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Environmental Toxicology

THE TOXICITY OF CREOSOTE-TREATED WOOD TO PACIFIC HERRING EMBRYOS AND CHARACTERIZATION OF POLYCYCLIC AROMATIC HYDROCARBONS NEAR CREOSOTED PILINGS IN JUNEAU, ALASKA

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Abstract

Polycyclic aromatic hydrocarbons (PAHs) from creosote exposure in the laboratory resulted in deleterious effects in developing Pacific herring (*Clupea pallasi*) embryos, and potentially toxic concentrations of PAHs were measured using passive water samplers at 1 of 3 harbor field sites in Juneau, Alaska, USA. Aqueous **<ZAQ;1>**total PAH concentrations of 4.6 µg/L and 8.4 µg/L from creosote exposure resulted in skeletal defects and ineffective swimming in hatched larvae in the laboratory (10% effective concentrations) and were the most sensitive parameters measured. Hatch rates also suffered from creosote exposure in a dose-dependent manner: at exposures between 5 µg/L and 50 µg/L total PAH, 50% of the population failed to hatch. Comparisons between laboratory and field deployed passive samplers suggested that for at least 1 harbor in Juneau, concentrations sufficient to induce teratogenic effects were found directly on

creosoted pilings, within 10 cm of them, and sometimes at a distance of 10 m. Total PAH concentrations generally decreased with distance from creosoted pilings. Creosote pilings contribute to the PAH load within a marina and can rise to PAH concentrations that are harmful to fish embryos, but at a scale that is localized in the environment.

Keywords: Aquatic toxicology, Environmental toxicology, Polycyclic aromatic hydrocarbons (PAHs), Water quality, Ecotoxicology, Herring, Fish embryos, Creosote

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INTRODUCTION

Placement of creosote-treated wood in marine structures such as docks raises environmental concerns about its toxicity to nontarget organisms such as Pacific herring embryos that spawn nearshore on or near creosoted pilings [1–4]. Creosote is toxic to marine borers and can preserve wood for up to 100 yr in the marine environment [5]. The chemical composition is complex and includes polycyclic aromatic hydrocarbons (PAHs) [6–9], which are known to be toxic to developing fish embryos at low parts-per-billion (ppb) concentrations [7,10–12]. The sensitivity to low ppb concentrations of PAH causes concern for embryos that might be exposed to low level leachate from creosote-treated pilings, including those not attached to treated pilings.

Literature on embryo toxicity investigating the effects of the 1989 *Exxon Valdez* spill and the 2010 *Deepwater Horizon* blowout has demonstrated that low ppb (μg/L) PAH concentrations were harmful to embryos of several different species, ranging from cold-water species of salmon

and herring to warm-water species of blue fin tuna and mahi-mahi [11–19]. The same toxic PAHs are found in both creosote-treated woods and crude oils, although in different proportions [8,20].

In the present study, we used embryos from Pacific herring (*Clupea pallasi*), because spawning often occurs in harbors, near and on creosote structures, and because previous studies have demonstrated that herring embryos are sensitive to PAHs from crude oil at concentrations as low as 1 µg/L to 5 µg/L [1–4,10,21]. These concentrations can result in malformations and genetic damage, and higher concentrations are associated with more obvious morphological defects such as retarded growth, precocious hatching, reduction in swimming ability, and mortality [11,13,21]. Chronic, low-level aqueous total PAH **<ZAQ;2>**(TPAH) concentrations (<1 µg/L) can accumulate in developing embryos, negatively affecting fitness in later life stages, which may have population-level consequences [20,22].

Information on the toxicity of creosote is limited, but the most recent and relevant study on the effects of creosote exposure to Pacific herring embryos is that of Vines et al. [10]. In laboratory exposures of creosote-contaminated water to embryos, Vines et al. [10] estimated the concentration lethal to 50% of herring embryos (LC50) was 50 µg/L diffusible creosote, determined by ultraviolet fluorescence. Field observations demonstrated 100% mortality in embryos attached to creosote pilings. Some embryos were removed from pilings to clean water, but 76% of those embryos failed to develop beyond the first few days of incubation. Most of the larvae that hatched after creosote exposure (93%) exhibited reduced heart rates and arrhythmia, consistent with embryos of other species exposed to crude oil PAHs [10,11,13,16,17,20,21]. Morphological abnormalities, especially scoliosis, were observed in embryos exposed to creosoted pilings [10].

The objective of the present study was to expand the Vines et al. [10] study in 2 ways: first, determine the minimum aqueous TPAH concentrations from creosote that are damaging to herring embryos, and second, determine environmental exposure using passive field sampling in harbors at varying distances from creosote-treated pilings. We used a constant flowing water exposure system that mimics the environmental exposure as much as possible, combined with identification and quantification of the individual PAH in the exposures. Rather than using aged piling pieces with unknown and varying weathering for the laboratory toxicity study, we exposed herring embryos to wood that was impregnated with creosote (according to current best management practices) and minimally weathered, providing a relevant toxicity data set for embryonic creosote exposure to new creosote piling structures.

MATERIALS AND METHODS

Herring embryo collection and preparation

Gravid Pacific herring were caught with jigs and beach seines during March and April 2011 near Juneau, Alaska (Seymour Canal, USA; 57.5°N, 133.8 °W). Fish were maintained in tanks at the National Oceanic and Atmospheric Administration Auke Bay Laboratory until 18 May 2011, when they were used in the present study. The mean and standard error for the gonadal somatic index was 18 ± 0.15 for females and 14 ± 0.37 for males, indicating that the fish were in a ripe spawning state [23].

Eggs from 16 females were crossed with 8 males to yield 16 replicates of 100 to 200 eggs in each of 8 treatments. The eggs were removed from longitudinally cut ovaries with a spatula and swirled in water to adhere them to glass slides as described by Carls et al. [11]. The slides were subsequently placed in slide racks, suspended in 1-L beakers, and fertilized within 30 min with a few milliliters of milt. Each male fertilized the eggs of 2 females. After 5 min, the eggs

were moved into clean seawater. When all fertilizations were complete, the slide racks were moved into their respective treatments. At 24 h postfertilization, excess embryos were removed from the margins of slides with a razor blade where deposition was more than 1 layer deep. Exposures lasted until the onset of hatching (15 d), then each slide was removed from treatment and placed in a 100-mm ×15-mm petri dish filled with clean seawater. The petri dishes were maintained in a walk-in cooler at ambient seawater temperature. Hatch rates were recorded, and fresh seawater was provided every 1 d to 3 d.

To test the effect of creosote exposure on fertilization, an additional 5 slides per ovary were prepared as described, except that gametes were fertilized in their respective treatment solutions. These slides were individually housed inside plastic bottles outfitted with a Nitex screen to allow water flow and were not relocated at the onset of hatch.

To measure PAH uptake, additional eggs were applied to a Nitex screen and fertilized as described. These were placed on the bottoms of treatment aquaria in 3 different treatments: 6.5 L and 26 L creosote-treated wood and the water control. Subsamples of embryos were collected for hydrocarbon analysis on experimental days 1, 2, 4, 8, and 15.

Exposure conditions

Embryos were exposed to creosote effluent generated by continuously flowing fresh, filtered seawater past variable volumes of creosote-treated boards nested within polyvinyl chloride (PVC) pipes. Fresh, filtered seawater from Auke Bay was gravity-fed from a 500-L Living Stream® fiberglass tank into a PVC manifold equipped with 9 valves and outlets to 8 separate generator columns.

Twenty creosote-treated boards were donated by J.H. Baxter & Co. through Oregon State University. They were treated according to the American Wood Preservers Association UC5 best

management specifications for marine applications (J. Farley, J.H. Baxter & Co., Eugene, OR, USA, personal communication). Ten of the boards were end-sealed using untreated Douglas fir boards glued to the end using Gluvit and leached inside a large container using a free-flowing seawater system. The weathering process was measured by taking effluent samples and analyzing them using the GCMS methods specified in the *PAH extraction and measurement* section of the *Methods* section. The weathering data were used to determine the volume(s) of wood needed for the exposure series to be between 1 µg/L and 75 µg/L TPAH over a 2-wk period. The controls and the first 4 treatment generator columns were 20 cm in diameter × 60 cm tall; the upper 2 were 30 cm × 122 cm. The 2west treatments (0.3 L and 0.8 L creosote-treated wood) were created with a single, previously leached board cut into a 5-cm and a 15-cm piece, respectively. The 15-cm board had 1 end sealed, and the 5-cm board had neither end sealed. The other creosote treatment generator columns (3.2 L, 6.5 L, 13 L, and 26 L creosote-treated wood) contained 1 board (end-sealed and weathered), 2 boards (cut in half without ends sealed and unweathered), 4 boards (half end-sealed and weathered), and 8 boards (unsealed and unweathered), respectively. The generator columns were run with flowing seawater for 2 d prior to the toxicity experiment start.

Seawater flowed into the base of the generators, past the boards, and out through Tygon tubing to the bottoms of 10-L polycarbonate aquaria. The aquaria were nested within a 500-L Living Stream® tank supplied with running seawater that functioned as an insulating water bath to aid in temperature control. Water samples (3.8 L) were collected from effluent outlets on exposure days 0, 1, 2, 4, 8, 12, 15, and 30 for TPAH analysis to document dose levels and changes over time.

Flow rates were 480 ± 3.4 mL/min; dissolved oxygen was > 90% saturated (measured

using a YSI 55/12 FT portable dissolved oxygen meter on days 8 and 13); nitrate and ammonia were below detection (measured with an API aquarium test kit on days 10 and 13); salinity was 31 psu (measured with a YSI 30 Model 30/10 FT portable meter on days 8, 10, and 13); and mean temperature was 6.4°C to 6.5 °C in most treatments, 6.8 °C in the wood control, and 7.1 °C to 7.5 °C in the 2 upper treatments. Temperatures in the 2 upper treatments and the wood control were significantly different from each other and from remaining treatments ($p \leq 0.05$).

Embryo and larval measurements

Fertilization success, hatching success, frequency of skeletal defects, and swimming performance were measured for the developing embryos. Fertilization success was measured on day 12 by counting the number of eyed and uneyed embryos using photographs obtained with a Canon EOS Rebel T2i camera with an ESF 18–55-mm lens. Beginning at the onset of hatch (day 15), the remaining responses were observed and quantified on 25% to 75% of the slides daily. Numbers of hatched larvae (live and dead), **<ZAQ;3>**dead, eyed embryos, and skeletal defects in hatched larvae were recorded. A skeletal defect was noted if the spine had any kinks apparent to the naked eye. For live larvae, swimming performance was observed and classified as normal, abnormal, or moribund. Normal swimming was defined as swimming in a typical S pattern, whereas abnormal swimming included sporadic twitching and swimming in circles. All larvae alive at the time of observation were preserved in 10% neutral buffered formalin and placed in uniquely labeled glass vials after a lethal dose of tricain methanesulfonate solution was administered in accordance with the University of Alaska Institutional Animal Care and Use Committee guidelines.

Passive water sampler preparation

Passive water samplers were used in the field and the laboratory to characterize

hydrocarbon exposures. Passive water samplers were manufactured, deployed, retrieved, extracted, and analyzed for PAHs as described by Carls et al. [24]. They were constructed from low-density polyethylene (thickness, ~98 µm; Brentwood Plastics). For field deployment, the passive samplers were 4.9 cm wide × 50 cm or 70 cm. To compare field and laboratory data, smaller strips (approximately 2.5 cm × 4.9 cm) were submerged in treatment effluent. Before deployment, all strips were hydrocarbon cleaned by sonicating in pentane for 15 min, then soaking in pentane for 3 intervals of 30 min. Field deployed strips were loaded into pucks (cylindrical stainless steel housings having perforated stainless steel tops and bottoms [50 cm]) or attached to stainless steel halibut clips (70 cm), depending on the application. Both pucks and clips were cleaned prior to use by soaking and agitating in methylene chloride, followed by drying. Clips were used for 1-m and 10-m samples. Pucks were used for all 0.1-m samples, ensuring that the passive samplers were not in direct contact with creosoted pilings. After the pucks were loaded with the sampling strips, they were double-wrapped in aluminum foil and double heat sealed in Ziploc® freezer bags. A laboratory blank was taken from each batch of 15 prepared pucks and analyzed to verify predeployment cleanliness. Retrieved pucks and clips were also double-wrapped in aluminum foil and double-bagged. They were either frozen or processed immediately. To ensure field cleanliness, a trip blank (packed and transported with all other samplers but never opened) was analyzed in addition to a field blank that was opened and exposed to the air for approximately 2 min. The strips were unloaded from pucks and clips with hydrocarbon clean tools, placed in hydrocarbon-free jars, and frozen pending analysis.

PAH extraction and measurement

Embryo tissue was extracted with methylene chloride in a Thermo Scientific Dionex ASE system ™ [25]. Mean tissue sample mass was 1.7 ± 0.08 g. Samples were purified by both silica

gel/alumina column chromatography and high-performance liquid chromatography before analysis on a gas chromatograph–mass spectrometer.

Water samples were extracted within 1 h of collection with 75 mL methylene chloride twice after adding 6 deuterated PAH standards for recovery calculation according to the method of Short et al. [25]. The extracts were stored at −20 °C until concentrated and exchanged for hexane on a steam bath.

Six deuterated PAH recovery standards in hexane were added to the passive sampler strips before extraction. The strips were placed in hydrocarbon-clean glass vessels, covered in aluminum foil, and sonicated in 80:20 pentane:methylene chloride for 20 min, followed by a 30-min soak period in triplicate. After the final sonication, extracts were dehydrated over sodium sulfate, concentrated on a steam bath, and exchanged for hexane. Concentrated samples were passed through a 1.5-g silica gel column to remove extraneous compounds.

All extracts were spiked with an internal instrument standard for concentration calculation and run in single ion monitoring mode according to Short et al. [25] on an Agilent 7890A gas chromatograph equipped with a model 5975C mass selective detector at National Oceanic and Atmospheric Administration Auke Bay Laboratories Ted Stevens Marine Research Institute. Measured PAHs were naphthalenes (N0 to N4), biphenyl, acenaphthylene, acenaphthene, fluorenes (F0 to F4), dibenzothiophenes (D0 to D4), phenanthrenes (P0 to P4), anthracene, fluoranthene, pyrene, fluoranthene/pyrenes (FP1 to FP4), benzo(a)anthracene, chrysenes (C0 to C4), benzo(b)fluoranthene, benzo(k)fluoranthene, benzo(e)pyrene, benzo(a)pyrene, perylene, indeno(1,2,3-cd)pyrene, dibenzo(a,h)anthracene, and benzo(ghi)perylene. Total PAH is reported as the sum of 44 individual PAHs and their alkylated homologues ranging in size from naphthalenes to benzo(ghi)perylene.

Passive sampler field deployments

To characterize environmental hydrocarbon exposures in the field, passive water samplers were deployed at 2 docks and 1 harbor in Juneau, Alaska, during October 2011 and summer 2012. The summer sampling involved both continuous and monthly deployments. The same field sites were sampled during both parts of the study. Each sampling period lasted for 14 d to compare data with the laboratory toxicity experiment.

The sites were chosen because they were spatially and functionally different and represented 3 different creosote exposure scenarios. They were Aurora Harbor (AH; 1423 Harbor Way), the Auke Bay Marine Station dock (ABMS; 11305 Glacier Hwy), and the National Park Service Indian Point Field Office dock (IPFO; 3100 National Park Rd). All 3 locations were built in the early to mid-1960s. Aurora Harbor is the largest public harbor in Juneau; it has approximately 260 creosoted pilings in a partially enclosed area approximately 0.1 km^2 and is used by approximately 400 vessels, many of them residential. The ABMS dock (48 creosote pilings) is located on the northeast side of Auke Bay, within a relatively developed marine area. The IPFO dock located in Indian Cove, just outside Auke Bay, has 97 creosote pilings and is used solely by the National Park Service.

Replicate passive samplers were deployed 0.1 m, 1 m, and 10 m from creosote-treated pilings once a month for 14 d in October 2011 and from June 2012 to October 2012, except that deployment and retrieval occurred every 2 wk at ABMS to collect a continuous data set. The 0.1-m and 1-m samplers were deployed among pilings. Four passive sampling strips also were fastened subtidally on both ends to creosoted pilings at ABMS to measure hydrocarbons leaching from the surface of the piling. Placement at AH was within the confines of covered houseboat slips to minimize hydrocarbon signals from boat motors. Tidal currents are significant in Juneau

area, with a mean tide of over 5 M twice per day.

Modeling and statistical analyses

Dose–response data were modeled against mean TPAH concentrations with logistic regression. The data were over-dispersed; variability was greater than expected from the logistic model; thus the quasibinomial family was used instead of the binomial family, and the resulting 95% confidence intervals are slightly larger to account for additional variability. The LC50s and median effective concentrations (EC50s) were determined by logistic regression and the dose.p function in the MASS package in R. The lowest concentration with a significant response was determined using analysis of variance (ANOVA) and Tukey contrasts. Differences in temperature between treatments were similarly estimated. The R Studio statistical software (Ver 0.94.110) was used for all analyses.

To compare TPAH concentrations in passive samplers, concentrations were modeled as a function of distance and with ANOVA for comparisons. Data were approximately normally distributed, and variances were similar for most sets of observations. Concentrations were log-transformed prior to analysis. Results were considered significant if $p \leq 0.05$. Hydrocarbon sources were determined visually (parent homologs are more abundant than substituted homologs in pyrogenic sources, such as creosote) and were verified with a source model [26,27]. The source model determines whether composition in 6 homologous groups (naphthalenes, fluorenes, dibenzothiophenes, phenanthrenes, fluoranthene-pyrenes, and chrysenes) is consistent with a pyrogenic source or a petrogenic source, or is indeterminate.

RESULTS

Bioassays: TPAH concentrations and composition

Aqueous TPAH concentrations from creosote generally decreased throughout the

exposure period (Figure 1; Supplemental Data, Figure S2); mean concentrations were calculated from the periodic measurements throughout the 14-d exposure period. The means for the 14-d exposure period were 1.5 µg/L, 3.0 µg/L, 4.0 µg/L,6.2 µg/L, 16 µg/L, and 30 µg/L TPAH and were used for statistical analyses. The mean TPAH concentrations for both the non–creosote-treated wood and water controls were ≤0.15 µg/L. Leaching rates of TPAH between treatments were tested using analysis of covariance, and no differences in slope as functions of treatment and time existed for naphthalene, phenanthrene, chrysene, or benzo(e)pyrene, suggesting that leaching rates were approximately equal across treatments.

Relative PAH composition also changed over time (Figure 2). Smaller PAHs were lost more rapidly than larger molecules in each treatment (Supplemental Data, Figure S2). As a result, proportionately more parent compounds were lost (e.g., naphthalene concentrations fell below methylnaphthalene during the exposure period). Over time, the relative proportion of total naphthalenes decreased, and the relative proportion of fluoranthene-pyrenes increased. Despite the changes in relative proportion, the treatments remained dominated by naphthalenes and phenanthrenes, comprising 76% and 66% of the TPAH dose for days 1 and 15, respectively. All compounds heavier than pyrene comprised less than 10% of the dose. Although the dose compositions were dominated by 2-ringed and 3-ringed compounds, heavier, 5-ringed compounds such as benzo(a)pyrene and perylene were also present at trace levels (≤0.26 µg/L).

Uptake of PAHs by embryos

The uptake of PAHs into embryo tissue was rapid; the highest concentrations were measured after 1 d of exposure to creosote-treated wood (Figure 1). Total PAH concentrations in tissue were approximately 100 times greater than in exposure water. Tissue concentrations were distinct from each other and declined over time similar to the exposure levels. Mean TPAH

concentrations in tissue were significantly greater in the exposed embryos ($p \leq 0.001$) over the hatching period (mean of 89 ng/g, 380 ng/g, and 2100 ng/g wet wt in the water control, 6.2 µg/L TPAH [6.5 L creosote-treated wood], and 30 µg/L TPAH [26 L creosote-treated wood], respectively; Figure 1).

Biological responses to creosote exposure

Fertilization rates were not affected by exposure to creosote. Mean fertilization was 84% in creosote treatment solutions and 81% in clean seawater, as measured in success of reaching the eyeing stage. Logistic regression confirmed that eyeing rates were independent of TPAH concentration ($p = 0.44$). These were short-term exposure tests of approximately 5 min, designed to isolate the fertilization process as the primary variable.

In contrast, the longer creosote exposures reduced hatching success in a dose-dependent manner ($p = 0.01$). However, the precise measure of survival rate was confounded by overcrowding, and variance was high even when the overcrowded slides of embryos were excluded from the analysis. Therefore, using only the slides with 100 embryos or fewer, the LC50 for hatching success estimate ranged from 5 µg/L to 50 µg/L TPAH ($n = 37$).

The frequency of skeletal defects in hatched embryos and subsequent impaired swimming ability increased with dose ($p \leq 0.001$; Figure 3); the EC50 for skeletal defects was 18 ± 0.8 µg/L TPAH, and swimming ability was affected at a slightly higher concentration of 22 ± 1.3 µg/L TPAH. Skeletal defects are visually apparent and the most sensitive of the effects to quantify (Figure 4). The effective concentrations for 10% of the population (EC10; a safer exposure level) were 4.6 ± 0.78 µg/L TPAH for skeletal defects and 8.4 ± 0.73 µg/L TPAH for swimming effects. These effects were independent of the crowding problem because the controls, which were equally crowded, did not show any such abnormalities. Yolk-sac edema

was frequent (Figure 4), but was not quantified.

Environmental conditions: PAH concentrations and trends in passive samplers

The PAHs were detected in passive samplers at all 3 harbors that were sampled monthly (14-d deployments, June–October). Concentrations generally increased as summer progressed (higher water temperature, higher boat activity, more direct sun on pilings), reaching concentrations as high as 290 000 ng/g device, and then declined as fall approached (Figure 5). The ABMS site had higher exposures to TPAH, indicated by the 5 highest concentrations and the mean of all samplers at 20 000 ng/g device, compared with the other 2 sites (IPFO, AH), which had exposures of 9400 ng/g and 2100 ng/g device, respectively. Total PAH concentrations in passive samplers also varied with distances from creosoted pilings (Figures 5 and 6). Blanks were consistently several orders of magnitude (10–1000 times) less than the field samplers (Figures 5 and 6). Total PAH concentrations in most cases were greatest adjacent to the creosoted pilings at ABMS and declined with distance (in 10 of 14 cases, $p_{\text{regression}} < 0.1$ in 7 of these; Figure 6). Conversely, in 1 outlier (of 14), slopes increased significantly. The incidences of pyrogenic composition in passive samplers were at least 72%, 88%, and 100% at the 3 harbor sites (ABMS, IPFO, and AH, respectively), consistent with creosote pilings as a major contributing source. Pyrogenics were identified by modeling in 71% of the laboratory-exposed passive samplers and none of the blanks.

Comparison of passive sampler data from the field and the laboratory

Laboratory-deployed passive samplers were compared with those deployed in the field. As mentioned in the *Biological responses to creosote exposure* section, the EC10 for skeletal defects was 4.6 µg/L TPAH. For comparison with field data, we chose the passive sampler that corresponded to a mean of 4.0 µg/L (passive sampler in the laboratory closest to the EC10 of 4.6

µg/LTPAH) as the threshold dose to compare field passive samplers. This laboratory passive sampler had a TPAH value of 41 000 ng/g device. Seven passive samplers located at the ABMS had TPAH concentrations greater than or equal to the comparison value. One passive sampler from the IPFO site also exceeded the comparison value. With the exception of 1, these were located 0.01 m from a creosoted piling. The exception was 10 m from a piling. In addition, all 4 samples located on the surface of creosoted pilings also exceeded the EC10 value.

DISCUSSION

The present study confirms that PAHs leached from creosote-treated wood are harmful to Pacific herring embryos in the low ppb (µg/L) range and that environmental concentrations of PAH, probably from creosote pilings, can approach harmful levels in marinas and harbors. The highest environmental concentrations were relatively local and near pilings, in a habitat used by some spawning herring.

The EC50s for spinal deformations and swimming effects were 18 µg/L and 22 µg/L TPAH, respectively, significantly lower than the LC50 of 50 µg/L reported by Vines et al. [10]. The spinal deformations are reported as an EC50; but in reality they are lethal effects, as larvae with bent spines cannot swim effectively and hence can neither capture prey nor avoid predation. The swimming performance EC50 of 22 µg/L corroborates the significance of the spinal deformations. Safe exposure concentrations would be less than the EC10 for spinal deformations, 4.6 µg/L TPAH.

The toxic effects of creosote exposure are not surprising, given the complex chemical composition of toxic compounds used to preserve wood, which include the PAH. Earlier studies with PAH exposure to fish embryos have detected low ppb PAH (µg/L) effects on survival, spinal deformations, heart rate, heart development and swimming performance, and delayed

effects on growth and survival to adults [11,20,28–30]. Creosote contains many of the same PAHs found in crude oils, but in different proportions, and it also contains other toxic compounds [6,8]. Although PAHs may not be solely responsible for the toxicity of creosote leachate, given their toxicity in the low ppb range to embryos, they are likely to be major contributors; measuring PAH concentrations of different doses over time provides the best available analysis of a complex mixture of toxicants in the creosote leachate.

Passive samplers detected PAHs in all 3 marinas, with the highest concentrations near creosote pilings. Passive samplers are sensitive because they sample continuously over a 14-d deployment period, but results can be confounded by multiple sources. Creosote pilings were implicated as a significant source because the highest concentrations were detected close to pilings and the PAH composition modeling indicated a pyrogenic source, consistent with creosote pilings as the source in passive samplers from both the laboratory tests and the environmental sampling.

The highest concentrations in several passive samplers deployed in marinas exceeded the 41 000 ng/g TPAH accumulated in a passive sampler placed in the 4.0 μg/L exposure during the controlled laboratory tests. Herring embryos had EC10s of 4.6 μg/L and 8.4 μg/L for spinal deformations and swimming performance, respectively. Thus, some marina water at 1 site (ABMS) was sufficiently contaminated to cause negative biological consequences. Environmentally, the significance of creosote pilings is probably relatively local, as suggested by the higher concentrations achieved close to the pilings and decreasing with distance from the pilings, but they would add to the PAH load coming from multiple sources in a marina. New pilings would probably have higher leaching rates than older pilings.

Creosote-treated wood in the aquatic environment is a long-lived point source of PAHs.

The PAHs migrate out of impregnated wood and into the surrounding water where they undergo weathering processes and may adsorb onto sediment and persist for an unknown amount of time [31]. Wave and tidal action on creosoted pilings and the hardware around them, such as metal rings, can cause rubbing and subsequent release of fresh creosote into the surrounding water and sediment. In addition, some of the compounds found in creosote—such as anthracene, fluoranthene, pyrene, and benzo(a)pyrene—are phototoxic, presenting additional risk to translucent herring embryos and larvae such as Pacific herring [20,32]. Pacific herring are sensitive to shoreline development and creosote structures located there because their eggs are spawned nearshore, where they develop, hatch, and grow until they are large enough to migrate offshore [1,3,10,33].

In conclusion, creosote leachate resulted in EC50s of 18 22 μg/L and 22 μg/L total TPAH (spinal deformation and reduced swimming performance, respectively) in Pacific herring embryos. A safe PAH exposure level would be less than 4.6 μg/L PAH, the EC10 for spinal defects, the most sensitive parameter measured. Spinal defects in herring larvae are lethal, because they are not correctable and severely limit larval ability to acquire prey or avoid predation. Creosote pilings contribute to the PAH load within a marina and can rise to PAH concentrations that are harmful to fish embryos but at a scale that is localized in the environment.

Supplemental Data—The Supplemental Data are available on the Wiley Online Library at DOI: 10.1002/etc.xxxx.

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Data Availability—Data, associated metadata, and calculation tools are available from the corresponding author (duncan846@gmail.com).

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Figure 1. (**A**) Aqueous and total polycyclic aromatic hydrocarbon (TPAH) concentrations in creosote treatments and (**B**) TPAH concentrations in embryos (wet weight). Note that the *y*-axes are a log scale.

Figure 2. Example aqueous polycyclic aromatic hydrocarbon (PAH) compositions in (**A**) laboratory exposure water, (**B**) embryo tissue, (**C**) laboratory passive sampler, and (**D**) field passive samplers (surface of piling and 10 cm from a piling).

Figure 3. Swimming ability of newly hatched embryos decreased with creosote exposure, and the incidence of skeletal defect increased with dose. Logistic regression line with 95% confidence bands (dashed lines). TPAH = total polycyclic aromatic hydrocarbon.

Figure 4. Photographs of (**A**) typical morphology of Pacific herring larvae from creosote treatments: control group having no visible skeletal defects and (**B**) 26-L creosote-treated wood (30 µg/L total polycyclic aromatic hydrocarbon) group having skeletal defects/scoliosis. Note shorter length and yolk-sac edema of deformed larvae.

Figure 5. Total polycyclic aromatic hydrocarbon (TPAH; ng/g device) as a function of distance (m) from creosoted pilings at 3 field locations: (**A**) Auke Bay Marine Station (ABMS), (**B**) Indian Point Field Office (IPFO), and (**C**) Aurora Harbor (AH). Lines denote 41 000 ng/g TPAH corresponding to the effective concentrations for 10% of the population for skeletal defect (4.6 µg/L).

Figure 6. Relationship between total polycyclic aromatic hydrocarbon (TPAH) concentration and distance by site and date: (**A**) Auke Bay Marine Station (ABMS), (**B**) Indian Point Field Office (IPFO), and (**C**) Aurora Harbor (AH). Slopes where $p_{\text{regression}} < 0.1$ are illustrated as solid lines. Data were log-transformed before regression.

<<ENOTE>> AQ1: Correct expansion of “TPAH”?

<<ENOTE>> AQ2: Correct definition of TPAH (here, and in all figure captions)?

<<ENOTE>> AQ3: Please check: Does this mean “dead embryos, eyed embryos”? If so, please add “embryos” after “dead”

<<ENOTE>> AQ4: Ref 4: Please insert the last date this website was accessed, prior to manuscript acceptance.

<<ENOTE>> AQ5: [0]Ref 5: Please insert the last date this website was accessed, prior to manuscript acceptance.

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