FINAL REPORT

ASSESSMENT OF EXPOSURE TO AROMATIC COMPOUNDS IN FISH FROM THE LOWER COLUMBIA RIVER, BY USE OF APPROPRIATE BIOMARKERS

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UNITED STATES DEPARTMENT OF COMMERCE
National Oceanic and Atmospheric Administration
NATIONAL MARINE FISHERIES SERVICE

Environmental Conservation Division
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2725 Montlake Boulevard East
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January 19, 1996

Don Yon
Oregon Department of Environmental Quality
Water Quality Division
811 SW 6th Ave
Portland, OR 97204

Dear Don,

Enclosed is our final report titled 'Assessment of exposure to aromatic compounds in fish from the Lower Columbia River, by use of appropriate biomarkers'. This study was conducted under the intergovernmental agreement between NOAA and the Lower Columbia River Bi-State Program (contract numbers 85-95 and C9500212, for Oregon DEQ and Washington DOE, respectively). There were no changes requested or suggested in the peer review comments which we received on January 12, but you should note that we have made slight editorial changes to this final document resulting from our own internal review process. However, our results, conclusions, and recommendations are unchanged.

We were glad to be able to participate in this investigation, and look forward to continuing to work with your program on issues related to habitat quality in the Lower Columbia River.

Sincerely,

Tracy K. Collier, Ph.D.
Ecotoxicology Branch Manager

Enclosure

cc Stein F/NWC2
Varanasi F/NWC

Background and Rationale

There is considerable evidence that aromatic compounds (ACs) and their derivatives are responsible for a variety of serious biological effects in fish exposed to such compounds. While much of this information is derived from laboratory studies, there is an increasing body of evidence from field studies which supports such relationships between AC exposure and biological damage (Casillas *et al.*, 1991; Johnson *et al.*, 1988; Landahl and Johnson, 1993; Myers *et al.*, 1987; Rhodes *et al.*, 1987). However, certain classes of ACs (e.g. polycyclic aromatic hydrocarbons) are subject to extensive metabolism and depuration by many species of fish (Varanasi *et al.*, 1989). Accordingly, direct measurement of tissue concentrations of parent compounds does not generally provide a useful indicator of exposure of fish to all classes of ACs. In recent years, however, methods have been developed for determining such exposure, based on our increased understanding of the mechanisms and pathways of AC metabolism in fish.

One such method, the measurement of fluorescent aromatic compounds (FACs) in bile, relies on the fact that many fluorescent metabolites of PAHs are primarily excreted via the hepatobiliary system in fish. This method has the added advantage of being able to be 'fine-tuned' for different classes of PAH, such as 2-3 ring, 3-4 ring, or 4-5 ring structures (Krahn *et al..*, 1992; Krahn *et al..*, 1984). Another method relies on the fact that hepatic cytochrome P4501A (CYP1A), together with the associated monooxygenase activity aryl hydrocarbon hydroxylase (AHH), can be rapidly induced in many fish species after exposure to a wide variety of organic contaminants, including PAH (Collier and Varanasi, 1991; Payne *et al.*, 1987; Payne and Penrose, 1975). Thus the induction of CYP1A represents an early biological effect which is also useful in estimating PAH exposure of fish. Moreover, the induction of CYP1A is initiated by the interaction of compounds with the *Ah* receptor, a step believed to be integral to the development of toxic effects, including

reproductive dysfunction, teratogenicity, mutagenicity, and carcinogenicity. An additional concern is based on our knowledge that electrophilic intermediates of certain ACs (e.g. high molecular weight PAHs) can form covalent adducts with nucleophilic centers within biological macromolecules, including hepatic DNA. Recently a method utilizing ^{32}P-postlabeling of deoxynucleotides and chromatographic separation of adducted nucleotides from normal nucleotides has been developed which is highly sensitive for detecting bulky hydrophobic DNA adducts in organisms exposed to ACs (Gupta and Randerath, 1988; Varanasi *et al.*, 1989b). Each of these methods has been developed for use in determining AC exposure of fish by the staff of the Environmental Conservation Division (ECD), of the Northwest Fisheries Science Center. The time- and dose- responsiveness of each of these measures has been determined after exposure to either single ACs or to complex mixtures of ACs and other organic contaminants (Collier and Varanasi, 1991; Stein *et al.*, 1993; Stein *et al.*, 1994) and each of these measures has been extensively field tested by the ECD, both in Washington State (Collier *et al.*, 1995; Collier *et al.*, 1992; Krahn *et al.*, 1986; Stein *et al.*, 1992; Varanasi *et al.*, 1986) and elsewhere (Collier *et al.*, 1993a,b; Krahn *et al.*, 1992; Varanasi *et al.*, 1989). The results of these studies have shown that the combined use of several indicators is warranted, because these different methods all rely on different aspects of AC toxicokinetics, and provide useful information concerning possible toxic effects of AC exposure (Collier *et al.*, 1993; Stein *et al.*, 1992).

The objective of the current investigation was to assist in the Lower Columbia River Bi-State Program Fish Health Study, by using biochemical methods (e.g. biomarkers) to determine exposure to aromatic compounds, of fish sampled by other participants in the Program (Tetra Tech). This was done by measuring biliary levels of fluorescent aromatic compounds (FACs) and hepatic activities of cytochrome P4501A (CYP1A) dependent enzyme activities, in largescale suckers

collected from several sites in the Lower Columbia River. Levels of DNA adducts will be measured at a later date. The work performed was a cooperative effort between the Oregon/Washington Lower Columbia River Bi-State Program and NOAA, National Marine Fisheries Service, Northwest Fisheries Science Center, Environmental Conservation Division (ECD).

Sample Collection

Largescale suckers were captured by electrofishing as described in the Sampling Plan prepared by Tetra Tech (Draft Report TC 0110-01). Blood was collected from the caudal blood vessels from each fish captured, then fish were killed by a blow to the head with a wooden club. Fish were examined for any external abnormalities, and then necropsied according to the protocols provided by the ECD. Liver and bile were removed from each fish and immediately flash frozen in liquid nitrogen (-196°C). Samples were subsequently placed in a liquid nitrogen dry shipper which was recharged periodically during the sampling period to maintain cryogenic temperature. Initial collection of samples for biomarker analyses (liver and bile) was conducted with technical support of ECD personnel (locations I-1 and I-2, sampled Jan. 30-31, 1995). Tetra Tech personnel were taught necropsy techniques for removal and freezing of liver and bile, and subsequently performed all necropsies at sites sampled after Jan. 31, 1995. Table 1 provides a summary of the number of biomarker samples collected at each location. All fish were captured at what were described as either Industrial/Urban or Backwater locations (Tetra Tech, Update on the Fish Health Work Assignment TC 0110-01). No fish were captured at any of the Main channel locations surveyed. At the completion of the sampling effort the charged dry shipper, with samples inside, was turned over to the ECD by Tetra Tech, on Feb. 10, 1995. Liver samples were stored in

a -80°C ultra-cold freezer and bile samples were stored in a -20°C freezer until further sample preparation and analyses were performed.

Sample Analyses

Fluorescent aromatic compounds (FACs) were measured in the bile, using the HPLC method of Krahn *et al.*, (1984), with minor modification (Collier and Varanasi, 1991). Wavelength pairs were 380/430 nm excitation/emission, to measure 5-ring, high molecular weight compounds such as BaP and its metabolites (hereafter designated $FACs_{BaP}$), 256/380 nm for identifying 3-ring compounds such as phenanthrenes ($FACs_{PHN}$), and 290/335 nm for identifying 2-ring compounds such as naphthalenes ($FACs_{NPH}$). External standards of BaP, PHN and NPH were used, and the fluorescence response converted to the respective equivalents. In addition, levels of FACs were normalized based on levels of biliary protein, in order to account for the feeding status of each fish (Collier and Varanasi, 1991). Quality assurance procedures for the determination of FACs in bile included periodic calibration of instruments and analysis of duplicate samples, analyses of a reference bile material with every sample set, and participation in interlaboratory comparison studies. The AHH assays were performed following the method of Collier *et al.*, (1986) with modification as described in Collier *et al.*, (1995). Assays were performed in triplicate using ^{14}C-benzo[a]pyrene (BaP) as the primary substrate. For quality assurance, the acceptable percent coefficient of variation (% CV) within each triplicate was less than 20%, and if a single outlier existed in the triplicate it was removed in order to reduce the % CV to less than 20%. Samples were rerun only if the % CV within the remaining duplicate was still greater than 20%. Protein content of microsomal suspensions was measured using the method of Bradford, 1976. The method of Lowry *et al.*, (1951) was used to determine protein levels in

bile. Bovine serum albumin was used as a standard for both types of protein measurements.

Results

All tissues collected for CYP1A were analyzed (79 total) along with all bile samples where a sufficient volume was collected to allow analysis (30 total). Mean levels of CYP1A (measured as hepatic AHH activities), and levels of biliary FACs are given in Table 2, and Figures 1 and 2 provide bar charts of the mean values, by site, in descending order. Analyses of variance (ANOVAs) were performed on log-transformed data followed by Fisher's Protected Least Significant Difference (PLSD) test to determine if there were any significant differences among sites (significance level was set at 0.05). Levels of biliary FACs and hepatic AHH activities, by site and gender, are depicted in Figure 3. Results from all ANOVAs performed are given in Table 3. Appendices A and B provide the raw data and final values for individual samples.

No between-site differences were observed for either the levels of biliary FACs or for hepatic AHH activities, based on ANOVAs of these data sets (Table 3). Another set of ANOVAs were run, with all sites designated as either 'backwater' or 'industrial', (designations received from Tetra Tech), and again there were no significant differences between these two types of sites for either biomarker measured. Many of the females sampled showed signs of ovarian development, and because spawning is known to affect these biomarkers in females of some fish species, ANOVAs were performed to test for differences between male and females. Sex differences were noted, especially for AHH activities, where levels were significantly lower in females compared to males. Because of this sex difference, ANOVAs were also run to test for between-site differences, using values from female fish only (the majority of the fish were females). These ANOVAs also

showed essentially no significant between-site differences for all measures, except that $FACs_{BaP}$ had an overall significance level of 0.05 for between-site differences. Fisher's PLSD analysis of this result showed that values for females sampled at site B-8 were lower than values from females sampled at sites B-6, B-9, I-1, and I-7 (see Figure 3 for data presentation). We also examined the data to see if there were any relationships between individual measurements of FACs in bile and hepatic AHH activities, and found no significant correlations, based on linear regression (significance level set at 0.05, results of statistical analysis not shown).

Interpretations and Conclusions

Overall, the results of this study do not provide evidence for marked exposure of largescale suckers to aromatic compounds in the lower Columbia River. There were generally no significant between-site differences for either levels of biliary FACs or hepatic AHH activities, and no significant differences between sites designated as 'industrial' compared to sites designated as 'backwater'. These data are difficult to interpret, however, due to the small number of samples collected at each site, the apparent sex difference in biomarker response (likely due to advanced reproductive status of females), and the absence of any site clearly designated as a reference site.

The levels of biliary FACs in largescale suckers, though not showing significant between-site differences, were comparable to levels previously measured in other fish species from moderately contaminated areas (Stein *et al.*, 1992). This may be partially due to the relatively low levels of biliary protein measured in largescale suckers, compared to biliary protein content of other fish species (T. Hom, personal communication). Thus, when values are corrected for biliary protein content, species with low protein values may tend to show higher levels of biliary FACs compared to other species with higher biliary protein contents. In a previous

study of FACs in bile of white sturgeon following the 1984*Mobiloil* oil spill in the lower Columbia River, levels of FACs were reported without protein normalization. The mean levels of biliary FACs reported in that study (Krahn *et al.*, 1986) for reference sites upriver of the grounding site (near St. Helens, OR, at river mile 87), were approximately 130, 9600, and 24,000 ng/g bile, for $FACs_{BaP}$, $FACs_{PHN}$, and $FACs_{NPH}$, respectively. At an impacted site 57 miles downstream from the spill site, these same respective measurements were 2100, 210,000, and 200,000. Values obtained for largescale suckers, in the current Bi-State study, were between these values (overall mean values, uncorrected for protein content of bile, were 470, 26,000 and 110,000). Thus, these values in largescale sucker were 2-3 fold higher than reference values for white sturgeon, but substantially lower than values for sturgeon from a putatively oil-impacted site. However, with no significant between-site differences seen in levels of biliary FACs, and in the absence of dose-response data for largescale suckers, it is difficult to conclude that the FAC data from this study as shows evidence of exposure of largescale suckers to ACs.

Hepatic AHH activities in largescale suckers were considerably lower than previously reported for other benthic fish species from moderately and severely contaminated sites. For example, the highest mean AHH activity measured in the current study was 123 (pmoles BaP metabolized per mg protein per minute), and in a study of several sites in Puget Sound, in three species of flatfish, the highest mean activities ranged up to 800, with the lowest mean value at a reference site of 95 (Stein *et al.*, 1992). Induction of hepatic AHH activity has been shown to be the one of the most sensitive biomarkers of organic contaminant exposure in benthic fish species (Collier *et al.*, 1988; Collier and Varanasi, 1991). Accordingly, lack of significant induction of this biomarker argues against substantial exposure of the fish sampled in this study. There was some depression of AHH activity in females, likely due to reproductive status, and females made up the majority of the fish

sampled, making the results of this biomarker measurement difficult to interpret. However, in the limited number of males that were analyzed from five of the sites, there was no evidence of increased AHH activities.

Recommendations

Suggestions for modification of sampling protocol and experimental design for future studies, to allow for a better resolution of possible low levels of exposure, would include the following:

- *A priori* determination of suitable reference sites for comparison. This might be helped by more sampling effort at main channel sites, to determine if these sites may serve as reference sites.
- Earlier sampling, to minimize sampling of females undergoing gonadal maturation. That was the intention of this study, but unforeseen delays in obtaining sampling permits precluded earlier sampling.
- Increased sampling effort, to assure collection of minimum numbers of fish of both sexes at each site.
- Chemical analyses of stomach contents and surficial sediments, to determine the presence of aromatic contaminants in the fish's habitat.
- Consideration of other species for sampling, including piscivorous species such as northern squawfish, which may have a greater potential for bioaccumulation of persistent halogenated aromatic contaminants.

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