Cytochrome P450 1A and related measurements in juvenile chinook salmon (*Oncorhynchus tshawytscha*) from the Fraser River[1]

J.Y. Wilson, R.F. Addison, D. Martens, R. Gordon, and B. Glickman

Abstract: Juvenile chinook salmon (*Oncorhynchus tshawytscha*) were captured at six sites on the upper Fraser, Nechako, and Thompson rivers, British Columbia, Canada. Biological responses were measured in the liver to assess the effects of contaminants on the fish before they began migration downstream. Both ethoxyresorufin-*O*-deethylase (EROD) activity and CYP 1A concentrations were significantly enhanced, being two- to three-fold higher in Fraser River samples compared with those fish from reference sites on the Nechako River. DNA adduct concentrations were two- to four-fold higher in Fraser River fish, although liver histopathology appeared unaffected. Polychlorinated dibenzo-*p*-dioxins (PCDDs), polychlorinated dibenzofurans (PCDFs), and polychlorinated biphenyls (PCBs) in the carcasses contributed to total contaminant burdens of less than 1 pg·g^{-1}. Polycyclic aromatic hydrocarbon (PAH) metabolites were undetectable in nearly all samples of bile. There were strong correlations between EROD activity, CYP 1A induction, and DNA adduct concentrations but no clear correlation between these responses and PCDD, PCDF, or PCB concentrations.

Résumé : Des juvéniles de saumon quinnat (*Oncorhynchus tshawytscha*) ont été capturés à six sites du cours supérieur du Fraser, de la Nechako et de la Thompson (Colombie-Britannique), Canada. Nous avons mesuré les réponses biologiques dans le foie pour évaluer les effets des contaminants sur les poissons avant le début de leur dévalaison. L'activité de l'éthoxyrésorufine-*O*-déséthylase (EROD) et les concentrations du CYP 1A étaient nettement accrues, puisqu'elles étaient deux à trois fois plus élevées dans les échantillons du Fraser que chez les poissons des sites témoins de la Nechako. Les concentrations des adduits d'ADN étaient de deux à quatre fois plus élevées chez les poissons du Fraser, mais l'histopathologie hépatique ne semblait pas touchée. Les dibenzo-*p*-dioxines polychlorées (PCDD), dibenzofuranes polychlorés (PCDF) et les polychlorobiphényles (PCB) présents dans les carcasses contribuaient aux charges totales de contaminants, qui étaient inférieures à 1 pg·g^{-1}. Les métabolites d'hydrocarbures aromatiques polycycliques (HAP) étaient indétectables dans pratiquement tous les échantillons de bile. Nous avons noté de fortes corrélations entre l'activité EROD, l'induction du CYP 1A et les concentrations d'adduits d'ADN, mais aucune corrélation claire entre ces réponses et les concentrations de PCDD, de PCDF ou de PCB.

[Traduit par la Rédaction]

Introduction

The upper Fraser River and its tributaries are spawning grounds and overwintering sites for chinook salmon (*Oncorhynchus tshawytscha*). Overwintering juveniles may be affected by contaminants from industrial and municipal discharge (Servizi et al. 1993), which may result in decreased survival during smoltification. Rogers et al. (1989) found high concentrations of 2,3,7,8-tetrachlorodibenzo-*p*-dioxin (TCDD) and 2,3,7,8-tetrachlorodibenzofuran (TCDF)

(68 and 370 pg·g^{-1}, respectively) in chinook at one site on the Fraser River, although bleached kraft mills have altered practices so that contaminant discharges have decreased since this study was completed. The chinook in the upper Fraser River watershed spend over 1 year in the river system before migrating downstream to smoltify. They are a good sentinel species in which to investigate contaminant toxicity, since they have been exposed to contaminants continuously throughout early development.

Ethoxyresorufin-*O*-deethylase (EROD) activity and CYP

J.Y. Wilson.[2] Department of Fisheries and Oceans, Contaminants Science, Institute of Ocean Sciences, P.O. Box 6000, Sidney, BC V8L 4B2, Canada, and Biology Department, University of Victoria, P.O. Box 1700, Victoria, BC V8W 2Y2, Canada.
R.F. Addison. Department of Fisheries and Oceans, Contaminants Science, Institute of Ocean Sciences, P.O. Box 6000, Sidney, BC V8L 4B2, Canada.
D. Martens and R. Gordon. Department of Fisheries and Oceans, Contaminants Science, Cultus Lake Laboratory, 4222 Columbia Valley Road, Cultus Lake, BC V2R 5B6, Canada.
B. Glickman. Biology Department, University of Victoria, P.O. Box 1700, Victoria, BC V8W 2Y2, Canada.

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[2]Author to whom all correspondence should be sent at the following address: MS#32 Woods Hole Oceanographic Institution, Woods Hole, MA 02543, U.S.A. e-mail: jwilson@whoi.edu

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1A induction have been used as biological monitoring tools in a number of studies. EROD activity is a measure of the catalytic activity of the CYP 1A protein, and therefore, we expect these measurements to be well correlated, although in practice, this is not always true (Peters et al. 1997). The induction of EROD activity or CYP 1A protein can be used as a biomonitor of exposure to organic contaminants, since it occurs after exposure to certain chemical classes, including polychlorinated dibenzo-*p*-dioxins (PCDDs), polychlorinated dibenzofurans (PCDFs), and nonortho- (NO-) and monoortho- (MO-) polychlorobiphenyls (PCBs). Induction of the CYP 1A system has been linked with higher order effects in fish (discussed in Addison 1996) and thus serves as a useful measure of toxic effects in the field.

Measurement of DNA adducts by the ^{32}P postlabeling assay is a useful tool for assessing polycyclic aromatic hydrocarbon (PAH) exposure and toxicity. This method is particularly useful for complex chemical exposure, as it integrates all adducts into one measurement. PAH–DNA adducts are formed by binding of reactive intermediates, created during metabolism of the parent hydrocarbons by cytochrome P450 1A, to DNA and other macromolecules in the cell. DNA adduct concentrations and aryl hydrocarbon hydroxylase (AHH) activity, an enzyme activity similar to EROD and also catalyzed by CYP 1A, have been shown to be strong risk factors for early toxicopathic hepatic lesions in subadult English sole (*Pleuronectes vetulus*) (Myers et al. 1998).

In this paper, we describe the concentrations or activities of CYP 1A, EROD, and DNA adducts in juvenile chinook from several sites in the Fraser River. The biological responses should reflect contaminant burdens, and if pulp mill effluent exposure is significant in chinook, the response should be consistent in terms of spatial distribution of responses downstream. These biological responses were chosen to assess the effect of contaminants on juvenile chinook just before migration. We show that EROD activity, CYP 1A concentrations, and DNA adduct concentrations are not well correlated with concentrations of PCDDs, PCDFs, or PCBs, which are among the expected agents responsible for these effects.

Materials and methods

Sample collection and study sites

Juvenile (1+ years) chinook salmon were captured at six sites on the upper Fraser, Thompson, and Nechako rivers, British Columbia, Canada, during March and April 1995 (Fig. 1). The reference sites were located on the Nechako River, and the sites on the Fraser and Thompson rivers were expected to be impacted from the discharge of pulp mill effluent. Fish were captured by electrofishing, and the liver and gallbladder were removed and frozen in liquid nitrogen for transport. The carcasses were placed on dry ice. Livers for histopathology were placed in Bouin's fixative.

The Nechako River has no known source of contamination, and fish from these sites were low in contaminants in previous studies (R. Gordon and D. Martens, unpublished data). Three bleached kraft pulp mills at Prince George discharge to the Fraser above the confluence of the Nechako, and a sewage outfall from the city enters below the confluence. One bleached kraft mill, a thermomechanical pulp mill, and a sewage outfall discharge waste into the Fraser at Quesnel. There is a bleached kraft mill, other industrial discharges, and a sewage outfall at Kamloops, upstream of the

study site, but the effluent travels through Kamloops Lake before it enters the Thompson River.

In March, fish from the Thompson River were caught near Spences Bridge (Thompson on Fig. 1). In April, fish were caught at the remaining sites. Reference fish from the Nechako River were caught at Swanson's Creek and near Fort Fraser. These sites are so near each other that they are only denoted by one location (control site) on Fig. 1. On the upper Fraser River, fish were caught just below the diffuser at Northwood Pulp and Timber, Prince George (Northwood on Fig. 1), at Stoner and Longbar above Quesnel, and at Soda Creek below Quesnel. The locations of the study sites and some discharges are outlined on Fig. 1.

The delay in sampling between the Thompson River site and the other sites was due to the delay in ice breakup on the river at the more northern sites. Ice must clear from the sides of the river, as chinook habitat is limited to the shallow waters where the flow is slower. An effort was made to collect 20 fish per site, although this was not possible at all sites.

Biological measurements

Microsomes were prepared from pools of two frozen liver samples according to Hodson et al. (1991). Microsomes were analyzed for protein concentration, EROD activity, and CYP 1A protein. Protein concentrations were determined by the method of Bradford (1976) using bovine serum albumin (Sigma, St. Louis, Mo.) as a standard. EROD activity was determined by the methods of Hodson et al. (1991) using 7-ethoxyresorufin and resorufin (Molecular Probes, Eugene, Oreg.) as substrate and standard, respectively. CYP 1A protein was determined by western blotting as described by Arlotto and Parkinson (1989) using an antibody raised against a synthetic peptide (Myers et al. 1993). The primary antibody has been shown to recognize CYP 1A proteins in diverse species (Lin et al. 1998). CYP 1A protein was expressed as a ratio to the intensity of rat 1A1 standard from the same membrane. CYP 1A protein is referred to as integrated density and is not converted to picomoles of protein because there is a difference in the affinity of the primary antibody for the standard and samples. All analyses were done in triplicate and averaged. The primary antibody and rat 1A1 standard used for these analyses were prepared by Dr. Stelvio Bandiera (Pharmaceutical Sciences, University of British Columbia, Vancouver, B.C.).

DNA adducts were analyzed according to Reichert and French (1994). DNA was extracted from the pellet formed during the $10\,000 \times g$ centrifugation for microsome preparation using a phenol–chloroform extraction technique (Reichert and French 1994). The adducts were enriched by the nuclease P1 method, which enzymatically alters normal (unadducted) nucleotides to prevent them from being labeled. Adduct concentrations were determined using phosphor imaging techniques.

Livers for histopathology were embedded in paraffin, sectioned at 7 μm, and stained with hematoxylin and eosin. Sections were examined under a light microscope. Samples were coded for analysis so that histopathological examinations were blind.

Contaminant analysis

PCDDs, PCDFs, NO-PCBs, and MO-PCBs were analyzed in the carcasses (minus liver and gallbladder) using a succession of extraction and column chromatographic cleanup steps followed by high-resolution gas chromatography – high-resolution mass spectrometry (HRGC/HRMS) as described elsewhere (Anonymous 1992*a*, 1992*b*; MacDonald et al. 1997). The criteria for identification and quantification and the quality control measures used for the HRGC/HRMS analysis of all the compounds of interest were based on procedures described elsewhere (Anonymous 1992*a*, 1992*b*). Details of chromatography and of instrument operating conditions are described in MacDonald et al. (1997). Because in-

Fig. 1. Fraser River watershed in British Columbia, Canada. Study sites, pulp mill effluent discharges, and sewage outfalls are marked according to the legend. The Nechako River site is the control site and approximates both sites used for the reference site. Major towns and cities are marked with a circle for reference.
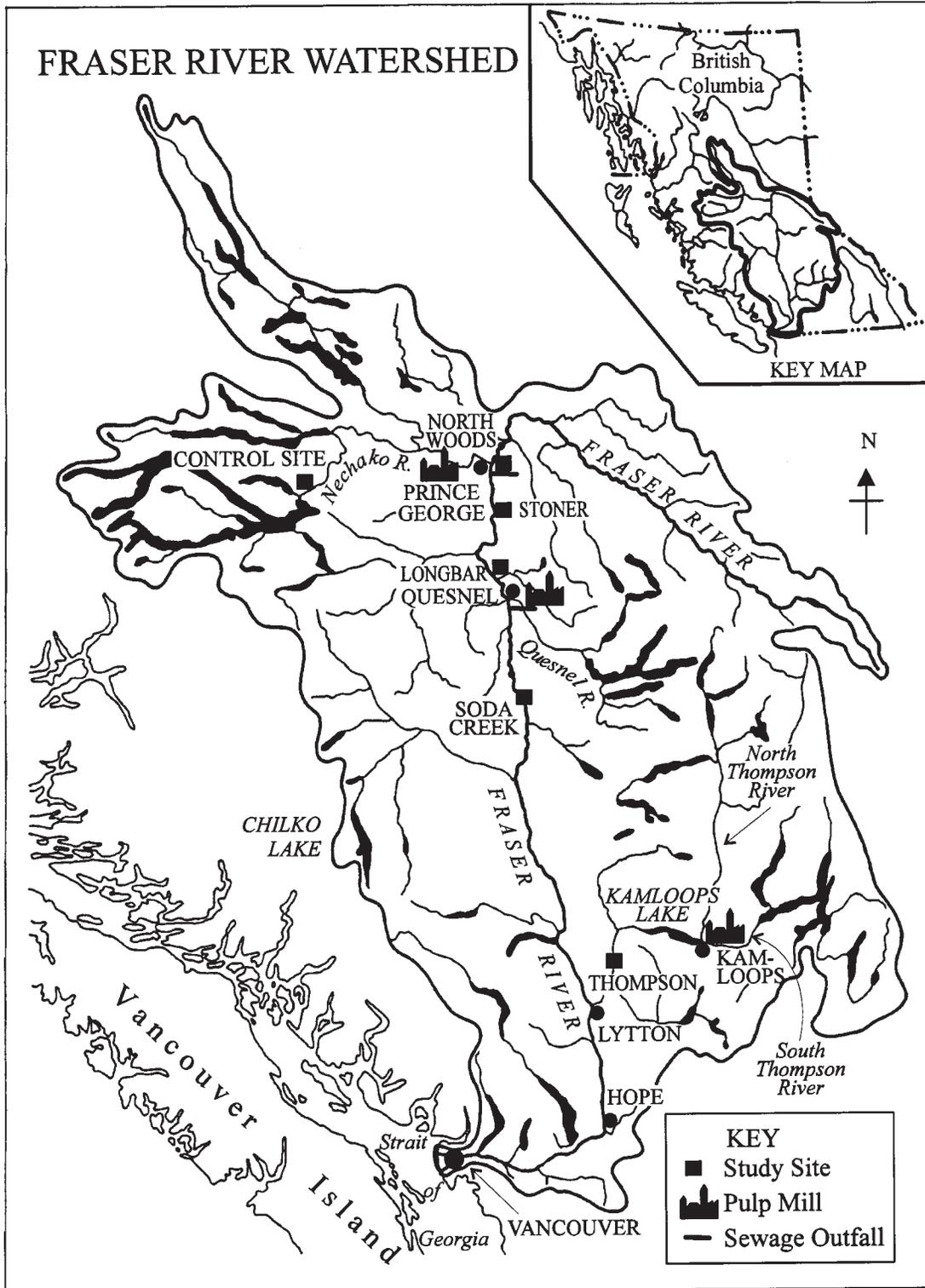
sufficient biomass was available for analysis of individual fish, two pools of fish were analyzed per site. Data are reported as picograms per gram and have been corrected for the percent recovery of the reference standard. Toxic equivalent quotients (TEQs) were calculated from toxic equivalency factor (TEF) data (NATO-CCMS 1988; Ahlborg et al. 1994)

Bile sacs were centrifuged to break them open and the bile was removed for analysis. Two pools of bile were analyzed per site. In

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some cases, dilution of the bile sacs with water was required to attain enough sample for analysis. PAH metabolites were analyzed by synchronous scan fluorimetry for 1-hydroxypyrenol equivalents according to Ariese et al. (1993). Protein concentrations in the bile were analyzed using the Bradford assay (Bradford 1976), modified for a microplate reader.

Statistical analysis

All statistical analyses were performed using Statistica 5.1 (Statsoft, Tulsa, Okla.). Differences among site means were analyzed using ANOVA and Fisher's least significant difference test, when Levine's test for homogeneity of variance was nonsignificant and examination of mean versus standard deviation plots showed no significant relationship. Pearson's correlations were used to examine relationships between variables. Data are reported as mean ± SD, except for contaminant data (see Table 2), which are based on an average of two pools.

Results

General characteristics

The juvenile chinook were approximately 18 months of age with a mean fork length and weight of 8.19 cm and 6.94 g, respectively (Table 1). The weight data were not normally distributed ($P = 0.02$, Shapiro–Wilks W test), in part because there was a significant difference between the mean weights of the two Nechako collections (Swanson's Creek and Fort Fraser, Table 1). There were significant differences in the lengths and weights among sites. The fish could not be separated by sex via gonadal development and thus were grouped together. A few individuals had internal parasites and the largest number were in the fish caught from the Nechako River near Fort Fraser (not kept for identification). Fish collected from different sites did not exhibit any difference in organ appearance, weight, or length except for a few fish from Soda Creek, which had an increase in mesenteric fat. In previous years' sampling, increased mesenteric fat was associated with pale, mushy livers, increased weight, the occasional fin clip, and very different contaminant profiles, suggesting that they were hatchery-reared fish (R. Gordon and D. Martens, unpublished data). In this study, fish with increased mesenteric fat had none of these characteristics (change in liver appearance, increased weight, fin clips) and contaminant profiles were not different. Fish collected from Soda Creek were considered to be from wild stock.

Biological measurements

The EROD activities, CYP 1A data, and DNA adduct concentrations were not distributed normally ($P < 0.001$, Shapiro–Wilks W test), and thus, the log-transformed data were used for all analyses. The site means for these measurements (geometric means) are shown in Fig. 2. There was no difference between the two collections from the Nechako River, and they were thus combined for further analyses. EROD activities (Fig. 2a) were generally increased at downstream sites with a maximum at Soda Creek. The EROD activity at the Thompson River site was similar to that at the Nechako site. Fish from the Nechako River had lower CYP 1A densities than those from all other sites ($P < 0.001$) (Fig. 2b). Fish from Northwood and Thompson River were found to have CYP 1A densities significantly different from those of Stoner and Longbar with P values of <0.02 and <0.01, re-

Table 1. Lengths and weights of chinook salmon.

Study site	Length (cm)	Weight (g)
Nechako River		
Swanson's Creek ($n = 11$)	8.68 (0.52)	7.67 (1.13)
Fort Fraser ($n = 17$)	9.53 (0.62)	10.67 (1.64)
Combined ($n = 28$)	9.20 (0.71)	9.49 (2.07)
Northwood ($n = 16$)	6.79 (1.02)	3.68 (1.72)
Stoner ($n = 20$)	7.16 (0.71)	4.66 (1.55)
Longbar ($n = 20$)	7.71 (0.55)	5.99 (1.20)
Soda Creek ($n = 20$)	8.66 (0.74)	8.78 (2.30)
Thompson River ($n = 20$)	8.96 (0.80)	7.34 (1.86)
All sites ($n = 124$)	8.19 (1.17)	6.94 (2.77)

Note: Data are reported as means (SD); n is the number of fish sampled at each site.

spectively. There was no difference in CYP 1A between Stoner, Longbar, and Soda Creek samples. The mean DNA adduct concentrations are shown in Fig. 2c. As was the case for CYP 1A, concentrations reached a maximum downstream at Stoner and Longbar.

Livers from all sites except Northwood, where there were not enough fish caught, were kept for histopathology. Three conditions were found: vacuolation, hepatocellular steatosis, and hydropic vacuolation (data not shown). The most frequent condition found was vacuolation, which was at all sites studied (frequencies ranged from 0.15 to 0.83). Hydropic vacuolation was found in fish from Nechako (frequency of 0.16) and Stoner (frequency of 0.13), while hepatocellular steatosis was found at Stoner, Longbar, and Soda Creek (frequencies ranged from 0.19 to 0.20). There was no clear pattern in the frequency of lesions among sites.

Correlations were performed on all biological response measurements with respect to length and weight to ensure that differences in response among sites were not related to differences in length or weight. There was no correlation between either mean length or mean weight and any other measurement (data not shown).

EROD, CYP 1A, and DNA adducts were analyzed on the same pool of livers for each sample. There was a significant correlation between EROD activity and both CYP 1A density and DNA adducts and between CYP 1A density and DNA adducts (Table 2).

Contaminants

There was very little bile in each gallbladder, so up to nine gallbladders were pooled, and in some cases, samples had to be diluted to yield the volumes needed for analysis. Hydroxylated PAH metabolites were found in only one sample from Soda Creek (trace concentrations) and in samples from the Thompson River (3883 ng·mL^{-1} or 139.7 ng·mg protein^{-1}). In all samples, protein concentrations were above zero and ranged from 6.69 to 17.21 mg·mL^{-1} (data not shown). The detection limit of this analysis was 760 ng pyrene-1-glucuronide equivalents·mL^{-1}.

Carcasses were pooled to attain ≈30 g of fresh tissue for analysis. PCDD, PCDF, NO-PCB, and MO-PCB concentrations were expressed in terms of TEQs. Specific TEQs were calculated for each contaminant class (PCDDs, PCDFs, NO-PCBs, and MO-PCBs) and then summed to calculate the to-

Fig. 2. Biological responses in chinook salmon liver. (*a*) EROD activity. Data are shown as pmol resorufin·mg protein^{-1}·min $^{-1}$. (*b*) CYP 1A integrated density. Data are shown as density units, relative to the intensity of staining of 0.5 pmol of rat 1A1 standard. See Materials and methods for details. (*c*) DNA adduct concentration. Data are shown as nmol adducts·mol normal nucleotide^{-1}. For all graphs, data shown are geometric means. Error bars represent SD. Bars with different letters differ significantly ($P < 0.05$).
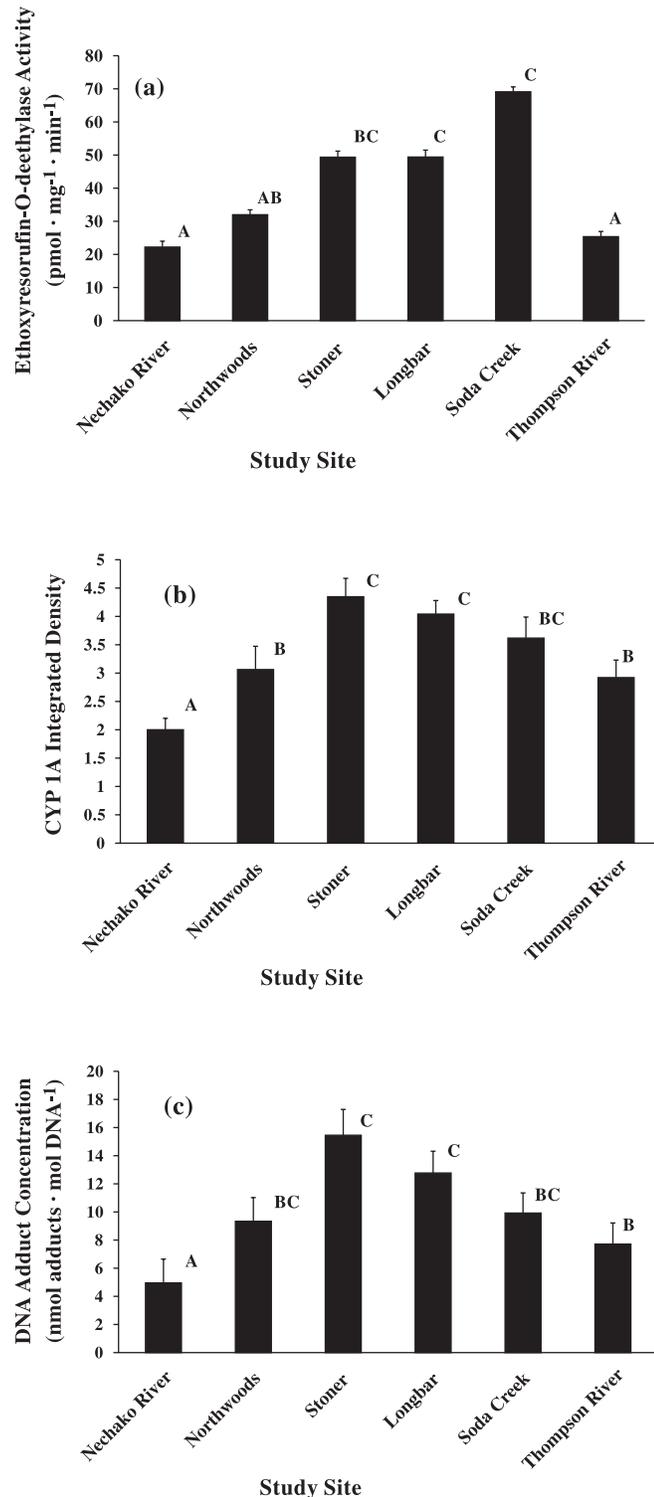
tal contaminant burden (total TEQ). The differences in TEQ values among sites were not tested statistically.

PCDD-specific TEQ concentrations in the fish ranged from 0.16 to 0.41 pg TCDD equivalents·g^{-1} (Table 3). Fish from Stoner and the Thompson River had the highest PCDD concentrations, while fish from the Nechako had the lowest. Fish from the two Nechako collections had similar contaminant concentrations and were combined for statistical analysis. Fish from Longbar, Soda Creek, and Northwood had very similar PCDD concentrations. PCDF-specific TEQ concentrations ranged from 0.09 to 0.26 pg·g^{-1} (Table 3), with Nechako fish being the lowest and fish from the Thompson River being the highest. Fish from Stoner, Longbar, and Soda Creek all had approximately the same PCDF concentrations.

Both MO- and NO-PCBs were detected with the HRGC/ HRMS analysis of carcasses. For the MO-PCBs, only eight congeners have established TEFs (PCB 123, 118, 114, 105, 167, 156, 157, 189), although 22 congeners were measured. For the NO-PCBs, only three congeners have established TEFs (PCB 77, 126, 169), while 18 congeners were measured. For the PCB-specific TEQ calculations, only those congeners with an established TEF are included. The MO-PCBs ranged from 0.02 to 0.06 pg TCDD equivalents·g^{-1} and showed very little variation between reference and exposed sites (Table 3). The NO-PCBs ranged from 0.09 to 0.16 pg TCDD equivalents·g^{-1} (Table 3). Fish from Northwood had the largest NO-PCB concentrations, but all other sites had comparable concentrations (0.08–0.12 pg·g^{-1}). The total TEQ was lowest in the Nechako River fish. The total TEQ ranged from 0.56 to 0.86 pg·g^{-1} at the other sites.

Multiple correlations between the different classes of organic contaminants were found (Table 4). PCDDs, PCDFs, and MO-PCBs were found to be strongly correlated, but NO-PCBs were found to be uncorrelated with the other contaminants. The total TEQ correlated with PCDDs, PCDFs, and MO-PCBs but not with NO-PCBs.

Contaminants and biological responses could only be compared using site means, as the pools were not equivalent. The Nechako collections were not pooled for this analysis. EROD activity, CYP 1A density, and DNA adducts were not correlated with any contaminant group or total TEQ (Table 5). There was no significant correlation between 1,2,3,6,7,8-HxCDD, 1,2,3,7,8,9-HxCDD, or 2,3,7,8-TCDF, the major PCDDs and PCDFs discharged by pulp mills (Yunker and Cretney 1996), and any biological response (data not shown).

Discussion

The relative EROD activities seen in this study are comparable with those previously reported in chinook, including juvenile migrating chinook from urban estuaries in Washington (Stein et al. 1995). Laboratory exposures to bleached kraft mill effluent (BKME) have shown levels of EROD activity similar to the highest in this study. After 60 and 144 days of exposure to 0.3–4% BKME (collected in 1989 from Northwood Pulp and Timber), juvenile chinook exhibited twofold induction over control exposures (Servizi et al. 1993). These effluent exposures were chosen to represent the exposures found at Stoner and Longbar in winter conditions.

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Table 2. Correlation matrix for biological measurements.

	EROD[a]	CYP 1A[b]	DNA adduct[c]
EROD	1.00	0.35	0.35
CYP 1A		1.00	0.40

Note: All correlations are significant ($P < 0.05$); based on $n = 60$ samples from all sites.
[a]EROD activities from Fig. 2a.
[b]CYP 1A densities from Fig. 2b.
[c]DNA adduct concentrations from Fig. 2c.

In previous years' sampling, EROD activity has been much higher in the Fraser River chinook (Rogers et al. 1989). However, temporal changes in the effluent characteristics (most notably PCDD and PCDF discharges) and methodology in measuring EROD activity interfere with the direct comparison of this work with field (Rogers et al. 1989) and experimental (Servizi et al. 1993) studies. It is unlikely that changes in methodology could account for the large decrease in EROD activity from Rogers et al. (1989).

CYP 1A density is not as commonly used in field studies as EROD activity, and the two measurements are rarely used together. Although the expectation is that these two markers would produce the same results, it is not proven that they are interchangeable. Peters et al. (1997) found increased EROD activity in the absence of measurable changes in CYP 1A in juvenile turbot (*Scophthalmus maximus*) exposed to benzo[a]pyrene. In this study, EROD and CYP 1A induction have covaried. This has also been seen in field studies of European flounder (*Platichthys flesus*) (Addison and Edwards 1988), longnose sucker (*Catostomus catostomus*), and mountain whitefish (*Prosopium williamsoni*) (Kloepper-Sams et al. 1994). EROD activity has also been found to correlate with levels of CYP 1A mRNA in dab (*Limanda limanda*) (Renton and Addison 1992). These studies indicate that measurement of various components of the CYP 1A system can be useful for biomonitoring.

Fish from the reference site had extremely low concentrations of DNA adducts that were distinguishable from all other sites. The concentrations found in these fish were very similar to concentrations of adducts in juvenile chinook from Washington State estuaries (Stein et al. 1995) and red drum (*Sciaenops ocellatus*) from Tampa Bay (McCain et al. 1996). The concentrations were lower than those found in field studies of English sole, rock sole (*Pleuronectes bilineatus*), and starry flounder (*Platichthys stellatus*) (Stein et al. 1990), winter flounder (*Pleuronectes americanus*) (Grondlund et al. 1991), and striped killifish (*Fundulus majalis*), gulf killifish (*Fundulus grandis*), and hardhead catfish (*Arius felis*) (McCain et al. 1996).

In most studies, correlations between biological measurements are not performed, but EROD (or AHH) activity and DNA adducts seem to covary in chinook (Stein et al. 1995), English sole, starry flounder (Stein et al. 1990), and striped killifish (McCain et al. 1996). Fish collected downstream from pulp mills showed a correlation between EROD activity and the ability to form benzo[a]pyrene–DNA adducts in vitro (Kantoniemi et al. 1996). Certainly, the finding that both CYP 1A and DNA adducts are risk factors for lesions in English sole (Myers et al. 1998) suggests that these measurements could covary. CYP 1A is not the only factor important in DNA adduct formation. Many studies now indicate that other factors are critical for the time course of adduct formation (Peters et al. 1997) and for species differences in DNA adduct formation (Stein et al. 1990; Dubois et al. 1995; Ploch et al. 1998). However, within a particular species, it would appear that increased metabolism via CYP 1A is important in determining the DNA adduct concentration.

EROD activity, CYP 1A density, and DNA adduct concentration were significantly correlated and followed similar trends among sampling sites. Fish from the Nechako River (reference site) had low concentrations or activities that were distinguishable from the other sites. Fish from Northwood and Thompson River had similar responses. The Thompson River fish were expected to have low responses because of dilution of the effluent by a lake and the distance from the pulp mill. Fish from Stoner, Longbar, and Soda Creek all had similar responses, even though they represent different distances from the point-source inputs for this system. There was no downstream gradient of response; otherwise, a decreased response in Longbar compared with Stoner would be seen. This suggests that non-point-source inputs play a significant role in the contaminant load of these rivers, that the fish are mobile in winter months, or that migration to the ocean had begun.

Of the three histopathological conditions found, only hepatocellular steatosis was at a lower frequency at the control site and higher at the Fraser River sites. It is interesting that this condition occurred in a pattern consistent with the other measurements; however, the lack of samples at Northwood makes it difficult to draw strong conclusions. This condition is a degenerative disorder suggestive of metabolic disorders (Myers et al. 1987) and is commonly associated with dietary deficiencies or toxic chemical administration. It is possible that the fish in this study were not old enough to show significant lesions resulting from low-level chronic exposure to contaminants. Similar to these results, BKME exposure has not lead to increased histopathological lesions in a variety of studies. Rainbow trout (*Oncorhynchus mykiss*) had no increase in lesions after exposure to 1.3–5.1% BKME in experimental streams (Hall et al. 1992). Mountain whitefish exposed to BKME showed no differences in histopathology compared with fish from control sites in the Wapiti and Smoky rivers (Kloepper-Sams et al. 1994). There was no correlation between histopathology and contaminants in longnose sucker during the same study (Kloepper-Sams et al. 1994).

Fish from the Northwood site were surprisingly low in EROD activity and CYP 1A protein and relatively low in contaminant burdens, considering its proximity to the pulp mill. This result could be due to several possibilities. First, the fish at Northwood may be unexposed (or exposed to low concentrations) due to inadequate mixing of effluent. Chinook prefer shallow, low-flow areas in the cobblestones, near the edge of the river, whereas the diffuser discharging effluent is in the middle of the river, where flow is higher. Second, the effluent from the pulp mill could be insignificant in comparison with the municipal waste effluent and other inputs. The pulp mill effluent would then represent a small proportion of the contaminant load on the river, and downstream from the Northwood site, other inputs would result in higher response. Third, chinook migration could have

Table 3. Contaminant burdens in 2,3,7,8-TCDD equivalents (TEQs).

Study site	PCDDs[a]	PCDFs[a]	MO-PCBs[b]	NO-PCBs[c]	Total TEQs[d]
Nechako River	0.156	0.092	0.017	0.085	0.328
Northwood	0.278	0.125	0.020	0.159	0.583
Stoner	0.402	0.169	0.047	0.104	0.722
Longbar	0.273	0.176	0.059	0.086	0.564
Soda Creek	0.277	0.161	0.045	0.093	0.576
Thompson River	0.411	0.261	0.062	0.125	0.859

Note: Data are reported as pg·g wet weight^{-1}; based on $n = 2$ pools. See text for details of calculation.
[a]2,3,7,8-substituted congeners only.
[b]Monoortho-PCBs; includes CB123, CB118, CB114, CB105, CB167, CB156, CB157, CB189.
[c]Nonortho-PCBs; includes CB77, CB126, CB169.
[d]PCDDs + PCDFs + MO-PCBs + NO-PCBs.

Table 4. Correlation matrix for contaminant concentrations.

	PCDDs	PCDFs	MO-PCBs	NO-PCBs	Total TEQs
PCDDs	1.00	0.861[a]	0.758[a]	0.442	0.973[a]
PCDFs		1.00	0.889[a]	0.225	0.916[a]
MO-PCBs			1.00	−0.016	0.817[a]
NO-PCBs				1.00	0.493
Total					1.00

Note: Based on mean TEQ values (see text for details); $n = 7$ site means.
[a]Correlation significant ($P < 0.05$).

Table 5. Correlation matrix for contaminant concentrations and biological measurements.

	EROD	CYP 1A	DNA adduct
PCDDs	0.38	0.71	0.71
PCDFs	0.25	0.53	0.48
MO-PCBs	0.52	0.72	0.64
NO-PCBs	−0.08	0.19	0.22
Total TEQs	0.35	0.69	0.66

Note: Contaminants are based on mean TEQ values (see text for details); $n = 7$ site means. No correlations are significant with $P < 0.05$.

started before or during sampling, and fish downstream would be exposed for longer periods than at Northwood, where the fish would be newly exposed to the effluent zone. This scenario is unlikely, since EROD induction can occur within 24 h (J. Wilson, personal observation), although maximal induction may take 2–5 days, depending on the species and contaminant (Addison et al. 1981; Collier and Varanasi 1991; Parrott et al. 1995).

PCDD, PCDF, and PCB concentrations were low in fish from all sites, with some congeners near the detection limit. In all cases, fish from the Nechako had the lowest concentrations of contaminants. Fish from the other sites had similar concentrations of all contaminant groups, and the trends seen in the biological responses were absent. There were no correlations between EROD activity, CYP 1A density, or DNA adduct concentrations and any chemical group or with the total contaminant burden. In addition, these measurements did not correlate with either 1,2,3,6,7,8-HxCDD or 2,3,7,8-TCDF, which are considered to be marker chemicals for pulp mill effluent exposure (Yunker and Cretney 1996). The lack of correlation with chemical class or toxic burden may be due to the difficulty of resolving small differences in low contaminant concentrations in the fish. This is in part

due to the lack of adequate replication in the contaminant data, but it is also due to errors introduced by the use of TEFs.

The contaminants were probably not high enough to cause the biological effects observed. For a significant EROD induction, TEQ values in chinook must be at least 3 pg·g^{-1} (Servizi et al. 1993), a level not achieved at any site. Even though the contaminant concentrations are low, the biological responses differ significantly from the control site. This information may indicate that exposure to other inducing chemicals may have occurred. Considering the presence of DNA adducts in the fish, PAH exposure is probable. PAH metabolites in bile were detected in fish from only one site. Although the protein analysis indicates that sample dilution was not excessive, the PAH metabolite concentrations may have dropped below detection following dilution. PAH metabolites were not detected by synchronous scan fluorimetry but were detected with high-performance liquid chromatography (HPLC) analysis for benzo[a]pyrene metabolites in BKME-exposed English sole (D. Brand, University of Victoria, Victoria, B.C., personal communication). Therefore, undetected PAH metabolites may reflect problems in methodology only. The presence of DNA adducts suggests that there are sources of PAHs in this watershed. The methods used for detection of adducts select for large, bulky hydrophobic adducts. PCB–DNA adducts can be formed in vitro by rat microsomes (McLean et al. 1996) and in cultured cells (Dubois et al. 1995). Thus, the presence of PCB–DNA adducts cannot be ruled out in this study, although the solvent systems were different from those used to isolate PCB–DNA adducts.

Conclusions

Juvenile chinook on the upper Fraser River had significant increases in EROD activity, CYP 1A density, and DNA

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adduct concentrations over control sites on the Nechako River. The EROD activity and CYP 1A data show a pattern of induction that increases at sites downstream of the Northwood mill. The reason for this pattern is not known, although it is possible that the fish at the Northwood site are outside the effluent plume. Non-point-source inputs may be significant along the Fraser, accounting for the similar responses at sites of varying distances from the point sources. DNA adducts were similar at all of the exposed sites, although they were significantly greater than at the control site. There were strong correlations between EROD activity, CYP 1A density, and DNA adduct concentrations in these fish. Liver histopathology revealed no clear differences between reference and nonreference sites.

PCDD, PCDF, and PCB concentrations in the fish were low and were not correlated with any of the biological effects. From previously established dose–response relationships, it seems that these contaminant concentrations cannot account for the biological effects observed in the fish. PAH exposure cannot be ruled out, and HPLC analysis for benzo[*a*]pyrene metabolites is suggested for further research. The presence of DNA adducts suggests that PAH exposure had occurred. There was a consistent pattern in the biological responses over sites indicating that there was a whole-organism response in juvenile chinook salmon in the Fraser River.

Acknowledgments

References

Addison, R.F. 1996. The use of biological effects monitoring in studies of marine pollution. Environ Res. **4**: 225–237.

Addison, R.F., and Edwards, A.J. 1988. Hepatic microsomal monooxygenase activity in flounder *Platichthys flesus* from polluted sites in Langesundfjord and from mesocosms experimentally dosed with diesel oil and copper. Mar. Ecol. Prog. Ser. **46**: 51–54.

Addison, R.F., Zinck, M.E., and Willis, D.E. 1981. Time and dose-dependence of hepatic mixed function oxidase activity in brook trout *Salvelinus fontinalis* on pholychlorinated biphenyl residues: implications for 'biological effects monitoring'. Environ. Pollut. **25**: 211–218.

Ahlborg, U.G., Becking, G.C., Birnbaum, L.S., Brouwer, A., Derks, H.J.G.M., Feeley, M., Golor, G., Hanberg, A., Larsen, J.C., Liem, A.K.D., Safe, S.H., Sclatter, C., Wærn, F., Younes, M., and Yrjänheikki, E. 1994. Toxic equivalency factors for dioxin-like PCBs. Chemosphere, **28**: 1049–1067.

Anonymous. 1992*a*. Reference method for the determination of polychlorinated dibenzo-*p*-dioxins (PCDDs) and polychlorinated dibenzofurans (PCDFs) in pulp and paper mill effluents. EPS 1/RM/19. Environment Canada, Ottawa, Ont.

Anonymous. 1992*b*. Internal quality assurance requirements for the analysis of dioxins in environmental samples. EPS 1/RM/23. Environment Canada, Ottawa, Ont.

Ariese, F., Kok, S.J., Verkaik, M., Gooijer, C., Velthorst, N.H., and Hofstraat, J.W. 1993. Synchronous fluorescence spectrometry of fish bile: a rapid screening method for the biomonitoring of PAH exposure. Aquat. Toxicol. **26**: 273–286.

Arlotto, M., and Parkinson, A. 1989. Identification of cytochrome P450a (P450IIAI) as the principal testosterone 7α-hydroxylase in rat liver microsomes and its regulation by thyroid hormones. Arch. Biochem. Biophys. **270**: 458–471.

Bradford, M.M. 1976. A rapid and sensitive method for the quantitation of microgram quantities of protein utilizing the principle of protein–dye binding. Anal. Biochem. **72**: 248–254.

Collier, T.K., and Varanasi, U. 1991. Hepatic activities of xenobiotic metabolizing enzymes and biliary levels of xenobiotics in English sole (*Parophrys vetulus*) exposed to environmental contaminants. Arch. Environ. Contam. Toxicol. **20**: 462–473.

Dubois, M., Pfohl-Leszkowicz, A., Grosse, Y., and Kremers, P. 1995. DNA adducts and P450 induction in human, rat and avian liver cells after exposure to polychlorobiphenyls. Mutat. Res. **345**: 181–190.

Grondlund, W.D., Chan, S., McCain, B.B., Clark, R.C., Jr., Myers, M.S., Stein, J.E., Brown, D.W., Landahl, J.T., Krahn, M.M., and Varanasi, U. 1991. Multidisciplinary assessment of pollution at three sites in Long Island Sound. Estuaries, **14**: 299–305.

Hall, T.J., Haley, R., Borton, D.L., Walsh, A.H., and Wolke, R.E. 1992. Histopathology of rainbow trout (*Oncorhynchus mykiss*) after long-term exposure to biologically treated bleached kraft mill effluent in experimental stream channels. Can. J. Fish. Aquat. Sci. **49**: 939–944.

Hodson, P.V., Kloepper-Sams, K.R., Munkittrick, K.R., Lockhart, W.L., Metner, D.A., Luxon, P.L., Smith, I.R., Gagnon, M.M., Servos, M., and Payne, J.F. 1991. Protocols for measuring mixed function oxygenases of fish liver. Can. Tech. Rep. Fish. Aquat. Sci. No. 1829.

Kantoniemi, A., Vahakangas, K., and Oikari, A. 1996. The capacity of liver microsomes to form benzo[*a*]pyrene–diolepoxide–DNA adducts and induction of cytochrome P450 in feral fish exposed to pulp mill effluents. Ecotoxicol. Environ. Saf. **35**: 136–141.

Kloepper-Sams, P.J., Swanson, S.M., Marchant, T., Schryer, R., and Owens, J.W. 1994. Exposure of fish to biologically treated bleached-kraft effluent. 1. Biochemical, physiological and pathological assessment of rocky mountain whitefish (*Prosopium williamsoni*) and longnose sucker (*Catostomus castostomus*). Environ. Toxicol. Chem. **13**: 1469–1482.

Lin, S., Bullock, P.L., Addison, R.F., and Bandiera, S.M. 1998. Detection of cytochrome P4501A in several species using antibody against a synthetic peptide derived from rainbow trout cytochrome P4501A1. Environ. Toxicol. Chem. **17**: 439–445.

MacDonald, D.D., Ikonomou, M.G., Rantalainen, A.-L., Rogers, I.H., Sutherland, D., and van-Oostdam, J. 1997. Contaminants in white sturgeon (*Acipenser transmontanus*) from the upper Fraser River, British Columbia. Environ. Toxicol. Chem. **16**: 479–490.

McCain, B.B., Brown, D.W., Hom, T., Myers, M.S., Pierce, S.M., Collier, T.K., Stein, J.E., Chan, S., and Varanasi, U. 1996. Chemical contaminant exposure and effects in four fish species from Tampa Bay, Florida. Estuaries, **19**: 86–104.

McLean, M.R., Robertson, L.W., and Gupta, R.C. 1996. Detection of PCB Adducts by the ^{32}P-postlabeling technique. Chem. Res. Toxicol. **9**: 164–171.

Myers, C.R., Sutherland, L.A., Haasch, M.L., and Lech, J.J. 1993. Antibodies to a synthetic peptide that react specifically with rainbow trout hepatic cytochrome P450 1A1. Environ. Toxicol. Chem. **12**: 1619–1621.

Myers, M.S., Rhodes, L.D., and McCain, B.B. 1987. Pathologic anatomy and pattern of occurrence of hepatic neoplasms, putative preneoplastic lesions and other idiopathic hepatic conditions in English sole (*Parophrys vetulus*) from Puget Sound, Washington. J. Natl. Cancer Inst. **78**: 333–363.

Myers, M.S., Johnson, L.L., Hom, T., Collier, T.K., Stein, J.E., and Varanasi, U. 1998. Toxicopathic hepatic lesions in subadult English sole (*Pleuronectes vetulus*) from Puget Sound, Washington, U.S.A.: relationships with other biomarkers of contaminant exposure. Mar. Environ. Res. **45**: 47–67.

NATO Committee on the Challenges of Modern Society (NATO-CCMS). 1988. International toxicity equivalency factor (I-TEF) method of risk assessment for complex mixture of dioxins and related compounds. NATO-CCMS Rep. 176.

Parrott, J.L., Hodson, P.V., and Dixon, D.G. 1995. Rainbow trout hepatic mixed-function oxygenase induction by polychlorinated dibenzo-*p*-dioxins (PCDDs) as a function of time and tissue concentration. J. Toxicol. Environ. Health, **46**: 301–316.

Peters, L.D., Morse, H.R., Waters, R., and Livingstone, D.R. 1997. Responses of hepatic P4501A and formation of DNA-adducts in juvenile turbot (*Scophthalmus maximus* L.) exposed to waterborne benzo[*a*]pyrene. Aquat. Toxicol. **38**: 67–82.

Ploch, S.A., King, K.C., Kohan, M.J., and Di Giulio, R.T. 1998. Comparative in vitro and in vivo benzo[*a*]pyrene–DNA adduct formation and its relationship to CYP1A activity in two species of ictalurid catfish. Toxicol. Appl. Pharmacol. **149**: 90–98.

Reichert, W.L., and French, B. 1994. The ^{32}P-postlabeling protocols for assaying levels of hydrophobic DNA adducts in fish. NOAA Tech. Memo. NMFS-NWFSC-14.

Renton, K.W., and Addison, R.F. 1992. Hepatic microsomal monooxygenase activity and P450IA mRNA in North Sea dab *Limanda limanda* from contaminated sites. Mar. Ecol. Prog. Ser. **46**: 65–69.

Rogers, I.H., Levings, C.D., Lockhart, W.L., and Norstrom, R.J. 1989. Observations on overwintering juvenile chinook salmon (*Oncorhynchus tshawytscha*) exposed to bleached kraft mill effluent in the upper Fraser River, British Columbia. Chemosphere, **19**: 1853–1868.

Servizi, J.A., Gordon, R.W., Martens, D.W., Lockhart, W.L., Metner, D.A., McBride, J.R., and Norstrom, R.J. 1993. Effects of biotreated bleached kraft mill effluent on fingerling chinook salmon (*Oncorhynchus tshawytscha*). Can. J. Fish. Aquat. Sci. **50**: 846–857.

Stein, J.E., Reichert, W.L., Nishimoto, M., and Varanasi, U. 1990. Overview of studies on liver carcinogenesis in English sole from Puget Sound; evidence for a xenobiotic chemical etiology II: biochemical studies. Sci. Total Environ. **94**: 51–69.

Stein, J.E., Hom, T., Collier, T.K., Brown, D.W., and Varanasi, U. 1995. Contaminant exposure and biochemical effects in outmigrant juvenile chinook salmon from urban and nonurban estuaries of Puget Sound, Washington. Environ. Toxicol. Chem. **14**: 1019–1029.

Yunker, M.B., and Cretney, W.J. 1996. Dioxins and furans in crab hepatopancreas: use of principal components analysis to classify congener patters and determine linkages to contaminantion sources. *In* Environmental fate and effects of pulp and paper mill effluents. *Edited by* M.R. Servos, K.R. Munkittrick, J.H. Carey, and G.J. Van der Kraak. St. Lucie Press, Delray Beach, Fla.