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Semipermeable Membrane Devices Link Site-Specific Contaminants To Effects: Part II – A Comparison Of Lingering Exxon Valdez Oil With Other Potential Sources Of CYP1A Inducers In Prince William Sound, Alaska

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**SEMIPERMEABLE MEMBRANE DEVICES LINK SITE-SPECIFIC
CONTAMINANTS TO EFFECTS: PART II – A COMPARISON OF LINGERING
EXXON VALDEZ OIL WITH OTHER POTENTIAL SOURCES OF CYP1A
INDUCERS IN PRINCE WILLIAM SOUND, ALASKA**

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Abstract

We deployed semi-permeable membrane devices (SPMDs) on beaches for 28 days at 53 sites in Prince William Sound (PWS), Alaska, to evaluate the induction potential from suspected sources of cytochrome P450 1A (CYP1A)-inducing contaminants. Sites were selected to assess known point sources, or were chosen randomly to evaluate the region-wide sources. After deployment, SPMD extracts were analyzed chemically for persistent organic pollutants (POPs) and polycyclic aromatic hydrocarbons (PAH). These results were compared with hepatic CYP1A enzyme activity of juvenile rainbow trout injected with the same extracts prior to clean-up for the chemical analyses. Increased CYP1A activity was strongly associated with PAH concentrations in extracts, especially chrysene homologues but was not associated with POPs. The only apparent sources of chrysene homologues were lingering oil from *Exxon Valdez*, asphalt and bunker fuels released from storage tanks during the 1964 Alaska earthquake, creosote leaching from numerous pilings at one site, and PAH-contaminated sediments at Cordova Harbor. Our results indicate that PWS is remarkably free of pollution from PAH when nearby sources are absent as well as from pesticides and PCBs generally.

Keywords: Pollution monitoring, oil spills, sources, PAH, PCB, SPMD

1. Introduction

Oil from the T/V *Exxon Valdez* (EVO) has been surprisingly persistent on some beaches in western Prince William Sound (PWS), after deposition following the spill in 1989. Estimated loss rates of $< 4\% \text{ yr}^{-1}$ for the buried oil imply persistence for decades (Short et al. 2007), presenting a potentially long-term reservoir of bioaffective contaminants to wildlife. However, distinguishing any long term biological effects of this oil from those of other contaminant sources is highly problematic.

Several species that interact with the intertidal zone in the spill-impacted part of PWS have shown prolonged expression of cytochrome P450 1A (CYP1A), a substrate-inducible enzyme that is a sensitive biomarker of exposure to planar contaminants such as polycyclic aromatic hydrocarbons (PAH), certain polychlorinated biphenyls (PCBs) and other persistent organic pollutants (POPs). Expression of CYP1A in PWS nearshore vertebrates such as sea otters (*Enhydra lutris*) harlequin ducks (*Histrionicus histrionicus*) and crescent gunnels (*Pholis laeta*) has been highest in animals taken from locations where EVO has been most persistent, and has appeared to decrease as the oil dispersed (Bodkin et al. 2002, Jewett et al. 2002, Trust et al. 2000). These patterns suggest that exposure to remaining EVO, a known CYP1A inducer (Woodin et al., 1997), may be the source of the CYP1A induction observed in these animals.

Whether the EVO remaining on PWS beaches is sufficiently bioavailable to induce CYP1A in wildlife is a matter of some controversy. Once hardened into highly viscous tar mats and asphalt pavements, the resistance to mass transfer of PAH is so great that loss rates of PAH may become extremely low. However, sea otters and ducks can

disturb sediments while foraging (Kvitek and Oliver 1992; Calkins 1978), and any subsurface oil encountered could contaminate their pelage or plumage. Oil ingested during subsequent preening (Bodkin et al. 2002, Trust et al. 2000) provides an exposure pathway for eliciting CYP1A induction and potentially toxic effects (Schwartz et al. 2004).

Evidence of CYP1A induction alone is not sufficient to identify the source of the inducing agent when numerous potential sources may be present (Short and Springman 2006). In PWS, several sources of potential CYP1A inducers besides EVO must be considered. Putative sources include atmospheric transport of POPs (Stern et al. 1997), PAH associated with erosion of terrestrial hydrocarbon source rock outcrops transported hydraulically to the northern Gulf of Alaska and then by the Alaska Coastal Current to PWS where they are deposited on the seafloor (Page et al. 1996, Short et al. 2007), POPs associated with adult salmon returning to PWS to spawn (Krümmel et al. 2003, Ewald et al. 1998), small spills of diesel and bilge oils from marine vessels, residues of asphalt and bunker fuels derived from the Monterey Formation in California and released from storage tanks in PWS that ruptured during the 1964 Alaska earthquake (Kvenvolden et al. 1995), and operations associated with continuing industrial activity in PWS such as salmon hatcheries. Resolving contributions from these prospective sources to observed CYP1A responses in biota presents a considerable challenge.

One promising approach for evaluating the CYP1A induction potential of a contaminant source involves injecting fish with contaminants accumulated by semi-

permeable membrane devices (SPMDs) deployed at locations strongly affected by the candidate source (Springman et al. 2008). This approach does not require knowledge of the chemical identity of the suspected contaminant, although analysis may provide this. Rather, it examines the biomarker response from the bioavailable complex mixture without selective filtration if the accumulated contaminants are injected without cleanup. At a minimum, prospective contaminant sources that are not capable of eliciting CYP1A responses in biota can be identified at high confidence when the injected contaminants fail to elicit a response, provided the amount of contaminant injected is comparable with those likely to be accumulated through passive equilibrium partitioning by the same organisms exposed in the field.

Our objectives in this study are to distinguish which contaminant sources in PWS are capable of eliciting CYP1A activity in dosed fish and to identify which compounds of the sampled complex mixture are most responsible. To accomplish this, we deployed SPMDs at a variety of locations that included all the prospective contaminant sources mentioned above. In Part I of this study, we measured the CYP1A induction potential of contaminants extracted from deployed SPMDs and injected without clean-up into juvenile rainbow trout (*Oncorhynchus mykiss*, Springman et al. 2008). Here we present chemical analysis results of extracts from SPMDs deployed to evaluate the compounds most likely responsible for induction, and of additional SPMDs deployed to better characterize contaminant sources and distributions in PWS.

2. Methods

2.1 Study Sites

We deployed SPMDs at 53 sites within PWS, including known and suspected contaminant source sites and sites selected at random to characterize the pollution burden of the Sound (Fig. 1). Known contaminant source sites include boat harbors at Cordova (site 1, Table 1) and Chenega (site 2), patches of Alaska North Slope (ANS) oil remaining from the 1989 *Exxon Valdez* oil spill (sites 3 - 7) or of Monterey Formation bunker fuels released from ruptured fuel tanks at industrial “human activity” (HA) sites during the 1964 Alaska earthquake (sites 8 - 12), and salmon hatcheries (sites 13 - 17). Salmon streams (sites 18 - 22) are suspected as a source of contaminants released from decomposing carcasses of mainly pink salmon (*Oncorhynchus gorbuscha*) following spawning. One stream from each of the five largest Alaska Department of Fish and Game districts was selected based on three criteria: 1) large returns of salmon during our study period, 2) small stream channels and narrow deltas where fish (with putative contaminants) would be concentrated, and 3) accessibility via float plane.

To characterize region-wide, non-point contaminant sources, 30 sites were distributed at random among 5 sectors partitioning the Sound (depicted in Fig. 1). The number of sites within each sector was proportional to the total sector shoreline length (sites 23 – 52). This stratification reduced site clustering that could result from unrestricted random selection, ensuring site dispersion throughout the sound. Additionally, to better evaluate PAH availability, one SPMD was located at a long-term monitoring

station (Karinen et al., 1993) in Constantine Harbor (site 53). At this site, PAH associated with hydrocarbon-rich rocks eroded from geologic sources east of PWS have an intertidal sediment concentration of 561 ± 15.2 ng/g ($\pm$ standard error here and following) including the same suites of 2- to 4-ring PAH as are typically found in crude oils (Short and Babcock 1996). Finally, one site was located in Graves Harbor (site 54), a remote site in southeast Alaska ~ 300 km east of PWS and upcurrent of the geologic hydrocarbon sources east of PWS to serve as a regional control.

The ANS sites (3 – 7) correspond with the following respective beach segment identifiers that are used extensively in the literature on the *Exxon Valdez* oil spill, including Part I of this study (Springman et al. 2008): DI067A, EL056C, KN109A, KN114A, and KN136A. These sites are on Disk Island, Eleanor Island and Knight Island.

Random site locations were determined by considering the shoreline within a sector as a continuous line of total length L , generating a random number r between 0 and 1, and finding the location coordinates associated with the product $(r \cdot L)$. The SPMD was deployed at the nearest suitable location to the calculated coordinates.

Additional local control sites were selected for the ANS and HA sites to evaluate the spatial extent of contaminants from these sources. At the ANS sites, SPMDs were deployed within patches of beach that contained obvious subsurface oil, to promote accumulation of dissolved oil components. Associated local control deployments were

on apparently un-oiled beaches within 0.5 km of the oiled SPMD deployment location. At the HA sites, SPMDs were deployed within patches of surface asphalt (4 sites), or subsurface oil at one site (site 9), again to promote accumulation of dissolved oil components, and local control SPMDs were deployed along shore on un-oiled portions of the same beach, within 100 m either side of the oil deposit.

2.2 SPMD Deployment and Processing

All the SPMDs were deployed at mid-tide height on beaches, in one of 2 types of canisters. The SPMDs were obtained from Environmental Sampling Technologies (EST, St. Joseph, MO), and were usually deployed in cylindrical cages 30 cm long, each containing 5 SPMDs woven on stainless steel carriers stacked within the cage. Only 1 SPMD was deployed at the local HA control locations and at 21 of the random sites, contained within a canister housing 6 cm long. Each SPMD was 91.4 cm long and 2.5 cm wide, and contained 1 ml of ultra-high purity triolein. Canisters containing 5 SPMDs were used to increase the amount of accumulated contaminants available for chemical analysis and for injection into juvenile rainbow trout that were assayed for CYP1A induction (see Springman et al. 2008 for details), while those containing only 1 SPMD were used to extend the geographic range of contaminant surveillance and were only analyzed chemically.

At each deployment site, the cages were half-buried in pits excavated on shorelines at the mid-tide level of each site, with the other half exposed to the

atmosphere. After placement in a pit, the excavated material was replaced to the level of the beach surface, surrounding, but not covering, the cage. The grain sizes of sediments on most PWS beaches range from pebbles to boulders, with relatively little mass associated with sand and finer-grained sediments that could pass through the 2 mm diameter of the SPMD cage perforations (Hayes and Michel 1999). Hence, little sediment accumulated within the cages while deployed, and in no case more than partially covered the bottom-most SPMD in the cage. This deployment arrangement therefore exposed SPMDs to the atmosphere at lower tides, to surface seawater at higher tides, and to mainly interstitial water flow within the upper 15 cm of beach for the larger canisters, while minimizing biofouling and direct contact with beach sediments. Field blank cages were briefly exposed to the atmosphere during some of the deployments. Cages were deployed for 27 – 28 days, and then retrieved for dialysis of accumulated contaminants.

Contaminants accumulated by the SPMDs were recovered by dialysis into hexane at EST labs as described in Huckins et al. (2000). The dialysates from all SPMDs within a deployment canister were combined to create a composite sample. Following concentration of the dialysates to ~1 ml hexane, samples were partitioned into subsamples for analysis. For samples derived from 5 SPMDs, the sample was partitioned into 3 aliquots for chemical analyses each containing 5% of the sample, and 2 more aliquots of 42.5% each for injection into juvenile trout for determination of CYP1A activity. One each of the aliquots for injection and for chemical analysis was archived in the event of accidental losses. For samples derived from 1 SPMD, the sample was split

into 2 aliquots for chemical analysis of PAH and POPs with no sample spared for archives. This arrangement produced twice as much contaminant mass available for chemical analysis from the single-SPMD deployments compared with the deployments involving 5 SPMDs (0.5 vs. $(5 \times 0.05 = 0.25)$) assuming equivalent deployment conditions and performance, resulting in expected environmental detection limits for the single-SPMD deployments to be lower by a factor of 2.

2.3 Sample Analysis

The sample extracts were analyzed chemically for suites of 2- to 5-ring PAH (including alkyl-substituted and dibenzothiophene homologues, the latter more accurately denoted as polycyclic aromatic compounds but included here as PAH), selected legacy pesticides and selected PCBs listed in Table 1. The PAH were analyzed in sets of twelve plus one method blank, one spiked blank, and two reference samples at ABL following the method described in Short et al. (1996). Briefly, the hexane aliquot for PAH analysis was purified by 20 g:5 g silica:alumina column chromatography and gel permeation HPLC, following addition of a suite of perdeuterated PAH used as internal standards. Purified PAH were measured by gas chromatography/mass spectrometry (GCMS) operated in the selected ion monitoring mode, and were quantified on the basis of a 5-point calibration curve of the most similar PAH standard available.

The POPs were analyzed in sets of four to twelve SPMD samples plus one method blank and one spiked method blank. Method blanks were prepared by adding 0.5

ml of dichloromethane to a 2-ml vial. Spiked method blanks were prepared by adding 0.5 ml of dichloromethane and a spiking solution of analytes to a 2-ml vial; PCBs were spiked at approximately 0.3-1.3 ng sample⁻¹ and pesticides were spiked at approximately 6 ng sample⁻¹. An internal standard (PCB 103) was added to each method blank, spiked blank and SPMD sample in its original 2-ml vial. PCBs are identified here according to the scheme presented by Ballschmiter et al. (1992)

Samples were cleaned up by size-exclusion high-performance liquid chromatography using a 350-mm x 21.2-mm Envirosep ABC[®] preparatory column and a 60-mm x 21.2-mm Envirosep ABC[®] guard column, (Phenomenex, Inc., Torrance, CA), with dichloromethane as the mobile phase (Sloan et al. 2005). Extracts containing low amounts of PAH etc. were also cleaned up by gravity-flow silica/alumina chromatography using columns packed with 20 cc of silica gel on top of 10 cc of alumina wetted with dichloromethane, and the samples were eluted with 45 ml of dichloromethane (Sloan et al. 2005). Extracts containing high amounts of PAH etc. were cleaned up by gravity-flow acidic/basic silica chromatography. Columns were packed with 5 cc of neutral silica gel (lower layer), 5 cc of KOH impregnated silica gel (middle layer), and 15 cc of 40% (w/w) sulfuric acidic impregnated silica gel (upper layer) wetted with 60 ml of 1:1 dichloromethane:hexane. The samples were eluted with 45 ml of 1:1 dichloromethane:hexane 40 minutes after being loaded on the column to allow reaction with the acidic silica (Krahn et al. 1994, modified; see also Sloan et al. 2005 for further details).

All samples were analyzed by GCMS in selected-ion monitoring mode using a 60-m x 0.25-mm, 0.25- μ m film thickness DB-5[®] GC column (J & W Scientific, Folsom, CA) and a 10-m x 0.53-mm deactivated fused-silica guard column, with cool on-column injection and helium carrier gas (Sloan et al. 2005). The concentrations of analytes were calculated based on the internal standard and point-to-point (also known as piece-wise linear) calibration using six concentrations of calibration standards ranging from approximately 0.001 to 0.1 ng μ l⁻¹ for each analyte. The lower limit of quantitation (LOQ) was reported when the GCMS response for that analyte in that sample was below the GCMS calibration range. LOQs were calculated individually by analyte and by sample (Sloan et al. 2004).

Induction of CYP1A in juvenile trout livers following injection of sample aliquots was evaluated by the ethoxyresorufin-*O*-deethylase (EROD) assay, based on an original method by Pohl and Fouts (1980) and modified for a microplate spectrofluorometer (Hodson et al. 1996). Molar specific activity was reported as picomole resorufin per mg protein per minute (pmol mg⁻¹ min⁻¹). Details of the exposure conditions and assay methods are given in Springman et al. (2008).

The oil in the patches at the ANS sites had previously been confirmed as remnants from the 1989 Exxon Valdez oil spill (Short et al. 2004). The Monterey Formation source of the oil at the HA sites was confirmed on the basis of tricyclic and tetracyclic terpane analysis as described by Kvenvolden et al. (1995).

2.4 Data Analysis

Concentrations of PAH in the SPMDs are presented as $\text{ng g}_{\text{SPMD}}^{-1}$, with three figures significant. To facilitate comparison among samples, individual PAH concentrations were normalized to the total PAH (TPAH) concentration of the sample.

A rough approximation of ambient dissolved PAH concentrations may be inferred from concentrations measured in SPMDs. Sundberg et al. (2005) estimate dissolved concentrations corresponding with contaminant concentrations measured in SPMDs as:

$$C_w = C_{\text{SPMD}} \cdot k_{I(s)}^{-1} \cdot t^{-1} \quad (1)$$

where C_w and C_{SPMD} are the contaminant concentrations in water and the SPMD, $k_{I(s)}$ is the sampling uptake rate constant of the SPMD for the contaminant in $\text{l d}^{-1} \text{g}_{\text{SPMD}}^{-1}$, and t is the exposure time of the SPMD to the sampled medium. Luellen and Shea (2002) present estimates of PAH uptake rates as device sampling rates R_S (in l d^{-1}), where $R_S = m \cdot k_{I(s)}$, and m is the mass of the SPMD. The average value of R_S for the 2 – 4 ring PAH listed by Luellen and Shea (2002) is 4.4 l d^{-1} for SPMDs identical with ours, implying values of $k_{I(s)}$ near $1 \text{ l d}^{-1} \text{g}_{\text{SPMD}}^{-1}$, and $k_{I(s)}$ estimated for PCBs are also near $1 \text{ l d}^{-1} \text{g}_{\text{SPMD}}^{-1}$ (Meadows et al. 1998). Assuming an effective exposure time of 14 d (because the SPMDs were submerged half the time during 28 days and neglecting PAH absorbed from the atmosphere) implies aqueous TPAH concentrations on the order of $C_w = C_{\text{SPMD}} \cdot (14 \text{ d})^{-1} \text{ ng l}^{-1}$ when C_{SPMD} is given as $\text{ng g}_{\text{SPMD}}^{-1}$. Given the numerous assumptions involved, including neglect of partitioning with particulate organic matter, constant uptake rates during the exposure period and neglect of increased uptake from direct

contact with sediments infiltrating the deployment cages (Booij et al. 2003), this approximation is probably only accurate to within a factor of 3 – 5, which suffices to indicate the order of magnitude of the mean aqueous PAH concentrations.

3. Results and Discussion

3.1 Boat Harbors and Anchorages

The entrance to Cordova Harbor is constricted, promoting retention of contaminants released from the numerous potential sources within it, and leading to substantial accumulation of PAH by the SPMD deployed there. The TPAH concentration of the SPMD was 73,700 ng g⁻¹, equivalent to ambient PAH concentrations on the order of 5 µg l⁻¹ (eq. 1). Phenanthrenes/anthracenes and fluoranthenes/pyrenes were the most abundant homologous series, declining in abundance with increasing alkyl substitution within each series (Fig. 2A). This PAH distribution pattern reflects the composite input from multiple sources, including spilled fuels and lubricants, and combustion exhaust products from inboard and outboard motors.

The much smaller and less confined harbor at Chenega results in considerably lower PAH concentrations compared with Cordova harbor. The TPAH concentration in the Chenega SPMD, 1,690 ng g⁻¹, was less than 2% of the concentration accumulated during the Cordova harbor deployment, and equivalent to dissolved concentrations on the order of ~121 ng l⁻¹. The PAH distribution pattern from Chenega resembled that of the

Cordova deployment, with phenanthrenes/anthracenes and fluoranthenes/pyrenes the most abundant homologous series, and with declining abundance with increasing alkyl substitution within each series (Fig. 2B). Nearly identical patterns were evident at the two random sites where TPAH were greatest, in Sawmill Bay (site 26, about 3 km from the Chenega harbor site and within the same embayment), and at Windy Bay (site 44, an embayment on the eastern side of PWS enclosing a commercial oyster farm), where the TPAH concentrations were 374 and 442 ng g⁻¹, respectively (Table 2). These SPMD concentrations imply that embayments that are more open than the harbor at Cordova have TPAH concentrations on the order of 30 ng l⁻¹, likely from outboard motors and other small boat activity.

The concentrations of total PCBs and pesticides in the SPMDs indicate that these contaminants rarely exceeded 1 ng l⁻¹ in ambient seawater. At 32.9 ng g⁻¹, the total PCB concentration in the SPMD deployed at Chenega harbor was among the highest of any found during this study (41.8 ng g⁻¹ at nearby Sawmill Bay, site 26; Table 2). At Cordova harbor the SPMD concentration was only 6.31 ng g⁻¹. Elsewhere, 90% of the SPMD concentrations were below 7 ng g⁻¹ (median = 2.67 ng g⁻¹, *N* = 89). These concentrations substantially exceeded those of the laboratory blanks (range: <LOQ – 0.40 ng g⁻¹, *N* = 4) but not the field blanks (range: 2.00 – 16.3 ng g⁻¹, *N* = 8). The distribution of relative PCB concentrations was nearly identical among all the samples analyzed, including field blanks, with almost all the PCBs present within the congener range 17 – 153 inclusive (Fig. 3). This distribution pattern is similar to that evident in alpine lakes of Europe (Fernandez and Grimalt 2003, Van Drooge et al. 2004) or in open waters of the

Baltic Sea (Sundberg et al. 2005), indicating surface water absorption of gas-phase PCBs from the atmosphere. These concentrations imply total aqueous PCB concentrations were below $\sim 1 \text{ ng l}^{-1}$ throughout PWS, except near Sawmill Bay where concentrations approach 3 ng l^{-1} . As noted by Sundberg et al. (2005), these part per trillion concentrations are unlikely to stimulate CYP1A induction in exposed biota.

Pesticide concentrations in the deployed SPMDs were at least as low as those of the PCBs, and were within the range of concentrations found in field and dialysis blanks. The summed concentrations of the pesticides listed in Table 1 ranged from $<\text{LOQ} - 5.52 \text{ ng g}^{-1}$ ($N = 87$), compared with concentrations in field blanks ranging from $0.08 - 4.73 \text{ ng g}^{-1}$ ($N = 8$), and in dialysis blanks ranging from $0.03 - 2.92 \text{ ng g}^{-1}$. The similarity of these concentrations suggests that the detected pesticides were trace level residues incorporated during SPMD manufacture or processing, rather than an indication of ambient environmental concentrations in PWS. Because the concentrations of PCBs and pesticides were uniformly below thresholds likely to induce CYP1A in biota throughout PWS, we dismissed them as material factors and focused on PAH at the other sites considered in more detail below.

Injection of the SPMD extracts from the Cordova harbor SPMD elicited a strong 2 d EROD response of $36 \text{ pmol mg}^{-1} \text{ min}^{-1}$, compared with a weak response from the Chenega harbor SPMD ($5.8 \text{ pmol mg}^{-1} \text{ min}^{-1}$; Table 2). The 7 d EROD response from Chenega was $2.97 \text{ pmol mg}^{-1} \text{ min}^{-1}$, and all these responses were significantly ($P < 0.001$) greater than those from control and un-contaminated sites

3.2 ANS Sites

The SPMDs deployed at the oiled ANS sites (sites 3 – 7, Table 2) consistently accumulated substantial PAH burdens. The TPAH concentration in the SPMDs ranged from 8,920 – 263,000 ng g⁻¹, equivalent to aqueous concentrations of 640 – 19,000 ng l⁻¹ (eq. 1). These aqueous concentration estimates are comparable with direct measurements of dissolved-phase PAH in interstitial water that drained similar ANS oil patches in 2002, which ranged from 76 – 4,600 ng l⁻¹ (average = 1,200 ng l⁻¹; Payne et al. 2005). The distribution of PAH homologues showed the characteristic preponderance of alkyl-substituted PAH (Fig. 4A), which followed a weathering pattern diagnostic of ANS oil (Short and Heintz 1997), with chrysene homologues accounting for 5 – 9% of the TPAH. Accumulated PAH in replicate SPMD deployments at 2 of the 3 replicated sites agreed within a factor of 2, but at site 3 (at Disk Island), the PAH concentration in one replicate was ~ 19-fold greater than the other. The Disk Island replicates were separated by 25 m in an extensive deposit of peat, and the low-PAH replicate was located in a surface stream above the peat layer that continually flushed the SPMD, which may have suppressed PAH accumulation. Previous studies have shown that humic substances (including peat) increase the CYP1A induction of oil when the two are co-located, possibly by increasing the solubility of petroleum hydrocarbons (Matsuo et al. 2006). The stream at the Disk Island low PAH replicate transported the humic substances from the deployment site, and these results were reflected in the EROD activity elicited by extracts from these sites, particularly at 7 d.

The injected aliquots of the SPMD extracts from the oiled ANS sites consistently elicited strong 2 d EROD responses, and often these responses persisted through 7 d (Table 2). The 2 d EROD responses ranged from 27.5 – 59.4 pmol mg⁻¹ min⁻¹, and the 7 d responses ranged from 2.73 – 100 pmol mg⁻¹ min⁻¹. The EROD replicates also corresponded within factors of ~ 1.5 except for the 7 d responses at the Disk Island site (site 3), where the EROD response of the replicate in the stream-flushed peat was lower than that of the other less well flushed replicate by a factor of ~ 6 (Springman et al. 2008).

The SPMDs deployed at the local control sites within the ANS sites accumulated far less PAH than those deployed at the oiled sites. The TPAH concentrations ranged from 132 – 299 ng g⁻¹, implying ambient seawater concentrations below ~20 ng l⁻¹, consistent with results from direct measurements of background PAH in PWS seawater (Boehm et al. 2007, 2005; Carls et al. 2006, Neff et al. 2006). Extracts from these SPMDs consistently failed to elicit a significant EROD response following injection into juvenile trout (Table 2; Springman et al. 2008).

3.3 Human Activity Sites

Although PAH were readily accumulated by SPMDs deployed within patches of oil lingering from fuel tanks damaged by the 1964 Alaska earthquake, the induction of hepatic CYP1A following injection of extracted contaminants into trout was more

variable than results from the oiled ANS sites. The patches of remnant oil at 4 of the HA sites were surficial asphalt pavements, and the SPMDs deployed within them accumulated TPAH concentrations ranging from 392 – 20,900 ng g⁻¹ (Table 2). The distribution of PAH homologues resembled that found at the small boat harbors (compare Figs. 2B and 4B), with phenanthrenes/anthracenes and fluoranthenes/pyrenes the most abundant homologous series, declining abundance with increasing alkyl substitution within each series, and chrysenes accounting for 0.5 – 5% of the TPAH. Following injection into trout, these extracts consistently elicited low but significant 2 d EROD responses ranging from 2.91 – 6.40 pmol mg⁻¹ min⁻¹ (Table 2; Springman et al. 2008). These responses subsided substantially by 7 d, ranging from 1.75 – 2.74 pmol mg⁻¹ min⁻¹.

In contrast with the surficial asphalt patches at 4 of the HA sites, the SPMD at site 9 (at McClure Bay) was deployed in contact with the only occurrence of subsurface Monterey Formation oil we have found in PWS, despite extensive sampling (Short et al. 2004, 2006). Chemical analysis confirmed the Monterey Formation origin of this oil, which is still semi-liquid after 40 years. The distribution of PAH homologues at site 9 resembles those of the SPMDs deployed at the ANS sites, showing the preponderance of alkyl-substituted PAH characteristic of crude and heavy refined oils, with chrysene homologues accounting for 6.7% of the TPAH (Fig. 4). Although the TPAH concentration in the site 9 SPMD was not the greatest among the HA deployments, the combined chrysene homologues were (Table 2), and the associated EROD response was also much greater at 2 d (at 37.6 pmol mg⁻¹ min⁻¹) and at 7 d (at 15.7 pmol mg⁻¹ min⁻¹). These results illustrate how burial preserves CYP1A-inducing components of the oil, and

suggest that buried ANS oil may remain a potent source of CYP1A-inducing compounds for decades, given the current slow loss rate of subsurface ANS oil from beaches in PWS.

The SPMDs deployed at the local control locations within the HA sites show that the PAH contamination from the asphalt patches did not extend much beyond them. Just 100 m on either side of these patches, TPAH concentrations in SPMDs ranged from 41 – 229 ng g⁻¹, similar to results from the control locations within the ANS sites. As with the ANS local controls, extracts from the HA control SPMDs consistently failed to elicit a significant EROD response following injection into juvenile trout (Table 2; Springman et al. 2008).

3.4 Salmon Hatcheries

Substantial PAH were accumulated by the SPMDs deployed at 2 of the salmon hatcheries (sites 13 and 15, Table 2), only one of which induced CYP1A. The deployment at the hatchery in Sawmill Bay (site 13) was near several hundred creosote pilings. This SPMD accumulated a TPAH concentration of 15,200 ng g⁻¹, with a PAH homologue distribution similar to those elsewhere in Sawmill Bay (compare Figs. 2B and 2C), except for the greater contributions from chrysenes and 5-ring PAH. The higher concentrations and proportions of 4- and 5-ring PAH from site 13 resulted in a stronger EROD response (34.8 pmol mg⁻¹ min⁻¹) compared with extracts from the other sites within Sawmill Bay (sites 2 and 44, Table 2), and this response remained elevated through 7 d (at 4.90 pmol mg⁻¹ min⁻¹).

In contrast, a TPAH concentration of 10,900 ng g⁻¹ accumulated by the SPMD deployed at Noerenburg hatchery (site 15) failed to elicit a significant EROD response following injection into trout ($P > 0.92$ for 2 d results; Springman et al. 2008; Table 2). The distribution pattern of the accumulated PAH indicates a diesel oil source, because the homologue distribution is truncated beyond the phenanthrenes/anthracenes (Fig. 5), reflecting the effect of distillation during refining. The failure of this extract to induce CYP1A corroborates the low induction potency of lower molecular mass PAH noted previously (Sundberg et al. 2005, Barron et al. 2004). Hence diesel spills, although chronic in PWS as a result of marine vessel traffic associated with commercial fishing, tourism, and personal transportation, may be discounted as a significant cause of CYP1A induction in biota.

3.5 *Salmon Streams*

Concentrations of PAH accumulated by SPMDs deployed at the salmon streams during spring and summer ranged from 57 – 585 ng g⁻¹ ($N = 10$; Table 2), similar to ranges from other sites not impacted by obvious local sources of PAH. Despite these low TPAH concentrations, there was a small but significant increase in EROD response from the late summer deployments compared with those of late spring (Springman et al. 2008). The salmon streams sampled ($N = 5$) exhibited a high degree of inter-site consistency in spring (2 d mean value of 2.7 ± 0.062 pmol mg⁻¹ min⁻¹, CV = 0.052), which was repeated for the summer sampling with consistent, slightly elevated 2 d responses ranging from

3.24 – 5.73. This slight seasonal increase in EROD response was attributed to contaminants associated with decomposing salmon carcasses by Springman et al. (2008).

3.6 Random Sites, Constantine Harbor and the Regional Control Site

The TPAH concentrations of SPMDs deployed at the random sites were consistently low, and showed no evidence of a seasonal difference. The TPAH concentrations of the spring deployments ranged from 25 – 374 ng g⁻¹ (mean = 130 ng g⁻¹, $N = 31$), compared with a summer range of 40 – 442 ng g⁻¹ (mean = 126 ng g⁻¹, $N = 13$). Aside from the two sites where TPAH concentrations were greatest, (sites 26 and 44, discussed in sec. 3.1 above) and one site where naphthalenes account for over half the PAH detected (site 33, where TPAH = 332 ng g⁻¹), the TPAH concentrations were below 212 ng g⁻¹. This range of concentrations was similar to those found in the SPMD field blanks, where TPAH ranged from 63 – 213 ng g⁻¹, consisting mostly of naphthalenes with some phenanthrenes and fluoranthenes/pyrenes detected consistently, and dibenzothiophenes and chrysenes occasionally detected near MDLs. Because random deployment sites may be taken as characteristic of PWS as a whole, these results imply that ambient concentrations of TPAH in seawater within the Sound were usually less than ~ 15 ng l⁻¹, similar to results from the local control deployments at the ANS and HA sites. Collectively these results indicate that PWS is remarkably free of pollution from PAH, as well as from pesticides and PCBs, when nearby sources are absent.

The SPMD extracts from the random sites failed to induce significant EROD responses following injection into trout (Table 2). The 2 d EROD responses ranged from 1.70 – 4.78 pmol mg⁻¹ min⁻¹ (N = 9), compared with responses from field and laboratory blanks that ranged from 2.80 – 6.40 pmol mg⁻¹ min⁻¹ (N = 5). This confirms that EROD inducing agents were generally absent in PWS.

The low ambient PAH concentrations of PWS are probably typical of seawater in the northern Gulf of Alaska generally. Comparable TPAH concentrations were found in SPMDs deployed at Constantine Harbor and at the regional control site in northern southeast Alaska (sites 53 and 54 respectively, Table 2). The SPMD deployed at Constantine Harbor accumulated a TPAH concentration similar to those found in the field blanks (Table 2), despite exposure to interstitial water draining sediments containing a TPAH concentration of ~ 560 ng g⁻¹ (Short and Babcock 1996). This failure of the SPMD to accumulate TPAH from these sediments demonstrates that PAH associated with hydrocarbon source rock that is eroded by glaciers and then transported by the Alaska Coastal Current along the northern Gulf of Alaska into PWS do not induce CYP1A in fish and other biota, contrary to Huggett et al. (2003) (see Springman et al. 2008 for more discussion).

3.7 Identification of CYP 1A-Inducing Compounds in PWS

We sought to identify which components of the injected PAH mixtures are most closely associated with CYP 1A induction by regression analysis. We began with a

comparison of linear and non-linear regressions of EROD activity on TPAH using a variety of functions and ranking results according to the goodness-of-fit as determined by the adjusted multiple- R^2 and model parsimony. The relation $\text{EROD} = a [\text{TPAH}]^b$ (eq. 2) accounted for the variability of the data better than any other function evaluated, including those with as many as four parameters (Marquardt-Levenberg non-linear regression $R^2 = 0.87$, $N = 62$; Fig. 10A). When the TPAH concentration of the injection mixtures is low, EROD activity is consistently at the basal level, implying that PAH are the likely inducing compounds rather than some other unknown contaminants absorbed by the SPMDs but not measured by our chemical analysis.

The CYP 1A induction potencies of PAH vary depending on the number of aromatic rings, with 4- and 5-ring compounds much more potent than PAH with fewer rings (Barron et al. 2004). We therefore compared non-linear regressions of TPAH, total 4- & 5-ring PAH and total chrysenes with EROD activity to identify more precisely which PAH homologues in our injection mixtures best account for EROD induction. Using the same 62 samples that were used above to determine the functional form for EROD response to TPAH (eq. 2), the adjusted multiple- R^2 values for total 4- & 5-ring PAH and total chrysenes were 0.87 and 0.92 respectively. This indicates the chrysenes account best for the EROD activity for the mixtures we injected, whereas the total 4- & 5-ring PAH account no better than do the TPAH. The closer association of the chrysenes implies that other PAH do not materially contribute to EROD induction in the mixtures we injected. The regression equation for the chrysenes is $\text{EROD} = 58.3 [\text{chrysenes}]^{0.306}$. Comparison of a plot of this equation and associated data with the

original plot for TPAH (Fig. 6) illustrates the better fit with the chrysenes, caused mostly by 3 samples that had elevated TPAH but undetectable chrysenes and low associated EROD activities. Note that the functional form of the EROD response implies that increasing responses imply disproportionately larger increases in exposure concentrations, which should be considered when interpreting results of monitoring programs based on the SPMD-extract injection method presented here.

The close association of chrysenes and EROD response is not surprising, given the PAH composition of the sources we evaluated and the general absence of other CYP1A-inducing compounds in PWS. Compared with the model CYP 1A inducer benzo[*a*]pyrene, chrysene is more abundant in these sources (especially in ANS oil, where its concentration is more than 50-fold greater), and it is about as water-soluble (Mackay et al. 1992). This higher abundance offsets the lower CYP1A induction potency of chrysene, which is ~25% that of benzo[*a*]pyrene (Barron et al. 2004). In addition, alkyl-chrysenes are even more abundant in ANS oil and other PWS sources, although considerably less water soluble. Alkyl-phenanthrenes are both abundant in PWS sources and substantially more soluble than are correspondingly-substituted chrysenes (Mackay et al. 1992), but their CYP1A induction potencies are lower by at least one order of magnitude. Other PAH are either not as abundant or are not as potent (or both) as are the chrysene homologues. We therefore conclude that chrysenes are primarily responsible for CYP 1A induction in PWS, at least when exposure occurs through aquatic contact (ingestion of prey contaminated by CYP 1A-inducing compounds is an independent exposure route that is not assessed by the SPMD-extract injection method described

here). Hence, bioavailable sources containing substantial concentrations of chrysene homologues are likely to stimulate CYP1A induction in appropriate test organisms through aquatic exposure, and induction in resident biota exposed to these sources would not be surprising. Such sources are relatively few in PWS and are limited to specific anthropogenic impacts, the *Exxon Valdez* oil spill being the largest.

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Tables

Table 1. Names and abbreviations of contaminants analyzed in SPMDs.

Table 2. Concentrations of PAH, PCB and pesticides in semipermeable membrane devices deployed in Prince William Sound, Alaska during 2004, and injected into juvenile rainbow trout along with the consequent induction of hepatic CYP1A as measured by the EROD assay (see Methods). ANS = Alaska North Slope crude oil; Chry = total chrysenes; LOQ = limit of quantitation; SEAK = southeastern Alaska. Mean EROD responses are from Springman et al. (2008).

Figure Legends

Figure 1. Map of study region. Site numbers are also listed in Table 2. Dashed lines indicate Alaska Department of Fish and Game districts.

Figure 2. Relative PAH concentrations accumulated by SPMDs deployed at (A) Cordova harbor (site 1), (B) Chenega harbor, Sawmill Bay and Windy Bay (sites 2, 26 and 44), and (C) Sawmill Bay salmon hatchery (site 13). Vertical lines indicate ranges here and following. See Table 1 for PAH abbreviations.

Figure 3. Proportions of total PCB concentrations of all field SPMD samples analyzed for this study ($N = 94$). Numbers on abscissa identify PCB homologues (see Table 1).

Figure 4. Relative PAH concentrations accumulated by SPMDs deployed at oiled (A) ANS sites 3 – 7, (B) surface (sites 8 and 10 – 12) and (C) subsurface (site 9) Monterey Formation sites.

Figure 5. Relative PAH concentrations accumulated by the SPMD deployed at site 15 (W. H. Noerenburg salmon hatchery on Esther Island) (site 15, Table 2). See Table 1 for PAH homologue abbreviations.

793 Figure 6. EROD response following injection of contaminants expressed as (A) TPAH
794 and (B) total chrysenes accumulated by SPMDs into rainbow trout.

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Figure 1.

Figure 2.

Figure 3.

Figure 4.

Figure 5.

Figure 6.

Table 1.

PAH	PCB Homologue Number		Pesticide
Naphthalene	N0	17	Hexachlorobenzene
C1-Naphthalenes	N1	18	α -Hexachlorocyclohexane
C2-Naphthalenes	N2	28	β -Hexachlorocyclohexane
C3-Naphthalenes	N3	31	Lindane
C4-Naphthalenes	N4	33	α -Chlordane
Biphenyl	bip	44	cis-Nonachlor
Acenaphthene	ace	49	γ -Chlordane
Acenaphthylene	acn	52	Heptachlor
Fluorene	F0	66	Heptachlor epoxide
C1-Fluorenes	F1	70	Nonachlor III
C2-Fluorenes	F2	74	oxy-Chlordane
C3-Fluorenes	F3	82	trans-Nonachlor
C4-Fluorenes	F4	87	Aldrin
Dibenzothiophene	D0	95	Dieldrin
C1-Dibenzothiophenes	D1	99	Mirex
C2-Dibenzothiophene	D2	101	endo-Sulfan I
C3-Dibenzothiophene	D3	105	o,p'-DDD
C4-Dibenzothiophene	D4	110	o,p'-DDE
Phenanthrene	P0	118	o,p'-DDT
C1-Phenanthrenes/Anthracenes	P1	128	p,p'-DDD
C2-Phenanthrenes/Anthracenes	P2	138	p,p'-DDE
C3-Phenanthrenes/Anthracenes	P3	149	p,p'-DDT
C4-Phenanthrenes/Anthracenes	P4	151	
Anthracene	A0	153 + 132	
Fluoranthene	flu	156	
Pyrene	pyr	158	
C1-Fluoranthenes/Pyrenes	FP1	170	
C1-Fluoranthenes/Pyrenes	FP2	171	
C1-Fluoranthenes/Pyrenes	FP3	177	
C1-Fluoranthenes/Pyrenes	FP4	180	
Benz[a]anthracene	baa	183	
Chrysene	C0	187	
C1-Chrysenes	C1	191	
C2-Chrysenes	C2	194	
C3-Chrysenes	C3	195	
C4-Chrysenes	C4	199	

Benzo[<i>b</i>]fluoranthene	bbf	
Benzo[<i>k</i>]fluoranthene	bkf	205
Benzo[<i>e</i>]pyrene	bep	206
Benzo[<i>a</i>]pyrene	bap	208
Perylene	per	209
Indeno[1,2,3- <i>cd</i>]pyrene	icp	
Dibenz[<i>a,h</i>]anthracene	dba	
Benzo[<i>ghi</i>]perylene	bgp	

Hatcheries

(13)	15,200	780	3.91	1.33	1.58	0.081	0.0004	0.0001	34.8	4.90
(14)	149	<LOQ	2.84	2.37	0.02	<LOQ	0.0003	0.0002	1.99	1.49
(15)	10,900	<LOQ	3.64	1.02	1.13	<LOQ	0.0004	0.0001	2.53	2.15
(16)	329	<LOQ	2.93	4.39	0.03	<LOQ	0.0003	0.0005	4.88	1.55
(17)	233	<LOQ	5.51	2.88	0.02	<LOQ	0.0006	0.0003	2.53	2.00

Salmon

Streams

Spring

(18)	211	<LOQ	2.93	3.03	0.022	<LOQ	0.0003	0.0003	2.47	1.41
(19)	585	<LOQ	2.22	0.94	0.061	<LOQ	0.0002	0.0001	2.68	0.98
(20)	150	<LOQ	2.76	2.74	0.016	<LOQ	0.0003	0.0003	2.75	1.48
(21)	168	<LOQ	2.67	2.61	0.017	<LOQ	0.0003	0.0003	2.84	1.71
(22)	191	<LOQ	4.71	3.32	0.020	<LOQ	0.0005	0.0003	2.60	1.52

Salmon

Streams

Summer

(18)	57	<LOQ	3.11	2.76	0.006	<LOQ	0.0003	0.0003	3.48	1.13
(19)	99	<LOQ	1.87	1.79	0.010	<LOQ	0.0002	0.0002	3.24	1.53
(20)	125	<LOQ	7.02	4.33	0.013	<LOQ	0.0007	0.0004	5.73	4.79
(21)	103	<LOQ	2.49	2.78	0.011	<LOQ	0.0003	0.0003	3.96	3.00
(22)	107	<LOQ	6.67	3.02	0.011	<LOQ	0.0007	0.0003	3.60	1.76

Random

Sites

Spring

(23)	179	<LOQ	8.89	3.82	0.019	<LOQ	0.0009	0.0004	2.67	0.59
(24)	211	<LOQ	15.1	3.41	0.022	<LOQ	0.0016	0.0004	1.98	1.76
(25)	205	<LOQ	3.38	3.30	0.021	<LOQ	0.0004	0.0003	2.97	0.65
(26)	374	2	41.8	5.52	0.039	<LOQ	0.0043	0.0006	3.56	1.48
(27)	79	<LOQ	2.67	3.98						
(28)	49	<LOQ	2.49	3.60						
(29)	59	<LOQ	1.24	1.21						
(30)	33	<LOQ	2.09	3.41						
(31)	110	<LOQ	<LOQ	2.67						
(32)	113	<LOQ	0.68	0.89						
(33)	322	<LOQ	1.73	2.49						
(34)	93	<LOQ	2.40	3.52						
(35)	25	<LOQ	2.18	3.31						
(36)	70	<LOQ	1.78	2.50						
(37)	69	<LOQ	2.04	3.32						
(38)	35	<LOQ	2.44	2.43						
(39)	29	<LOQ	2.67	3.59						

Random

Sites

Summer

(40)	102	<LOQ	2.22	1.62	0.011	<LOQ	0.0002	0.0002	1.81	1.86
(41)	128	<LOQ	2.13	3.30	0.013	<LOQ	0.0002	0.0003	2.38	1.70
(42)	127	<LOQ	3.56	3.13	0.013	<LOQ	0.0004	0.0003	3.10	1.64
(43)	77	<LOQ	4.00	2.75	0.008	<LOQ	0.0004	0.0003	4.78	0.98
(44)	442	<LOQ	6.13	1.72	0.046	<LOQ	0.0006	0.0002	1.70	1.97
(45)	91	<LOQ	1.56	3.03						
(46)	48	<LOQ	2.49	2.82						

(47)	56	<LOQ	1.96	2.39						
(48)	71	<LOQ	2.49	3.25						
(49)	40	<LOQ	1.87	2.75						
(50)	138	<LOQ	4.31	2.78						
(51)	208	<LOQ	2.18	2.25						
(52)	104	<LOQ	5.78	3.96						
Constantine										
Harbor										
(53)	197	<LOQ	2.93	3.48	0.020	<LOQ	0.0003	0.0004	2.83	1.39
Regional										
Control										
SEAK										
(54)	258	<LOQ	3.11	4.61	0.027	<LOQ	0.0003	0.0005	2.59	1.87
Field										
Blanks										
(18)	109	<LOQ	2.31	1.35	0.011	<LOQ	0.0002	0.0001	3.80	1.61
(42)	132	<LOQ	16.3	1.05	0.008	<LOQ	0.0010	0.0001	3.57	4.82
	213	3	3.41	1.07						
	88	<LOQ	3.20	0.08						
	63	<LOQ	2.80	2.74						
(27)	127	<LOQ	2.00	1.56						
	120	3	2.84	4.73						
	83	<LOQ	2.76	2.11						
Lab										
Blanks										
	89	<LOQ	<LOQ	2.92	0.009	<LOQ	<LOQ	0.0003	2.80	1.17
	71	<LOQ	<LOQ	0.31	0.007	<LOQ	<LOQ	<LOQ	6.40	5.02
	63	<LOQ	0.40	0.58	0.001	<LOQ	<LOQ	<LOQ	2.80	1.17
	0	<LOQ	<LOQ	0.03						
	18	<LOQ	<LOQ	0.32						