthalene Meta (ug/mg protein) | Benzo[a]pyrene Meta (ug/mg protein) | Phenanthrene Meta (ug/mg protein) | Naphthalene Meta (ug/mg protein) | Benzo[a]pyrene Meta (ug/mg protein) | Phenanthrene Meta (ug/mg protein) | Naphthalene Meta (ug/mg protein) | Benzo[a]pyrene Meta (ug/mg protein) | Phenanthrene Meta (ug/mg protein) | Naphthalene Meta (ug/mg protein) | Benzo[a]pyrene Meta (ug/mg protein) | Phenanthrene Meta (ug/mg protein) |
| 72 | 1 | 9.4 | 140 | 6 | 23 | 77 | 4.7 | 20 | 72 | 2.2 | 15 | 130 | 6.2 | 25 |
| 62 | 1.9 | 11 | 27 | 3.6 | 6.2 | 79 | 3.2 | 20 | 66 | 1.9 | 13 | 57 | 5.4 | 17 |
| 58 | 0.7 | 7.1 | 57 | 2.5 | 9.8 | 87 | 2.7 | 27 | 96 | 1.4 | 18 | 95 | 6.1 | 19 |
| 150 | 1.3 | 25 | 65 | 2.3 | 12 | 64 | 5 | 25 | 65 | 1.8 | 14 | 54 | 8.5 | 18 |
| 86 | 1.8 | 13 | 26 | 2.3 | 5.8 | 86 | 6.7 | 27 | 74 | 3.7 | 14 | 72 | 5 | 17 |
| 78 | 3.3 | 14 | 160 | 1.3 | 28 | 150 | 9.8 | 46 | 64 | 0.9 | 14 | 92 | 6.9 | 22 |
| 58 | 4.6 | 11 | 79 | 3.1 | 14 | 67 | 4.3 | 26 | 73 | 1.1 | 14 | 49 | 2.8 | 14 |
| 91 | 2.9 | 13 | 86 | 2.6 | 16 | 84 | 4.2 | 20 | 61 | 0.7 | 12 | 55 | 6.4 | 19 |
| 72 | 0.8 | 12 | 29 | 0.5 | 5.7 | 85 | 6 | 24 | 63 | 1.1 | 14 | 58 | 7.3 | 17 |
| 63 | 2.4 | 12 | 76 | 5 | 15 | 83 | 6.6 | 32 | 55 | 1.9 | 11 | 78 | 4.9 | 21 |

| | | | | | | | | | | | | | | | |
|------------|--------|-------|-------|--------|-------|-------|--------|-------|-------|-------|-------|-------|--------|-------|-------|
| Mean | 79.00 | 2.07 | 12.75 | 74.50 | 2.92 | 13.55 | 84.20 | 5.32 | 26.70 | 68.90 | 1.67 | 13.90 | 74.00 | 5.95 | 18.90 |
| Cutoff | 1.83 | 1.83 | 1.83 | 1.83 | 1.83 | 1.83 | 1.83 | 1.83 | 1.83 | 1.83 | 1.83 | 1.83 | 1.83 | 1.83 | 1.83 |
| Stand Dev | 27.41 | 1.25 | 4.74 | 45.74 | 1.63 | 7.44 | 24.80 | 2.06 | 7.79 | 11.18 | 0.87 | 1.85 | 25.50 | 1.55 | 3.11 |
| N | 10.00 | 10.00 | 10.00 | 10.00 | 10.00 | 10.00 | 10.00 | 10.00 | 10.00 | 10.00 | 10.00 | 10.00 | 10.00 | 10.00 | 10.00 |
| 95% LPL | 26.31 | -0.33 | 3.64 | -13.44 | -0.21 | -0.76 | 36.53 | 1.36 | 11.72 | 47.41 | 0.00 | 10.34 | 24.98 | 2.96 | 12.93 |
| 95% UPL | 131.69 | 4.47 | 21.86 | 162.44 | 6.05 | 27.86 | 131.87 | 9.28 | 41.68 | 90.39 | 3.34 | 17.46 | 123.02 | 8.94 | 24.87 |
| Stand Err. | 41.64 | 1.41 | 6.91 | 51.37 | 1.91 | 8.81 | 41.70 | 2.94 | 13.18 | 28.58 | 1.06 | 5.52 | 38.90 | 2.83 | 7.87 |

Pairwise Mean Diff.(row - column)

| | Benzo[a]pyrene Meta (ug/mg protein) | Benzo[a]pyrene Meta In NAS | Benzo[a]pyrene Meta Out NAS | Benzo[a]pyrene Meta In SWM | Benzo[a]pyrene Meta Out SWM |
|-------------------------------------|-------------------------------------|----------------------------|-----------------------------|----------------------------|-----------------------------|
| Benzo[a]pyrene Meta (ug/mg protein) | 0 | -0.85 | -3.25 | 0.4 | -3.88 |
| Benzo[a]pyrene Meta In NAS | | 0 | -2.4 | 1.25 | -3.03 |
| Benzo[a]pyrene Meta Out NAS | | | 0 | 3.65 | -0.63 |
| Benzo[a]pyrene Meta In SWM | | | | 0 | -4.28 |
| Benzo[a]pyrene Meta Out SWM | | | | | 0 |

MSE = 2.32486666666667

Pairwise Comparison Probabilities (Bonferroni Correction)

| | Benzo[a]pyrene Meta (ug/mg protein) | Benzo[a]pyrene Meta In NAS | Benzo[a]pyrene Meta Out NAS | Benzo[a]pyrene Meta In SWM | Benzo[a]pyrene Meta Out SWM |
|-------------------------------------|-------------------------------------|----------------------------|-----------------------------|----------------------------|-----------------------------|
| Benzo[a]pyrene Meta (ug/mg protein) | 1.000 | 1.000 | 0.000 | 1.000 | 0.000 |
| Benzo[a]pyrene Meta In NAS | | 1.000 | 0.010 | 0.734 | 0.001 |
| Benzo[a]pyrene Meta Out NAS | | | 1.000 | 0.000 | 1.000 |
| Benzo[a]pyrene Meta In SWM | | | | 1.000 | 0.000 |
| Benzo[a]pyrene Meta Out SWM | | | | | 1.000 |

Pairwise Mean Diff.(row - column)

| | Naphthalene Meta (ref) | Naphthalene Meta In NAS) | Naphthalene Meta (Out NAS) | Naphthalene Meta In SWM) | Naphthalene Meta (Out SWM) |
|----------------------------|------------------------|--------------------------|----------------------------|--------------------------|----------------------------|
| Naphthalene Meta (ref) | 0 | 4.5 | -5.2 | 10.1 | 5 |
| Naphthalene Meta In NAS) | | 0 | -9.7 | 5.6 | 0.5 |
| Naphthalene Meta (Out NAS) | | | 0 | 15.3 | 10.2 |
| Naphthalene Meta In SWM) | | | | 0 | -5.1 |
| Naphthalene Meta (Out SWM) | | | | | 0 |

MSE = 846.688888888889

Pairwise Comparison Probabilities (Bonferroni Correction)

| | Naphthalene Meta (ref) | Naphthalene Meta In NAS) | Naphthalene Meta (Out NAS) | Naphthalene Meta In SWM) | Naphthalene Meta (Out SWM) |
|----------------------------|------------------------|--------------------------|----------------------------|--------------------------|----------------------------|
| Naphthalene Meta (ref) | 1.000 | 1.000 | 1.000 | 1.000 | 1.000 |
| Naphthalene Meta In NAS) | | 1.000 | 1.000 | 1.000 | 1.000 |
| Naphthalene Meta (Out NAS) | | | 1.000 | 1.000 | 1.000 |
| Naphthalene Meta In SWM) | | | | 1.000 | 1.000 |
| Naphthalene Meta (Out SWM) | | | | | 1.000 |

Pairwise Mean Diff.(row - column)

| | Phenanthrene Meta ref | Phenanthrene Meta In NAS | Phenanthrene Meta Out NAS | Phenanthrene Meta In SWM | Phenanthrene Meta Out SWM |
|---------------------------|--------------------------|-----------------------------|------------------------------|-----------------------------|------------------------------|
| Phenanthrene Meta ref | 0 | -0.8 | -13.95 | -1.15 | -6.15 |
| Phenanthrene Meta In NAS | | 0 | -13.15 | -0.35 | -5.35 |
| Phenanthrene Meta Out NAS | | | 0 | 12.8 | 7.8 |
| Phenanthrene Meta In SWM | | | | 0 | -5 |
| Phenanthrene Meta Out SWM | | | | | 0 |

MSE = 30.3251111111111

Pairwise Comparison Probabilities (Bonferroni Correction)

| | Phenanthrene Meta ref | Phenanthrene Meta In NAS | Phenanthrene Meta Out NAS | Phenanthrene Meta In SWM | Phenanthrene Meta Out SWM |
|---------------------------|--------------------------|-----------------------------|------------------------------|-----------------------------|------------------------------|
| Phenanthrene Meta ref | 1.000 | 1.000 | 0.000 | 1.000 | 0.162 |
| Phenanthrene Meta In NAS | | 1.000 | 0.000 | 1.000 | 0.351 |
| Phenanthrene Meta Out NAS | | | 1.000 | 0.000 | 0.028 |
| Phenanthrene Meta In SWM | | | | 1.000 | 0.483 |
| Phenanthrene Meta Out SWM | | | | | 1.000 |

Appendix for Section 20 NOAA Comment Letter – April 20, 2004



U.S. DEPARTMENT OF COMMERCE
National Oceanic and Atmospheric Administration
 NATIONAL OCEAN SERVICE
 OFFICE OF RESPONSE & RESTORATION
 COASTAL PROTECTION & RESTORATION DIVISION
 c/o California Department of Toxic Substance Control,
 Human and Ecological Risk Division
 8800 Cal Center Drive
 Sacramento, CA 95826

April 20, 2004

Mr. Tom Alo
 California Regional Water Quality Control Board
 San Diego Region
 9174 Sky Park Court, Suite 100
 San Diego, CA 92123-4340

2004 APR 30 P 1:33

SAN DIEGO REGIONAL WATER QUALITY CONTROL BOARD

Dear Mr. Alo:

NOAA appreciates the opportunity to provide comments to you on two reports associated with the investigation of the NASSCO and Southwest Marine Shipyards. Dr. Gary D. Marty prepared a September 2003 report entitled, Necropsy and Histopathology of Spotted Sea Bass Sampled from San Diego Harbor, for the Shipyard's consultant, Exponent. Details and results of this report were incorporated into the NASSCO and Southwest Marine Detailed Sediment Investigation (September 2003) submitted by Exponent for the NASSCO and Southwest Marine Shipyards. NOAA's Coastal Protection and Restoration Division has requested the assistance of Mark S. Myers, a fish biologist and pathologist with the ecotoxicology branch of the NOAA Northwest Fisheries Science Center, in reviewing and commenting on these reports. The fish histopathology section (Section 8.2), and the fish bile section (Section 8.3) of the Detailed Sediment Investigation were reviewed, and comments on these sections are included in this letter.

Necropsy and Histopathology of Spotted Sea Bass Sampled from San Diego Harbor

General comments:

- The necropsy procedure, tissue processing, and histopathologic analysis of tissues were conducted according to appropriate and accepted protocols, and no comments will be provided on these sections. The figures contain good quality micrographs that show excellent documentation of the lesions encountered, and they are well described.
- The fish species analyzed in this report is normally referred to as spotted sandbass, not as spotted sea bass. Please make this correction in the text.



- Based on NOAA's past experience with examination of spotted sandbass and barred sandbass from San Diego Harbor, very few toxicopathic lesions have been found in these species. This is especially true for the liver or kidney of spotted sandbass from south San Diego Bay. Based on this observation, it would have been preferable to sample and examine white croaker or black croaker. However, it appears that reasonable attempts to capture these better sentinel species were carried out.

Summary and comment on the major histopathological findings:

- Abundant hepatocellular lipofuscin, indicating degradation of cell organelles, was found in all fish caught in the NASSCO "inside" location and in both the "inside" and "outside" locations at Southwest Marine. This is a significant, contaminant-associated effect that appears to moderately to severely affect approximately 12 to 20% of fish from inside the shipyard sites. Data indicate that fish collected from the reference site were only mildly affected.
- Abundant hemosiderin, indicating increased destruction of red blood cells, was most commonly found in "outside" shipyard locations. No hemosiderin was found in fish at the "inside" shipyard sites. Some attempt should be made to analyze this difference.
- Five out of the 253 fish collected during the study had liver weights greater than 10 grams. In addition, these five fish were female or female-intersex fish, and all came from the NASSCO site. There should be further discussion in the text regarding this potentially important finding and its overall significance.
- There are fewer "cysts of unknown etiology" from inside sites than from outside or reference sites. Scientists at NOAA have also seen this lesion in numerous marine/estuarine species and refer to it as an "oocyte-like body". It appears to be an infectious organism of some sort and, like Dr. Marty; NOAA does not know it's precise diagnosis. NOAA agrees with Dr. Marty that it may represent a life-history stage of *Ichthyophonus sp.*
- The generation of new nephrons was greater in kidneys from fish collected at the reference site. This may indicate a higher growth rate in fish found at the reference site. The scores for renal nephritis were higher in fish from the NASSCO location, and the only severe case of renal nephritis was found at Southwest Marine. It should be noted in the document that growth and survival of fish may be impaired by renal nephritis.
- Lipofuscin scores in testis of fish, which is an indicator of impaired reproduction, were found to be higher "inside" the shipyard sites than those

found at the reference site. Approximately 5-12% of the collected fish were affected, and the only severe cases were seen in fish from inside the shipyard sites. In one case, a male with severe lipofuscin found at the NASSCO "inside" location also had no maturing sperm. In the ovaries, pigmented macrophage aggregates (PMAs) were found in about 20% of the fish and were highest in fish from "inside" both shipyard sites. PMAs in female fish from inside shipyard sites may be significant, but there is a need to account for fish age in these analyses. Site differences in PMAs for testis were not significant.

- According to Marty (p. 4) and Appendix 5, intersex gonads were found at similar frequencies in fish collected at the shipyard sites and reference site. This effect was most common in smaller females, except for "inside" NASSCO, which had several large female-intersex fish. Based on NOAA scientist's previous experience in histologically examining barred and spotted sandbass from southern California, a large number of intersex fish were identified. As mentioned by Marty (p. 8), this may not be a surprising observation considering that these two species are thought to be hermaphroditic (protogynous), and typically change sex from female to male with advancing age. However, this feature of spotted sandbass should be discussed further in the analysis.
- Although three fish collected in the study had carcinomas, NOAA agrees with Dr. Marty that the tumor development identified in these fish does not appear to be specifically related to exposure at the NASSCO or Southwest Marine sites.
- The document states on p. 8 that "more fish from the inside shipyard sites had evidence of tissue damage than did fish from the outside shipyard sites". Although the document states that the most striking differences were in the liver, review of the report also shows that the gonad and kidney had significant lesions. These lesions were distinct enough to be used to separate fish from the contaminated areas and reference area. Further discussion should be provided on the significance of this observation.
- The prevalence of renal nephritis is consistent with increased disease in fish from inside the NASSCO site. Lower scores in regenerative tubules are consistent with reduced growth, but there does not appear to have been an evaluation of the age of the fish in relation to this finding. There is a possibility of higher values in younger fish. In addition, higher values would be expected in situations where fish were exposed to renal toxicants. Further discussion should be provided on the significance of this observation, and the relationship to the age of the fish.

- In Appendix 1, type specimens for foci of cellular alteration (FCA) and cholangitis/biliary hyperplasia are shown but not discussed. Please see additional comments on this subject later in this letter. Dr. Marty or Exponent should provide a discussion and analysis of the significance of these lesions.
- In the discussion of the data from Appendix 4, there is no evaluation or interpretation in the main text of Marty's report of atresia of yolked follicles, and atresia of unyolked follicles in the ovary. Also, there is no inclusion of the lesions F-INT (female, intersex) or M-INT (male, intersex) in the summary of male and female type specimens. Please provide a discussion and analysis of the significance of these findings.
- In the discussion of the data from Appendix 6, there is not an evaluation or even a mention of the preneoplastic foci of cellular alteration observed in the liver, as well as cholangitis/biliary hyperplasia, which were diagnosed, in spotted sandbass from all of the sampling sites. Both of these lesion classes, but especially the foci of cellular alteration, have been extensively used in wild fish as histopathological biomarkers of exposure to contaminants such as PAHs. The highly selective and biased failure to report in the text that preneoplastic focal lesions were detected in the liver of spotted sandbass from all sites in this study is disturbing. Regardless of their stated rationale that the lesions were not discussed because there were no statistically significant differences in the prevalence of lesions among the sites, the lesions were identified during the histopathological examination, and their significance should have been evaluated in the discussion.

Upon independent review of the liver lesion data presented in Appendix 6, the following prevalence of foci of cellular alteration (clear cell foci, eosinophilic foci, basophilic foci) among the sampling sites were found: reference site (15.4%); inside NASSCO (18.0%); outside NASSCO (16.0%); inside Southwest Marine (9.8%); and outside Southwest Marine (16.0%). The same observations apply to the presence of cholangitis/biliary hyperplasia in the same fish, at the following prevalence: reference site (11.5%); inside NASSCO (34.0%); outside NASSCO (24%); inside Southwest Marine (19.6%); and outside Southwest Marine (20.0%). These data should be subjected to further statistical analyses that account for fish age (e.g. stepwise logistic regression analyses) to prove that there are/are not inter-site differences in risk of lesion occurrence. There is also a possible need for outside QA and review of the actual histologic slides to confirm/refute the presence of these focal lesions in the fish examined in this study.

Additional Work and Synthesis

In his report, Dr. Marty states the further need to synthesize the data to include fish age data (which has been done to a certain extent) and contaminant data. He also recommends Transmission Electron Microscopy of liver tissue to confirm lipofuscin, special stains to distinguish lipofuscin and hemosiderin (he did these special stains), and suggests doing CYP1A staining in liver to further document PAH exposure.

Review of Exponent Sediment Report, Section 8.2, Fish Histopathology

Some explanation should be included in this report as to why the spotted sandbass was collected rather than the white croaker, the original target species.

Lesions Elevated at Shipyard Locations

Based on NOAA's review of the histopathology report, it is clear that the authors of the Exponent report have been selective and have not fully reported Marty's findings and data from the appendices in Marty's report. Marty did find and report higher scores for liver lipofuscin in fish from the "inside" shipyard sites, higher scores for hepatic hemosiderin in fish from the "outside" shipyard sites, higher scores for renal nephritis in fish from "inside" NASSCO, and higher scores for shiny gill foci (gross lesion) in fish from "inside" Southwest Marine. However, he also found higher scores for lipofuscin in gonads of fish from the "inside" shipyard sites, as well as increased scores for pigmented macrophage aggregates in ovaries of fish from the "inside" shipyard sites. These lesions in the gonad are not discussed in the Exponent sediment report, and considering these lesions affect reproductive organs, they should have been discussed and evaluated.

In addition, NOAA's evaluation of the liver lesion data also suggests that the prevalence of cholangitis/biliary hyperplasia may be elevated compared to reference sites (11.5%), at the "inside" and "outside" shipyard sites, especially at the "inside" NASSCO site (34%).

The statement in the Exponent report that only 4 of the 70 lesions evaluated in the study were elevated in the shipyard sites compared to the reference site is overly simplistic, given that a large majority of the lesions were not toxicopathic in nature, and were in essence, incidental findings.

Lesions Elevated at the Reference Area

The relevance of lesions found at the reference site is oversimplified in Exponent's discussion and conclusion. The data presented in Table 8-18 are attempting to show the reader that the prevalence of some lesions were higher at the reference site, as compared to one or more of the shipyard sites, whether or

not these lesions have anything at all to do with exposure to contaminants. For example, renal tubular regeneration is higher at the reference site as compared to outside NASSCO, only; severe atresia of yolked oocytes is higher at the reference site as compared to inside SWM only. Other lesions with higher prevalence at the reference than at the shipyard sites are only gross lesions, none of which have an established relationship to contaminant exposure.

Significance of Lesions

NOAA reviewed the liver lesion data presented in Appendix 6 of the Marty report and found that a number of fish from both the reference site, and the "inside" and "outside" shipyard sites were affected by preneoplastic foci of cellular alteration, including basophilic, clear cell and eosinophilic foci. However, in the Exponent report it is falsely stated that only two fish in this study exhibited one of the liver lesions typically associated in other field studies with contaminant exposure. The two fish were from the reference site, and identified as affected with either a hepatocellular adenoma or a biliary carcinoma (both liver neoplasms).

Data presented in the appendices of the Marty report show that preneoplastic foci of cellular alteration were detected in fish from all of the sampling sites. The extent of these important preneoplastic focal lesions was not mentioned or discussed in the text of the Marty report. Although Marty diagnosed these lesions, and did not discuss the lesion data in his report text, the Exponent report directly states in the text (page 8-44, lines 8-13), and in Table 8-19, that these lesions did not occur in any fish examined. Even if no significant inter-site differences in the prevalence of these foci of cellular alteration were found, this is a significant omission of very important information. The existence of these lesions at any site indicates a harmful effect strongly linked to PAH exposure, whether that occurred at a reference or shipyard site. It is incorrect to state that these lesions were not detected in the study. The Exponent report should acknowledge the diagnosis of these lesions and should address their significance in the Sediment Report.

The existence of liver neoplasms and foci of cellular alteration in spotted sandbass from the "reference" site calls into question the appropriateness of the selected reference site. Based on information from other studies utilizing these lesions as histopathological biomarkers of contaminant exposure, these toxicopathic lesions rarely occur in fish from uncontaminated reference sites. The questionable appropriateness of the reference site is further shown by the very high levels of PAH metabolites measured in bile of spotted sandbass from the reference site. This issue is discussed in more detail in the section on fish bile near the end of this letter.

Evaluations of fish growth, condition, and spatial comparisons

NOAA recommends that the fish condition index be defined more precisely and be consistent with standard, accepted approaches. The condition index should be expressed as the weight in grams/(length in cm)³, and could be multiplied by 100 (Fulton's condition index). Also, fish growth in fisheries biology is typically assessed with formulas more complex than simple age at length curves. A more complex curve, like the Von Bertalanffy growth curve should have been used in the growth analysis. Based on the relatively low sample size, and the stratification by sex, it is not surprising that no clear trends in growth or condition factor were determined. However, these comparisons should be repeated using a proper condition index and the age-length relationships typically used to assess growth in fisheries biology studies. Exponent should provide these additional analyses and should discuss their significance.

Comparisons Based on Liver Lesions

A condition index commonly used in fish biology should be used here, as well as age-length relationships typically used in fish biology to assess growth (e.g., Von Bertalanffy growth curves). In the second paragraph, these results actually indicate that an adverse effect on fish growth was not associated with the presence of either abundant hepatic lipofuscin, or hemosiderosis. Relative to the condition index in fish with and without these lesions, the fact that these liver lesions tended to occur in older fish that typically possess higher condition indices helps to explain the fact that fish with the lesions had higher condition indices. These findings are not surprising. Similar comparisons of growth rates and condition factors in English sole, with and without toxicopathic liver lesions, and that have exceptionally strong and consistent associations with exposure to PAHs, have also rarely shown any effect of these lesions on growth or fish condition in wild fish.

Review of Exponent Sediment Report, Section 8.3, Fish Bile

The finding of levels of fluorescent aromatic compounds (FACs) at benzo[a]pyrene wave lengths in the range of 0.7-4.6 ug/g protein at the reference site clearly shows exposure to PAH levels far beyond what would normally be expected at a relatively uncontaminated reference site. In most new publications in which FACs data are presented, including those from studies done by the Northwest Fisheries Science Center, biliary FACs data are typically expressed in ng BaP equivalents/g protein, so that the protein-adjusted levels in fish from the present study ranged from 700-4600 ng/g protein, with a mean of 2070 ng/g protein. These levels are far beyond the level of 1000 ng BaP equiv/g protein that NOAA typically uses as a benchmark to define a response in fish from an area that is significantly contaminated by PAHs.

For example, previously reported biliary FACS data from barred sandbass from sites in San Diego Bay and vicinity (McCain et al., 1992), showed levels ranging from ~100 ng/g at the Dana Point reference site, to approximately 1600 ng/g at East Harbor Island, approximately 4000 ng/g at 28th Street Pier (near the Southwest Marine and NASSCO) sites, and approximately 5500 ng/g at National City. Except for the reference site value at Dana Point, which was considerably lower than the levels at the reference site for the present study, these levels in a closely related species, barred sandbass, are comparable to the levels detected from similar sites in the present study in spotted sandbass.

It would also be helpful in the presentation of the biliary FACs data if Figures 8-34 through 8-36 could be shown as means \pm 1 std. deviation or a 95% confidence interval, rather than as means, minimum and maximum. Presentation of the data in this suggested format is the more accepted format in scientific documents, and will enable the reader to interpret the statistical relationships among levels at the reference and shipyard sites, as well as to more critically evaluate the data with respect to some of the statements made on p. 8-49. For example, the statement is made that levels of bile breakdown products (actually, these are usually referred to as "metabolites") in fish from the shipyards are not significantly greater ($P < 0.05$) than concentrations at the reference area. This in fact may be the case, but it is not possible to critically evaluate this statement in the format in which the data are presented. Moreover, it is probably not valid to state that "concentrations in fish from within the shipyard leaseholds are generally less than concentrations in fish from outside the leaseholds", if in fact there is no statistically significant difference between "inside" and "outside" sites.

Report Conclusions

Exponent's report concludes that fish from in or near the shipyards are not affected by contaminant exposure. This conclusion is overly simplistic and ignores some important data and diagnoses related to effects associated with contaminants known to be found at the Shipyards. Exponent and/or Dr. Marty should re-evaluate the data as recommended in these comments, and submit the data and diagnosis for additional quality assurance evaluation by another histopathologist prior to making any definitive conclusion regarding the impact to fish from site-related contaminants.

Thank you for the opportunity to comment of this report. If you have questions related to these comments, please contact me at (916) 255-6686, or directly contact Mark Myers at (206) 860-3329.

Sincerely,



Denise M. Klimas
Coastal Resources Coordinator
Office of Response and Restoration

Reference:

McCain et al., 1992. Chemical contamination and associated fish diseases in San Diego Bay. *Environmental Science & Technology* 26(4): 725-733.

Cc:

Mark Myers, NOAA NMFS
Donald MacDonald, NOAA ORR
Scott Sobiech, USFWS
Katie Zeeman, USFWS
Bill Paznokas, CA F&G
Laura Hunter, Environmental Health Coalition