HYLEBOS FISH INJURY STUDY
Round II

Part 3: Exposure of juvenile chinook salmon to chemical contaminants specific to the Hylebos Waterway: Tissue concentrations and biochemical responses.

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EXECUTIVE SUMMARY

The Hylebos Waterway of Commencement Bay, an urban estuary in central Puget Sound, Washington, is severely contaminated with a variety of organic and inorganic chemicals. Juvenile salmon inhabit this waterway in the late spring and early summer during their migration from fresh water to the open ocean. Studies conducted in 1989 and 1990 revealed that juvenile chinook salmon migrating through Commencement Bay had elevated concentrations of polycyclic aromatic hydrocarbons (PAHs) and chlorinated hydrocarbons (CHs) or their derivatives in tissues, bile and stomach contents, and that they exhibited biochemical changes indicative of contaminant exposure. Round I of the Hylebos Fish Injury Study, conducted in 1994 and 1995, showed that both juvenile chum and chinook salmon sampled from the Hylebos Waterway were also exposed to a wide range of chemical contaminants (Collier et al. 1998). Furthermore, the levels of exposure and biochemical responses were comparable to levels that have previously been associated with impaired growth, immunosuppression, and increased mortality following pathogen exposure in juvenile salmon; biological effects that can have significant population impacts. Whether juvenile salmon exposed in the laboratory to anthropogenic chemical contaminants specific to the Hylebos Waterway would exhibit similar chemical and biochemical changes was unknown.

In 1996, Round II of the Hylebos Fish Injury Study continued with laboratory experiments designed to establish links between chemical exposure and injury as well as quantify the extent of the injury. Results of the first two laboratory experiments (disease challenge and growth) in Round II (see SAP, Appendix 1) show that juvenile salmon exposed to PCBs, PAHs, HCBD, and organic-solvent extracts of sediments from the Hylebos Waterway exhibit increased disease susceptibility (altered immunocompetence) (Part 1 of Round II Hylebos Fish Injury Study) and reduced growth (Part 2 of Round II Hylebos Fish Injury Study). Within the scope of Round II studies, we needed to further identify the chemical contaminants potentially responsible for causing injury and examine the persistence of chemical contaminants and biochemical responses which were measured in Round I.

The objectives of the present laboratory study (Part 3 of Round II Hylebos Fish Injury Study) were to determine: 1) classes of chemical compounds that produce alterations in chemical and biochemical measures of contaminant exposure in juvenile salmon tissues, 2) the persistence of chemicals in the tissues of exposed fish, and 3) the magnitude and persistence of biochemical responses as a result of exposure to chemical compounds characteristic of the Hylebos Waterway. To accomplish these objectives, juvenile salmon were injected intraperitoneally (i.p.) with a single dose of either 1) hexachlorobutadiene (HCBD), a signature compound of the Hylebos Waterway, 2) an extract made from Hylebos Waterway sediment using methylene

chloride as the extracting solvent (HWSE-M), 3) an extract made from Hylebos Waterway sediment using pentane as the extracting solvent (HWSE-P), 4) a model mixture composed of 10 high-molecular weight polycyclic aromatic hydrocarbons (PAHs) proportional to their concentrations in a sediment sample from the Hylebos Waterway, 5) the PCB mixture, Aroclor 1254, 6) a reference sediment extract made from Nisqually River estuary sediment (NQSE) using methylene chloride as the extracting solvent, or 7) acetone/Emulphor (A/E), the solvent vehicle. Bile, liver, and gutted-whole body were collected at different intervals up to 60 d post exposure and analyzed for chemical exposure including tissue concentrations of CHs (e.g., HCBD, chlorinated pesticides and PCBs); as well as biochemical responses including biliary levels of fluorescent aromatic compounds (FACs), hepatic cytochrome P4501A-dependent monooxygenase activity (CYP1A) and hepatic DNA adduct levels.

Results from this portion of the study (Part 3) conducted in Round II of the Hylebos Fish Injury Study showed that: 1) PCBs and PAHs induced alterations in chemical and biochemical measures of exposure in juvenile salmon after single-dose injection, 2) PCBs, cytochrome P4501A induction and PAH-DNA adducts persisted for up to 60 days in the tissues of exposed animals after single-injection exposure, 3) HCBD exposure resulted in increased tissue concentrations, but did not induce, as expected, any biochemical response at the tissue levels of exposure used in this laboratory study, and 4) the tissue levels of PCBs, HCBD, and the biochemical responses in juvenile salmon treated with the Hylebos sediment extracts were comparable to the levels and responses observed in salmon sampled directly from the Hylebos Waterway in Round I (Collier et al. 1998). These findings, in conjunction with the findings from the first two laboratory experiments of Round II [showing impaired disease resistance (Part 1) as well as impaired growth (Part 2) in juvenile salmon exposed to the same Hylebos sediment extracts], support the conclusion that juvenile salmon residing in the Hylebos Waterway will likely exhibit impaired growth and increased disease susceptibility as a result of their dependence on this contaminated habitat.

INTRODUCTION

Estuaries are important habitats for several Pacific salmonid species (*Oncorhynchus* spp.), including the fall-spawning chinook which use estuaries as juvenile rearing habitat (Thorpe 1994). The estuary acts as an intermediate environment for their physiological adaptation to ocean conditions, a profitable foraging ground and a refuge from predators. Results from recent studies of juvenile chinook salmon (*Oncorhynchus tshawytscha*) captured from the Duwamish Waterway, a highly contaminated urban estuary in Puget Sound, consistently showed elevated exposure to polycyclic aromatic hydrocarbons (PAHs) and chlorinated hydrocarbons (CHs) when compared to juvenile chinook salmon captured from the Nisqually River Estuary, a minimally contaminated non-urban area (McCain et al. 1990, Collier et al. 1998). Moreover, increased exposure to these chemical contaminants led to induction of cytochrome P4501A (CYP1A) enzyme activities and damage to hepatic DNA in juvenile chinook salmon as they migrated through the contaminated estuarine environments of the Duwamish Waterway as well as Commencement Bay in Puget Sound, Washington (Stein et al. 1995).

In Round I of the Hylebos Waterway Fish Injury Assessment, juvenile chum (*Oncorhynchus keta*) and chinook salmon from the Hylebos Waterway showed elevated PAH metabolites in bile and CHs in liver which were comparable to those found in juvenile chinook salmon from the Duwamish Waterway (Collier et al. 1998). Furthermore, the levels of exposure were similar to those associated with impaired growth, immunosuppression, and increased mortality following pathogen exposure in chinook salmon collected from the Duwamish Waterway (Arkoosh et al. 1991, Casillas et al. 1995a). In addition, juvenile chum and chinook from the Hylebos Waterway had concentrations of hexachlorobutadiene (HCBD) in the liver that exceeded those found in any of our previous field studies with salmon (Varanasi et al. 1993). Associated with these increased concentrations of chemicals were indications of early biological alterations and damage, as shown by increases in hepatic CYP1A-associated enzyme activity and increased levels of DNA adducts (Collier et al. 1998). Increases in both of these measures are well established as being linked to contaminant exposure (Collier and Varanasi 1991, Varanasi et al. 1992, Stein et al. 1992). DNA adducts represent reversible damage to genetic material that has been shown to be associated with hepatic lesions in fish (Myers et al. 1998). In the present study DNA adducts were used as an indicator of exposure to genotoxic polycyclic aromatic compounds based on our previous studies (French et al. 1996, Stein et al. 1992, 1994). However, from the findings in Round I, it was not clear which classes of contaminants found in the Hylebos Waterway contributed to these biological alterations; nor was it clear how long elevated body levels of contaminants or biochemical alterations would persist after fish moved out of the contaminated estuary and into marine waters.

The objective of the present laboratory study was to assess the time-responsiveness of tissue CHs, biliary FACs, hepatic CYP1A, and hepatic DNA adducts (measured in Round I) in juvenile chinook salmon exposed to contaminants specific to the Hylebos Waterway and sampled for up to 60 days following a single exposure. Specifically in July 1997, juvenile chinook salmon from a local hatchery were injected intraperitoneally (i.p.) with: 1) hexachlorobutadiene (HCBD), a signature compound of the Hylebos Waterway, 2) an extract made from Hylebos Waterway sediment using methylene chloride as the extracting solvent (HWSE-M), 3) an extract made from Hylebos Waterway sediment using pentane as the extracting solvent (HWSE-P), 4) a model mixture composed of 10 high-molecular weight polycyclic aromatic hydrocarbons (PAHs) proportional to their concentrations in a sediment sample from the Hylebos Waterway, 5) the PCB mixture, Aroclor 1254, 6) a reference sediment extract made from Nisqually River estuary sediment (NQSE) using methylene chloride as the extracting solvent, or 7) acetone/Emulphor (A/E), the solvent vehicle. The results from this study will provide a basis for evaluation of the relationship between the level of exposure to chemical contaminants specific to the Hylebos Waterway and the significant biological effects observed in juvenile chinook salmon in the previous laboratory studies, and will also allow for comparison to the results obtained for juvenile chinook salmon sampled directly from the Hylebos Waterway in Round I.

METHODS

Details of the experimental design and methods used in the present study are described in the addendum to the SAP (Appendix 1). The chemical compositions of the NQSE, HWSE-M, HWSE-P sediment extracts and their concentrations are listed in Table 1, 2, and 3, respectively. The composition of the PAH model mixture is listed in Table 4.

Exposure Dosage Assessment

All fish were injected intraperitoneally (i.p.) with PAH model mixture, HCBD, PCB mixture (Aroclor 1254), sediment extracts from the Hylebos Waterway or the Nisqually River estuary mixed with the solvent vehicle, acetone:Emulphor (1:1, v/v), or the solvent vehicle alone. The objective of administering HWSE-M, HWSE-P, a model mixture of PAHs, Aroclor 1254, and HCBD was to expose juvenile salmon to known quantities of compounds representative of chemical contaminants present in the sediment from the Hylebos Waterway.

The Nisqually River estuary sediment extract (NQSE) was prepared from sediment collected near the mouth of the Nisqually River. The chemical profile in this extract is representative of sediment from a non-urban site that is minimally contaminated (Table 1). The NQSE sediment extract was prepared using methylene chloride as the extracting solvent. The injection dosage for this extract was 200 g sediment extracted/ kg fish which is equivalent to administering 0.005 mg ΣAHs/kg fish, 0.0004 mg ΣPCB/kg fish, and < 0.001 μg HCBD/kg fish. Juvenile chinook salmon treated with NQSE served as a control group for the Hylebos sediment extract treated fish, providing a more appropriate control group to assess the influence of Hylebos-associated contaminated sediment extracts on the chemical and biochemical parameters measured in juvenile chinook salmon.

The Hylebos Waterway sediment extracts (HWSE-M and HWSE-P) were prepared from sediment collected near the mouth of the Hylebos Waterway from Stations HY-07, -08, and -09, as designated during the sediment injury studies conducted during Round I of the Hylebos Damage Assessment investigations. (see Appendix D in Collier et al., 1998). These sediment extracts were prepared using two different solvents to obtain test solutions that included specific chemical compounds present in sediment from the Hylebos Waterway. The composition of analytes in the two Hylebos Waterway extracts is listed in Tables 2 and 3. The HWSE-M sediment extract was prepared using methylene chloride as the extracting solvent. The chemicals present in the HWSE-M represent the full range of chemicals (AHs and CHs) that may be available to fish. The HWSE-P extract was prepared using pentane to reduce the proportion of high-molecular weight AHs (HMWAHs) extracted, thereby increasing the concentration of CHs relative to HMWAHs. Some CHs present in the HWSE-P were partly removed in this

preparation, but to a lesser degree than the PAHs. The HWSE-M was administered at a dose of 200 g sediment extracted/kg fish which is equivalent to 0.35 mg ΣPAHs/kg fish, 0.032 mg ΣPCBs/kg fish, and 0.038 mg HCBD/kg fish; whereas, the HWSE-P extract was administered at a dosage of 400 g sediment extracted/kg fish which is equivalent to 0.24 mg ΣPAHs/kg fish, 0.028 mg ΣPCBs/kg fish, and 0.045 mg HCBD/kg fish. A dose equivalent to 400 g of HWSE-P sediment extracted/kg fish was used so that the levels of measured chemical contaminant administered for the two Hylebos sediment extracts would be comparable.

A model mixture of polycyclic aromatic hydrocarbons (PAHs) containing 10 high-molecular weight PAHs was prepared to reflect the same ratios of these analytes as previously found in sediment sampled during Round I of the Hylebos Damage Assessment studies at Station HY-24 (Appendix D, Collier et al. 1998). The PAH model mixture in acetone/Emulphor was administered at a dosage of 6.3 mg ΣPAHs/kg fish. The effect of PCBs was evaluated using a commercial mixture (Aroclor 1254). The PCB mixture prepared in acetone/Emulphor was administered at a dosage of 28 mg ΣPCBs/kg fish. The HCBD solution prepared in acetone/Emulphor was administered at a dosage of 21 mg HCBD/kg fish. All test solutions, including controls, were each administered at a volume equivalent to 1.5 µl solution/g fish.

Experimental conditions , mortality and sampling strategies

Ambient water temperatures during the course of the study ranged from 11.6°C at 0 d to 12.8 °C at 60 d post exposure (Casillas et al. 1998). The mean (± SD) water temperature was 12.2± 0.4 °C. Temperature was kept relatively constant by drawing seawater from a depth of approximately 50 feet, thereby minimizing the influence of more rapid changes in surface-seawater temperatures.

Fish were fed to satiation twice a day, once in the morning and once in the afternoon with food pellets (Biodry 1000, Bioproducts).

Recorded mortalities of experimental fish ranged from 6% to 19% (18 to 53 animals) for each of the treatment groups (300 fish per group) during the experimental period (Table 5). The highest number of mortalities was observed in juveniles receiving either the HWSE-M or the PCB formulation (Aroclor 1254). In contrast, the lowest mortalities were observed in juveniles receiving the solvent vehicle (acetone/Emulphor). With the exception of HWSE-P, HCBD, and PCB treated groups, mortalities were low and stable in the second half of the experimental period (Table 5). During the course of the experiment, we could not account for approximately 12% of the injected fish and we assume that these fish either escaped from the tanks through the exiting water standpipe, or gaps in the tank covers (Appendix 3). Exposed fish from all 7 treatments were sampled at 6 d and 20 d post exposure; but shortly after that it became apparent that insufficient numbers of fish would be available for the 60 d sampling time-point. Hence, the

HWSE-M and NQSE treated groups were sampled at 33 d post exposure. the solvent vehicle group was sampled at 35 d post exposure, and the HCBD and HWSE-P groups were sampled at 56 d post exposure. The PAH and PCB groups were sampled at 62 d post exposure. All three biochemical parameters were analyzed from all samples collected, except for chemical concentrations in tissues where only samples collected at 6 d, 20 d from the time-response study and samples collected at 60 d from the growth study were analyzed. To compensate for the treatment groups that could not be carried out to the full 60 d in this experiment, appropriate samples of fish which received the same exposure were collected from the growth experiment, conducted simultaneously with the biomarker experiment (Casillas et al, 1998). Therefore, the results presented in this report were of samples from all seven treatments collected at 6 d and 20 d post exposure from the biomarker study, and 60 d post exposure from the growth study.

Prior to analysis, samples were composited to obtain sufficient tissue and fluid for the biochemical and chemical analyses. Liver and bile from 20 individual fish were composited for biochemical analyses such as PAH metabolites in bile, hepatic CYP1A activity, and levels of hepatic DNA adducts. For chemical analyses of CHs in whole body tissues, five individual gutted whole bodies were composited. At each time point, three composites of bile, livers, and whole gutted bodies were collected per treatment. Composited liver samples were flash-frozen in liquid nitrogen and subsequently stored at -80°C until analysis. Composited bile and whole body samples were kept on ice and subsequently stored at -20°C until analysis.

Statistical methods

Statistical significance of the biomarker responses at 6, 20, and 60 d post exposure was assessed using ANOVA (Super ANOVA, Abacus Concepts Inc., Berkeley, CA, 1989). Significance of results of treatment groups compared to the control groups of fish (fish receiving acetone/Emulphor or the reference Nisqually River estuary sediment extract) was evaluated using Dunnett's Multiple Comparison Test (Zar 1978) at $\alpha = 0.05$ for each variable independently.

RESULTS

Sediment Extract Chemistry

In this study, two different extraction methods were used to isolate non-polar toxic CHs and AHs (polar compounds were removed from the extracts) from a representative Hylebos Waterway sediment. The major difference in these methods was the solvent used in the extraction process (pentane or methylene chloride). In the pentane extract of the Hylebos Waterway sediment (HWSE-P), the proportion of high-molecular weight AHs (HMWAHs) was reduced relative to the CHs; therefore, the ratio of CHs to HMWAHs was increased from a ratio

of 1.7 in the HWSE-M to a ratio of 2.8 in the HWSE-P. As described in the SAP, it was anticipated that a fraction containing reduced levels of both HMWAHs and low-molecular weight AHs (LMWAHs) could be prepared; however, it was not practical to eliminate or significantly reduce the proportion of both HMWAHs and LMWAHs without losing the CHs as well. Nevertheless, using pentane rather than methylene chloride in the extraction process reduced the proportion of HMWAHs nearly 47% while maintaining the chlorinated butadienes at nearly 88% of original levels (Tables 2 and 3). Hence, this extraction approach provided an opportunity to evaluate whether there is an interaction between HMWAHs and CHs in affecting the tissue concentration of chemical contaminants and biomarker of exposure responses in juvenile chinook salmon.

Measurement of Contaminant Exposure

Analyses of chemical concentrations or biochemical parameters were not conducted on all treatment groups. Tissue levels of PAHs were not measured, because of the rapid metabolism of PAHs in the liver of fish (Varanasi et al. 1989). Similarly, DNA adduct levels were not measured in the HCBD and PCB-exposed fish, because PCBs have not been shown to form adducts with DNA (Nath et al. 1991), and HCBD is not expected to form DNA adducts. In addition, analyses for CH exposure (i.e., ΣPCBs) were not performed on fish exposed to PAHs only.

Chemical Analysis Results:

As mentioned above, tissue concentrations of chemical contaminants were measured in samples collected at 6 d and 20 d from the time-response study, and at 60 d from the growth study.

PCB Liver Levels : Liver PCB concentrations were significantly elevated at 6 d post exposure in juvenile chinook salmon receiving PCBs or either of the Hylebos Waterway sediment extracts (Fig. 1). At this time-point, the concentration of Σ PCBs in livers of the group receiving PCBs alone was 23,000 ppb compared to the basal levels of approximately 70 ppb Σ PCBs in the solvent-vehicle treated group. Although PCBs in the administered dosages of both extracts were low, ca. 0.03 mg Σ PCBs/kg fish, the level of PCBs in liver showed an increase of 2 times the PCB level of solvent-vehicle and reference sediment extract (NQSE) groups. There were no significant differences in the total PCB tissue levels observed between the HWSE-P and HWSE-M groups confirming that the actual dose of PCBs administered in either extract was similar. Liver PCB levels decreased by day 60 to about 10% of the observed maximum levels at 6 d in the HWSE-P and HWSE-M exposed groups, whereas the liver PCB level in the PCB treated group declined to 5% of maximum level at 6 d post exposure. Liver PCB levels in the

NQSE and solvent vehicle treated groups were near background levels, 70 ppb at day 6 and 20 ppb by the end of the experiment.

PCB Whole Body Levels: Total PCB levels in whole body followed the same trend as in the hepatic tissues, with the highest levels observed at 6 d post exposure for the PCB (14,500 ppb), HWSE-M and HWSE-P (both at ~ 50 ppb) treated groups as compared to control levels of A/E and NQSE treated groups (both at 28 ppb). Whole body PCB levels in HWSE-M and HWSE-P exposed fish declined to 70% to 80% of their respective maximum levels by 60 d (Fig. 2). Total PCB levels at 60 d in whole body tissues of PCB injected fish were still at 30% of maximum levels suggesting a shift of PCBs from the liver to body tissues.

HCBD Liver Levels: The highest HCBD level (2,900 ppb) was observed at 6 d post injection in the HCBD treated group, about 4 orders of magnitude higher than the control groups (Fig. 3). These levels, however, quickly declined at day 20 to 55 ppb, and returned to basal levels by 60 d. At 6 d post exposure, the sediment extract treated groups (HWSE-M and HWSE-P) exhibited significantly elevated HCBD levels, about 6 and 3 times as high as the A/E group, respectively. The levels observed at 6 d in the HWSE-M and HWSE-P treated groups were also 8 times and 4 times as high as the NQSE treated group, respectively. By day 20 the levels of HCBD in the livers of the sediment extract treated groups were at basal levels.

HCBD Whole Body Levels: At 6 d post exposure, HCBD levels in whole body tissue of both HWSE-M and HWSE-P treated groups were 110 times and 20 times as high as the A/E treated group, respectively, and 150 times and 30 times as high as the NQSE treated group (Fig. 4). While the concentration of HCBD in whole body tissues of the HWSE-M group was approximately 3 times as high as HCBD concentration in the hepatic tissues at 6 d, this was not observed in the HWSE-P treated group where HCBD levels were similar in both tissue types. Moreover, the mean concentration of HCBD in whole body tissue of HCBD only group was approximately 20% of the concentration in hepatic tissuemeasured at 6 d post exposure. The current findings also showed that HCBD in both tissues rapidly declined with time.

Biochemical Analyses

Although samples collected at all time points were analyzed for biliary FAC levels, hepatic CYP1A activity and DNA adducts, as mentioned above, results reported for the 6 and 20 d time-points are from samples collected in the time-response experiment; and 60 d data are from samples collected in the growth experiment that was conducted concurrently (Casillas et al. 1998).

Biliary FACs: Biliary FAC concentration was normalized to protein content in the bile sample. The variability in the bile metabolite levels associated with feeding status of sampled fish is reduced using this normalization procedure; the level of protein in bile reflects the water content of bile (Collier and Varanasi 1991). The concentrations of biliary FACs,

measured at benzo(a)pyrene wavelengths (380 nm, ex. and 430 nm, em.) in fish treated with the PAH model mixture revealed a maximum FAC level at 6 d post exposure (though this difference was not significant at the $p<0.05$ level). By 20 d there was no evidence of increased levels of biliary FACs in fish from the PAH treatment group (Fig. 5). Exposure to a single injection of either sediment extract at the dosages used in this laboratory experiment did not appear to result in any significant increases in biliary FAC levels.

Hepatic CYP1A Activity: Cytochrome P4501A (CYP1A) enzyme activity was responsive at the low administered dosages of contaminants in the Hylebos sediment extracts. Aryl hydrocarbon hydroxylase (AHH) activity ranged from 150 (A/E) to a high of 1100 (HWSE-M) pmol BaP equivalents/mg protein/min at 6 d post exposure (Fig. 6). The PAH, PCB, HWSE-M, HWSE-P, and NQSE treatment groups all exhibited elevated AHH activity. The HWSE-M treated group showed the highest activity (~ 7 times) at 6 d post exposure as compared to the A/E treated group. CYP1A activity in fish treated with HWSE-M was about 3 times as high as the NQSE treated group. The HCBD treated group did not show any induction of AHH activity. At 60 d post exposure, CYP1A activity in all treatment groups returned to basal levels except for the PCB treated group, which remained elevated (~ 4 times as high as the A/E and NQSE groups).

DNA Adducts: The levels of DNA adducts, at 6 d post exposure, in the HWSE-P, HWSE-M and PAH treated groups were significantly greater than those in the NQSE treated group (Fig.7). The increased DNA adduct levels in the PAH, HWSE-M and HWSE-P treated groups persisted through 20 d, but declined to approximately 20% of the level measured at 6 d by the end of the experiment. Hepatic DNA adduct profiles from the acetone/Emulphor (solvent vehicle) injected fish were uncharacteristic of those generated previously in fish injected with this solvent vehicle; therefore they were not used as the control group in the interpretation of the DNA adduct data. The reference sediment extract (NQSE) treated group was used as the control. Additional information on previous DNA adduct data for A/E treated fish is presented in Appendix 2. The DNA adduct profiles and levels in the NQSE treated fish were representative of those typically observed in fish collected from uncontaminated sites, injected with uncontaminated sediment extracts or the solvent vehicle, acetone/Emulphor (French et al. 1996, Stein et al. 1993). In addition, the chemical levels and other biochemical responses measured in the NQSE-treated fish were comparable to those from the A/E treated fish (Figs. 1-6).

DISCUSSION OF MAJOR FINDINGS

PCBs and PAHs are primarily responsible for the alterations in biomarker responses in juvenile chinook salmon.

Laboratory exposure of juvenile chinook salmon to individual classes of compounds characteristic of the Hylebos Waterway demonstrated that PAHs clearly induced CYP1A activity and increased levels of DNA adducts in livers of juvenile chinook salmon. Evidence for increases in biliary levels of FACs was also observed in juvenile chinook salmon at 6 d following exposure to PAHs. Levels of PAHs in the body and liver were not measured, because unlike PCBs, PAHs are rapidly metabolized by the liver and their metabolites can be detected in the bile of exposed animals (Varanasi et al. 1989). Our findings are largely consistent with previous laboratory studies showing that biliary FACs, CYP1A activity and DNA adducts are sensitive and responsive to PAH exposure (Stein et al. 1992, Collier and Varanasi 1991). Although the increase in biliary FAC levels due to exposure to PAHs at 6 d post exposure was higher than that for any of the other treatment groups, it was not statistically significant. This low response of FACs to PAH exposure was not expected. Species-specific differences may be a factor, as previous extensive studies of the time- and dose-responsiveness of biliary FACs in flatfish have shown the sensitivity of this measure to PAH exposure. However, without additional information on the time-course response of FACs in juvenile chinook salmon exposed to PAHs, any further explanation of the findings would be speculative.

Juvenile chinook salmon exposed to PCBs had elevated body and liver levels of PCBs as well as induction of hepatic CYP1A activity. This is consistent with other studies showing similar CYP1A induction by PCB mixtures (Safe 1994; Collier and Varanasi, 1991). Evidence of HCBD exposure was detected as increased concentrations of HCBD in liver and whole body of juvenile salmon. Unlike PCBs or PAHs, no evidence of hepatic CYP1A induction or altered biliary FACs was observed, upon exposure to HCBD, at any time during the 60 d monitoring period. It was not surprising that the biochemical measurements made in this study did not show any response following HCBD exposure because HCBD was not expected to induce a response in the biomarkers measured. The types of chemicals that induce a response in the three biomarkers measured have very different chemical structures than HCBD. Moreover, we are not aware of any other validated biochemical responses which could be expected to change following acute exposure to HCBD in fish. It is possible that there may have been kidney damage resulting from the HCBD exposure, as this compound is known to be nephrotoxic in mammals (Nakagawa et al. 1998). Histopathological examination of fixed kidney tissue from HCBD-exposed salmon could address this question.

The biochemical responses to the sediment extract exposures were consistent with the observations made with individual or model mixtures of chemical compounds. For example, significant increases in tissue levels of PCBs and hepatic CYP1A activity were observed in juvenile chinook salmon exposed to single injection of either Hylebos sediment extract. Because CYP1A activity was significantly elevated at 20 d post exposure in fish treated with PCBs, but not with PAHs, the elevated CYP1A response at the later time points in the extract treated fish is likely due to PCBs or other compounds similar in structure and persistence in fish tissues as PCBs that are present in the Hylebos Waterway sediment extracts. Similarly, because fish exposed to either of the two extracts exhibited elevated DNA adduct levels at 20 d post exposure in the same manner as those exposed to PAHs only, this elevated response was likely a consequence of PAH exposure. Biliary FACs were not elevated at the earliest time period monitored (6 d post exposure) following exposure to either HWSE. While this lack of a FAC response was not expected, as discussed above it is not appropriate to speculate as to the reason for this lack of response. The overall responses from exposure to either of the HWSEs (HWSE-M and HWSE-P) were similar, which is consistent with the similarity in the dosage and composition of chemical contaminants in these two extracts. Furthermore, the similarity in the response to the two extracts and lack of response to the reference sediment extract (NQSE) supports the conclusion that it is the chemical contaminants present in the Hylebos Waterway sediments, and not naturally occurring compounds, that were responsible for the increases in DNA adducts and CYP1A activity. The apparently higher HCBD concentration in whole body tissue observed at 6 d in the HWSE-M group compared to the HWSE-P group may be due to the fact that the levels being quantitated are approaching the detection limit, making accurate quantitation more difficult.

Overall, the current findings show that each class of toxic chemicals characteristic of the Hylebos Waterway produced a signature response from the suite of biochemical and chemical endpoints employed in this study. When we evaluated the responses of juvenile chinook salmon exposed to the complex mixture of contaminants in the two Hylebos Waterway sediment extracts, the collective biomarker and chemical endpoint response generally reflected the contribution of the individual component classes of this complex contaminant mixture. Thus, the integrated use of a suite of measurements allowed for identification of the compound classes likely responsible for specific responses. This information increases our ability to interpret chemical exposure history of juvenile salmon from the Hylebos Waterway.

Compounds such as HCBD, PCBs, and PAHs, characteristic of the Hylebos Waterway, persist for an extended period after exposure.

Measures of chemical concentrations in tissues of exposed animals explicitly reveal the persistence and magnitude of exposure to chemical contaminants that are not easily degraded by biological processes, and provide information for linking biological effects to chemical contaminants. In the current study, when juvenile chinook salmon were exposed to PCBs at a dose equivalent to 20% of the 96 hr LD_{50}, this exposure was evident in liver and whole body PCB levels at the end of the 60 d experimental period. At this time point, whole body PCB concentrations had declined to approximately 30% of maximum levels measured, and liver PCB concentration declined to 5% of the levels observed at 6 d post exposure. These findings demonstrate that appreciable levels of PCBs persist in the tissues for a significant period of time, weeks after the exposure from external sources is terminated. On the basis of these findings, it is evident that the potential for biological effects from PCB exposure may also persist for an extended period. This conclusion is supported by the observation of induced CYP1A activities at 60 d in the PCB treated fish.

Persistence of PCBs in tissues was also observed in fish receiving either of the Hylebos Waterway sediment extracts. Elevated liver PCB concentrations were observed in fish exposed to either HWSE-M or HWSE-P sampled at 6 d post exposure, and body concentrations of PCBs were significantly elevated for at least 20 d post exposure. The lower magnitude and the apparent shorter duration of the response (based on the tissue concentrations) in fish exposed to sediment extracts are expected with a single injection of much lower dose of PCBs present in either of the Hylebos Waterway sediment extracts (28 to 32 µg PCBs/kg fish) compared to fish exposed to PCBs only (28,000 µg PCBs/kg fish). The concentrations of PCBs measured in liver tissues of the extract exposed fish were comparable to levels observed in juvenile chinook and chum salmon collected in the field study (Round I). Thus, it is likely that juvenile salmon may experience the biological consequences of a short-term exposure to PCBs for at least 3 weeks after leaving the Hylebos Waterway.

Elevated HCBD levels persisted in whole body and liver tissue of juvenile salmon exposed to HCBD only, but not to the same extent as PCBs. Whole body HCBD concentrations were significantly elevated through 20 d, and by 60 d HCBD levels were still elevated but no longer statistically different (at the $p<0.05$ level) from controls. The decline in HCBD levels was even more rapid in liver tissue. Hepatic HCBD concentrations were significantly elevated at 6 and 20 d post exposure, but were approaching the limits of detection by 60 d post exposure in HCBD injected fish. Fish injected with either of the two extracts also exhibited evidence of HCBD exposure at 6 d post injection; however, in contrast to the HCBD only treated group, HCBD was not detectable by 20 d post exposure. These findings showing that HCBD levels

declined more rapidly than PCB levels suggest that either metabolism, excretion or both metabolism and excretion of HCBD was greater in juvenile chinook salmon.

The persistence of the hepatic CYP1A activity and increased DNA adduct levels observed in fish exposed to sediment extracts from the Hylebos Waterway can be largely attributed to specific classes of compounds based on the responses observed from exposure to PCBs or PAHs individually. For example, CYP1A activity remained elevated for up to 20 d post exposure in juvenile salmon exposed to sediment extracts from the Hylebos Waterway, in the same manner as in fish exposed to PCBs only, thus indicating that the persistence of CYP1A response in this study is likely due to exposure to PCBs. Other substances, such as dioxins and dibenzofurans, could also potentially be contributing to such induction. However, these types of compounds were not analyzed in the current set of studies. Similarly, hepatic PAH-DNA adduct levels in sediment extract treated fish also remained significantly elevated at 20 d post exposure at about 3 times as high as the levels observed in the NQSE group. This finding suggests that exposure to PAHs results in persistent levels of PAH-DNA adducts for 1 - 3 weeks after a short-term exposure in juvenile salmon. This finding is consistent with studies with English sole showing that DNA adducts in liver persist for > 3 weeks (Stein et al. 1993).

Overall, these findings support the conclusion that chemical tissue burdens of chlorinated contaminants as well as biochemical responses will likely persist after juvenile chinook salmon leave the estuary and exposure to toxic chemicals from sources in the Hylebos Waterway ceases. Furthermore, the collective findings for CYP1A and DNA-adducts suggest the potential for effects from exposure to PCBs and PAHs, and that juvenile salmon may experience the biological consequences of a short-term exposure to these toxic compounds which can persist for at least 1 - 3 weeks after leaving the Hylebos Waterway.

Responses of juvenile chinook salmon exposed to sediments extracts from the Hylebos Waterway in the laboratory were comparable to those observed in juvenile chinook and chum salmon from the Hylebos Waterway.

When we evaluated the biomarker responses due to exposure to a mixture of PAHs, PCBs, and HCBD in the laboratory, as represented by sediment extracts of Hylebos Waterway sediments, the collective response was comparable to the responses observed in juvenile salmon sampled directly from the Hylebos Waterway. The current findings showed that total PCBs and HCBD concentrations in liver of sediment-extract injected fish observed at 6 d post injection were similar to those measured in salmon captured in the Hylebos Waterway during Round I of the Fish Injury Study (Collier et al. 1998). Thus, biomarker responses of juvenile salmon injected with a single dose of the sediment extracts in the laboratory reflected the exposure of naturally outmigrating juvenile salmon to chemical contaminants present in the Hylebos

Waterway. More importantly, the current laboratory results suggest that a relationship exists between alterations in the CYP1A and DNA adduct responses and exposure of juvenile chinook salmon to chemical contaminants present in this Waterway. Furthermore, the findings from this study indicated that exposure to chemical contaminants from the Hylebos Waterway elicit chemical and biochemical changes which can persist for up to 3 weeks after exposure ceases. The findings from the disease challenge (Part 1) and growth (Part 2) experiment components of Round II of the Hylebos Fish Injury Study also indicated that juvenile salmon exposed to the same test solutions used in this study exhibited impaired growth and increased disease susceptibility (Arkoosh et al. 1998a, Casillas et al. 1998). In previous studies, fish naturally exposed to a similar suite of contaminants from the Duwamish Waterway also exhibited immune dysfunction (Arkoosh et al. 1991, 1994) as well as increased disease susceptibility (Arkoosh et al. 1998b) and impaired growth (Casillas et al. 1995b). Hence, we conclude that juvenile salmon from the Hylebos Waterway have an increased risk of deleterious biological effects (altered immune function, increased disease susceptibility, and reduced growth) due to exposure to the chemical contaminants present in this Waterway.