1223 Ref

SETAC PRESS

Environmental Toxicology and Chemistry, Vol. 20, No. 3, pp. 624–631, 2001
Printed in the USA
0730-7268/01 $9.00 + .00

Ref

POLYCYCLIC AROMATIC HYDROCARBONS, ALIPHATIC HYDROCARBONS, TRACE ELEMENTS, AND MONOOXYGENASE ACTIVITY IN BIRDS NESTING ON THE NORTH PLATTE RIVER, CASPER, WYOMING, USA

THOMAS W. CUSTER,*† CHRISTINE M. CUSTER,† KIMBERLY DICKERSON,‡ KENNETH ALLEN,‡
MARK J. MELANCON,§ and LARRY J. SCHMIDT†
†U.S. Geological Survey, Upper Midwest Environmental Sciences Center, 2630 Fanta Reed Road, La Crosse, Wisconsin 54603
‡U.S. Fish and Wildlife Service, Ecological Services, 4000 Morrie Avenue, Cheyenne, Wyoming 82001
§U.S. Geological Survey, Patuxent Wildlife Research Center, 12011 Beech Forest Road, Laurel, Maryland 20708

(*Received* 9 *May* 2000; *Accepted* 31 *July* 2000)

Abstract—Tree swallow (*Tachycineta bicolor*) and house wren (*Troglodytes aedon*) eggs and chicks were collected near a refinery site on the North Platte River, Casper, Wyoming, USA and at a reference site 10 km upstream. Total polycyclic aromatic hydrocarbon (PAH) concentrations in swallow and wren chicks were higher at the refinery site than at the reference site. Polycyclic aromatic hydrocarbon concentrations in sediment and chick dietary samples were consistent with these findings. The general lack of methylated PAHs in sediment, diet, and bird carcasses suggested that the PAHs were derived from combustion and not from petroleum. The predominance of odd-numbered aliphatic hydrocarbons and the low ratios (≤0.25) of pristane:n-C_{17} and phytane:n-C_{18} in chick and diet samples also suggested that swallow and wren chicks were not being chronically exposed to petroleum. Mean ethoxyresorufin-*O*-dealkylase and benzyloxyresorufin-*O*-dealkylase activities in tree swallow livers averaged nine times higher at the refinery site than at the reference site and were probably induced by exposure to PAHs. Trace element concentrations in eggs and livers of swallows and wrens were similar or greater at the reference site than at the refinery site. Selenium, strontium, and boron concentrations were elevated in eggs and livers of swallows and wrens at both the refinery and reference sites.

Keywords—Aliphatic hydrocarbons Ethoxyresorufin-*O*-dealkylase activity Metals Polycyclic aromatic hydrocarbons Tree swallows

INTRODUCTION

Oil seepage into the North Platte River adjacent to a former petroleum refinery near Casper, Wyoming, USA (Fig. 1), was documented by the U.S. Environmental Protection Agency [1]. Sediment samples from the river where oil seeps occurred contained high levels of polycyclic aromatic hydrocarbons (PAHs) [1]. Whether current levels of PAHs in river sediments were elevated and being accumulated into the aquatic food chain was unknown.

The composition of individual PAHs and aliphatic hydrocarbons (ALHs) in biotic and abiotic samples can be used to determine whether these hydrocarbons originated from petroleum, combustion, or natural sources. Samples with a high proportion of methylated PAHs are indicative of a petroleum source, whereas the lack of methylated PAHs is indicative of a combustion source [2]. Elevated (>1.0) ratios of selected ALHs, such as pristane:n-C_{17} and phytane:n-C_{18}, are indicative of chronic exposure to petroleum [3]. Pristane and phytane, which occur frequently in petroleum, are apparently metabolized less readily than n-C_{17} and n-C_{18} and thus they tend to accumulate in tissues when the exposure to petroleum is chronic [3]. Because odd-numbered ALHs are more prevalent in plants than ALHs found in petroleum or produced as a result of combustion, a preponderance of odd-numbered ALHs is indicative of recent biological origin. In contrast, a more even distribution of odd-to-even ALHs is indicative of petroleum or combustion [2,3].

In birds, PAH and trace element concentrations in tissues and the induction of hepatic monooxygenase activity may be indicators of PAH exposure. Although PAHs are rapidly metabolized in birds [4], PAHs have been detected in bird tissues in relation to sources of petroleum [5] and combustion [6]. Trace element concentrations in soil and small mammals were higher at sites treated with petrochemical waste compared to reference sites [7]. Induction of hepatic monooxygenase activity after exposure to PAHs has been documented in birds in both the laboratory [8–11] and the field [12].

Cavity-nesting birds, especially tree swallows (*Tachycineta bicolor*) are being widely used as indicators of local contamination [13–16]. Tree swallows readily use nest boxes, so study sites can be established at specific locations of interest. They feed near their nest box [17] on emergent aquatic insects [18]; therefore, residues in the tissues of tree swallows reflect sediment contamination for those chemicals that transfer into the biota [19].

The objective of this study was to determine whether concentrations of PAHs were present and elevated in cavity-nesting birds breeding near a refinery on the North Platte River compared to a reference site upstream. If PAHs were present, we wanted to determine whether their source was petroleum or combustion. We also wanted to determine whether hepatic monooxygenase activities were induced or trace element concentrations were elevated in birds nesting near the refinery.

MATERIALS AND METHODS

Sample collection

On April 16–17, 1998, tree swallow boxes were positioned on metal posts along the North Platte River near Casper, Wy-

* To whom correspondence may be addressed (tom_w_custer@usgs.gov).

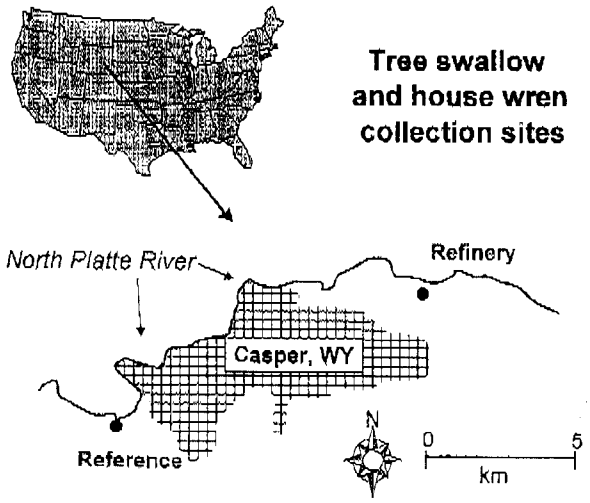

Fig. 1. Two sites on the North Platte River (an upstream reference and downstream refinery site) near Casper, Wyoming, USA, where tree swallows, house wrens, and sediment were collected in 1998.

oming (Fig. 1), near a refinery (42°51′58″N, 106°15′49″W; n = 20 boxes) and a reference site, the Wyoming Game and Fish headquarters (42°49′18″N, 106°22′50″W; n = 14 boxes). The boxes were checked once a week until eggs hatched and chicks had fledged. The species and number of eggs or young present was recorded at each visit. Tree swallow eggs and chicks were collected from the refinery (four egg samples and four chick samples) and reference site (three eggs and two chicks). House wrens (*Troglodytes aedon*) also used the boxes at these sites and house wren eggs and chicks were collected from the refinery (three eggs and three chicks) and reference site (three eggs and three chicks).

A sample consisted of two or three eggs per box and one to three sibling 12-d-old chicks. Based on date of egg laying, the estimated date of hatching, and morphologic features, the tree swallow chicks were estimated to be 12 ± 1 d old and house wren chicks were estimated to be 12 ± 2 d old. The day of hatching was considered as day 0. Egg contents were pooled by nest, emptied into chemically clean jars, and later analyzed for trace elements. Within 2 h of collection, chicks were weighed (±0.1 g) and decapitated with a sharp pair of scissors.

Immediately after death, the liver was removed from tree swallow chicks, weighed (±0.1 g), and about 0.3 g from one chick per brood was placed into a cryotube for measurement of monooxygenase activity. A few drops of glycerin were added to the cryotube and it was immediately placed into liquid nitrogen. The cryotubes were later transferred from the liquid nitrogen to an ultracold freezer (−80°C) for storage until processing. Monooxygenase activity was not measured in wrens because of a lack of baseline information for this species.

For chicks of both species, the livers and carcass remainders were each pooled by nest and each placed in chemically clean jars; contents of the upper gastrointestinal tract were pooled by species and site. All samples were frozen until transported to the Upper Midwest Environmental Sciences Center (La Crosse, WI, USA) for storage in a freezer. The gastrointestinal tract contents and carcass remainders were analyzed for PAHs and ALHs; livers were analyzed for trace elements.

Composite sediment samples were collected from the North Platte River from the top 20 cm of sediment within 1 m of shore near each site. Sediment samples from five to six locations along the river at each site were combined in a stainless steel bowl and mixed. A stainless steel spoon, rinsed in deionized water and hexane between samples, was used to make the collections. For petroleum hydrocarbon analyses, one sediment sample and a duplicate were placed in chemically cleaned amber glass jars and frozen. For trace element analyses, a second sediment sample and a duplicate were placed in plastic bags and frozen.

Chemical analysis

Egg contents, chick livers, and sediment were analyzed for trace elements by Research Triangle Institute (Research Triangle Park, NC, USA). Samples were freeze-dried, weighed, and then homogenized in a blender. Subsamples of freeze-dried livers were digested in stages with heat and nitric–perchloric acid and then analyzed for selenium (Se) and arsenic (As) by graphite furnace atomic absorption spectrophotometry, and for aluminum (Al), barium (Ba), beryllium (Be), boron (B), cadmium (Cd), chromium (Cr), copper (Cu), iron (Fe), lead (Pb), magnesium (Mg), manganese (Mn), molybdenum (Mo), nickel (Ni), strontium (Sr), vanadium (V), and zinc (Zn) by inductively coupled plasma–atomic emission spectrophotometry. Separate subsamples were digested by nitric acid reflux and analyzed for total mercury (Hg) by cold vapor atomic absorption spectrophotometry. Nominal levels of detection (μg/g dry weight) were as follows: Al = 8, As = 1.0, B = 3.0, Ba = 1.0, Be = 2.0, Cd = 0.1, Cr = 1.0, Cu = 1.0, Fe = 20, Hg = 0.2, Mg = 20, Mn = 0.5, Mo = 1.0, Ni = 1.0, Pb = 0.5, Se = 1.0, Sr = 0.5, V = 1.0, and Zn = 2.0. The number of spikes, duplicates, and blanks was 10% of the total number of samples analyzed. Concentrations were not adjusted for recovery, which averaged 91% overall for the various trace elements. Concentrations of trace elements are reported on a dry-weight basis; approximate wet-weight values can be calculated by using percent moisture values of 80.2% for eggs, 70.3% for livers, and 34.0% for sediment from this study.

Aliquots of chick carcasses, chick diet, and sediment were analyzed for PAHs and ALHs by Mississippi State Chemical Laboratory (Mississippi State University, MS, USA). Samples were digested in 6 N aqueous potassium hydroxide for 24 h at 35°C. Digestate was cooled and then neutralized with glacial acetic acid. The mixture was then extracted three times with methylene chloride and the extracts were combined and concentrated to near dryness before reconstituting in petroleum ether for transfer to a 20 g 1% deactivated silica gel column topped with 5 g of neutral alumina. Polycyclic aromatic hydrocarbons and ALHs were separated by eluting ALHs from the column with 100 ml of petroleum ether; PAHs were eluted using 100 ml 40% methylene chloride:60% petroleum ether (v/v) followed by 50 ml methylene chloride. The ALH eluate was then concentrated to an appropriate volume for quantification by capillary column flame ionization gas chromatography (GC). The silica gel eluate containing the PAHs was concentrated and reconstituted in methylene chloride to be subjected to gel permeation chromatography cleanup before quantification by GC and fluorescence high-performance liquid chromatography. Polycyclic aromatic hydrocarbon analytes included 1,2,5,6-dibenzanthracene; 1,2-benzanthracene; acenaphthalene; acenaphthene; anthracene; benzo[*a*]pyrene; benzo[*b*]fluoranthene; benzo[*e*]pyrene; benzo[*g,h,i*]perylene; benzo[*k*]fluoranthene; biphenyl; C_1-,C_2-,C_3-,C_4-chrysenes;

C_1-,C_2-,C_3-dibenzothiophenes; C_1-fluoranthenes and pyrenes; C_1-,C_2-,C_3-fluorenes; C_1-,C_2-,C_3-,C_4-naphthalenes; C_1-,C_2-,C_3-,C_4-phenanthrenes; chrysene; dibenzothiophene; fluoranthene; fluorene; indeno[1,2,3-cd]pyrene; naphthalene; perylene; phenanthrene; and pyrene. Aliphatic hydrocarbon analytes included n-C_{10} through n-C_{34} alkanes, phytane, and pristane. All ALHs and all named PAHs were run against standards of the same material. All the other C_1-,C_2-,C_3, or C_4- compounds were calculated against either a C_1-,C_2-,C_3-, or C_4- compound that was in the standard run or against the parent compound (e.g., C_2-naphthalene against 2,6-dimethyl naphthalene; C_1-chrysene against chrysene).

Minimum detection limits for PAHs and ALHs were 0.01 μg/g wet weight. For the calculations of the pristane:n-C_{17} and phytane:n-C_{18} ratios, one half of the detection limit (0.005 μg/g wet weight) was assigned to pristane or phytane, if the concentration was below the detection limit. The number of spikes, duplicates, and blanks was 9% of the total number of samples analyzed. Concentrations were not adjusted for recovery, which averaged 78% and 71%, respectively, for the various PAHs and ALHs. Concentrations of PAHs and ALHs are reported on a wet-weight basis. Total PAHs and total ALHs are the sum of the individual PAHs and ALHs, respectively.

Monooxygenase activity

Hepatic microsomes were prepared from homogenates of thawed liver samples by differential centrifugation. The 11,000-g supernatant was centrifuged at 40,000 rpm for 60 min to obtain the microsomal pellet. Each 100,000-g pellet was resuspended in 2.0 ml/g of tissue weight of 0.05 M Na/KPO_4, 0.001 M disodium ethylenediaminetetraacetate, pH 7.6. Benzyloxyresorufin-O-dealkylase (BROD) and ethoxyresorufin-O-dealkylase (EROD) were assayed by the methods of Burke and Mayer [20] as adapted to a fluorescence microwell plate scanner [21]. The 260-μl total assay volume contained microsomes equivalent to 0.65 mg liver, 2.5 μM substrate, and 0.125 mM NADPH in 0.066 M Tris buffer, pH 7.4. Protein concentrations were determined by a 50% reduced-volume Lowry assay [22]. Both BROD and EROD activities were calculated as pmol product/min/mg microsomal protein.

Statistical procedures

Measures of PAHs, ALHs, trace elements, and monooxygenase activity were log-transformed (using base 10 logarithms) to satisfy the homogeneity of variance assumption of analysis of variance (ANOVA). Two-way ANOVA (site, species, and interaction) was used to compare PAHs, ALHs, and trace elements between sites and species. If the overall two-way ANOVA was significant, then p values in text and tables are p values for the main variables. If not, the p values in text and tables are for the overall model. The ANOVAs were used if ≥50% of samples had detectable values; one half of the detection limit was assigned to samples below the detection limit. When less than one half of the PAHs and ALHs were detected, only the range of values is presented. When less than one half of individual trace elements were detected, the number not detected is presented. Because higher concentrations of PAHs were measured at the refinery site, we used one-tailed t tests to determine if monooxygenase activities were greater at the refinery than reference site.

RESULTS

Aromatic and aliphatic hydrocarbons

Twelve different PAHs were detected in tree swallow and house wren carcasses and diet samples at the refinery site (Table 1). In contrast, only one PAH, 1,2-benzanthracene, was detected in one wren carcass at the reference site. The profiles of PAHs in sediment samples were qualitatively similar between the refinery and the reference site (Table 1). Except for the 2-methylnapthalene detected in the swallow diet and C_1-phenanthrenes detected in the house wren diet, all of the PAHs in carcasses, diet, and sediment were not methylated (Table 1). Total PAHs were significantly higher at the refinery than at the reference site and higher in wrens than in swallows, but no significant interaction was found (overall $F_{1,1,1,8} = 5.5$, $p = 0.02$; site $F_{1,8} = 9.6$, $p = 0.01$; species $F_{1,8} = 5.8$, $p = 0.04$; site × species $F_{1,8} = 1.6$, $p = 0.24$; Table 2).

Odd-numbered ALHs were more predominant than even-numbered ALHs in the chicks of swallows and wrens, the diet of swallows and wrens, and the sediment at the refinery (Fig. 2) and reference site. Concentrations of n-C_{11}, n-C_{13}, n-C_{15}, and n-C_{17} were significantly higher in tree swallow than in house wren carcasses ($p = 0.001$, 0.01, 0.004, 0.003, respectively; two-way ANOVA overall $p < 0.05$); n-C_{15} was also higher at the reference than at the refinery site ($p = 0.03$). Concentrations of n-C_{20}, n-C_{29}, n-C_{31}, and n-C_{33} were significantly higher in wren than in swallow carcasses ($p = 0.03$, 0.004, 0.002, 0.007, respectively; two-way ANOVA overall $p < 0.05$). Other ALHs were not significantly different (two-way ANOVA overall $p > 0.05$) between sites or species and all site–species interactions were not significantly different ($p > 0.05$).

Total ALHs were not significantly different between sites or species (overall $F_{1,1,1,8} = 2.2$, $p = 0.17$; Table 2) and no consistent trend was found in total ALHs in the pooled diet samples between sites or species (Table 1). Small sample sizes may have contributed to these nonsignificant findings. The pristane:n-C_{17} ratio was <0.25 in carcass, diet, and sediment samples (Table 1). The pristane:n-C_{17} ratio in carcasses did not vary between sites, but was significantly larger in wrens than swallows; no significant interaction was found (overall $F_{1,1,1,8} = 8.7$, $p = 0.01$; site $F_{1,8} = 1.9$, $p = 0.20$; species $F_{1,8} = 23.5$, $p = 0.001$; site × species $F_{1,8} = 0.3$, $p = 0.59$; Table 2). The phytane:n-C_{18} ratio was ≤0.25 in carcass and diet samples (Table 1). The phytane:n-C_{18} ratio did not vary between sites, but was significantly larger in swallow than in wren carcasses; no significant interaction was found (overall $F_{1,1,1,8} = 4.7$, $p = 0.04$; site $F_{1,8} = 1.6$, $p = 0.24$; species $F_{1,8} = 8.9$, $p = 0.02$; site × species $F_{1,8} = 0.9$, $p = 0.38$; Table 2). The phytane:n-C_{18} ratio was ≤1.0 in sediment collected at the refinery and ≤0.45 in sediment collected at the reference site.

Trace elements

Arsenic, Be, Cd, Cr, Mo, Pb, Ni, and V concentrations were not detected in eggs; Al was detected in one swallow egg at the reference site. Boron (two-way ANOVA, overall $p = 0.49$), Fe ($p = 0.79$), Mn ($p = 0.97$), Se ($p = 0.33$), Sr ($p = 0.27$), and Zn ($p = 0.27$) concentrations in eggs did not differ between sites or species (Table 3). Barium ($p = 0.04$), Cu ($p = 0.005$), and Mg ($p = 0.003$) concentrations were higher (two-way ANOVA overall $p < 0.05$) in wren than in swallow eggs; Hg concentrations were higher in swallow than in wren eggs (p

Table 1. Range of polycyclic aromatic hydrocarbon and aliphatic hydrocarbon concentrations in tree swallow and house wren carcasses, pooled diet samples, and sediment from two sites on the North Platte River near Casper, Wyoming, USA, in 1998

	Concentration (μg/g wet wt)									
	Refinery					Reference				
	Carcass		Diet			Carcass		Diet		
Analyte	Tree swallow (n = 4)	House wren (n = 3)	Tree swallow (n = 1)	House wren (n = 1)	Sediment (n = 1)	Tree swallow (n = 2)	House wren (n = 3)	Tree swallow (n = 1)	House wren (n = 1)	Sediment (n = 1)
Polycyclic aromatic hydrocarbons										
1,2-Benzanthracene	3ND–0.03	1ND–0.04	ND	0.06	ND	ND	2ND–0.02	ND	ND	ND
2-Methylnapthalene	ND	ND	0.02	ND	ND	ND	ND	ND	ND	ND
Benzo[*a*]pyrene	ND	ND	ND	0.06	ND	ND	ND	ND	ND	0.01
Benzo[*b*]fluoranthene	ND	ND	ND	0.04	ND	ND	ND	ND	ND	0.01
Benzo[*e*]pyrene	ND	2ND–0.04	0.07	0.14	ND	ND	ND	ND	ND	0.01
Benzo[*g,h,i*]perylene	ND	ND	ND	0.02	ND	ND	ND	ND	ND	ND
Benzo[*k*]fluoranthene	ND	ND	ND	ND	0.02	ND	ND	ND	ND	0.01
C_1-Phenanthrenes	ND	ND	ND	0.02	ND	ND	ND	ND	ND	ND
Chrysene	3ND–0.02	2ND–0.01	0.12	0.18	0.02	ND	ND	ND	ND	0.02
Fluoranthene	ND	ND	ND	0.02	0.01	ND	ND	ND	ND	0.03
Naphthalene	ND	ND	0.01	ND	ND	ND	ND	ND	ND	ND
Perylene	ND	ND	ND	ND	0.05	ND	ND	ND	ND	0.08
Phenanthrene	ND	2ND–0.02	0.02	0.16	0.01	ND	ND	ND	ND	ND
Pyrene	ND	2ND–0.01	0.04	0.07	0.02	ND	ND	ND	ND	0.01
Total aromatics	2ND–0.03	0.04–0.06	0.28	0.77	0.11	ND	2ND–0.02	ND	ND	0.13
Aliphatic hydrocarbons[b]										
n-C_{17}	0.22–1.1	0.02–0.03	2.2	0.14	0.06	0.76–1.2	0.03–0.88	1.8	0.1	0.04
n-C_{18}	0.02–0.08	0.07–0.16	0.64	0.04	0.02	0.05–0.08	0.07–0.21	0.04	0.04	0.01
Pristane	3ND–0.02	ND	ND	0.02	ND	ND–0.02	2ND–0.01	0.02	0.02	0.01
Phytane	2ND–0.01	ND	ND	ND	0.01	ND	ND	ND	ND	ND
Total aliphatics	5.0–8.1	5.6–8.1	74.6	18.7	1.48	8.1–9.5	6.8–9.3	5.6	35.2	0.97
Pristane:n-C_{17} ratio	0.01–0.02	0.15–0.22	0.002	0.16	0.08	0.01–0.01	0.01–0.18	0.01	0.20	0.21
Phytane:n-C_{18} ratio	0.06–0.25	0.03–0.2	0.08	0.12	0.94	0.06–0.10	0.02–0.08	0.13	0.13	0.41

[a] The number before ND (not detected) is the number of samples below the detection limit.
[b] The range in concentrations of n-C_{10} to n-C_{16} and n-C_{19} to n-C_{34} are not presented.

= 0.03). Barium concentrations in eggs were also higher (p = 0.02) at the reference site (7.7 μg/g dry weight) than at the refinery (3.8 μg/g dry weight) site. No significant site–species interactions were found for any of these two-way ANOVAs.

Arsenic, Ba, Be, Pb, and V concentrations were not detected in livers. Aluminum, Cd, Cr, Hg, and Ni were detected in five or fewer livers and were not compared between sites or species. Copper (two- way ANOVA, overall p = 0.58), Mg (p = 0.42), Se (p = 0.13), Sr (p = 0.12), and Zn (p = 0.16) concentrations in livers did not differ by site or species (Table 3). Molybdenum concentrations were higher (0.03) at the reference (2.6 μg/g dry weight) than at the refinery (1.8 μg/g) site (two-way ANOVA overall $p < 0.05$). Boron (p = 0.004), Fe (p = 0.01), and Mn (p = 0.004) concentrations were higher in wren livers than in tree swallow livers (two-way ANOVA overall $p < 0.05$). No significant site–species interactions were found for any of these two-way ANOVAs.

Mercury, Mo, and Be were not detected in sediment samples at the refinery and reference sites (Table 3). Trace element concentrations in sediment were of a similar magnitude at the refinery and reference sites.

Table 2. Geometric mean total polycyclic aromatic hydrocarbon concentration (μg/g wet wt), total aliphatic hydrocarbon concentration, pristane:n-C_{17} ratio, and phytane:n-C_{18} ratio in carcasses of tree swallow and house wren chicks at two sites along the North Platte River, Casper, Wyoming, USA, in 1998

	Geometric mean			
	Species		Site	
Analyte	Tree swallow (n = 6)	House wren (n = 6)	Refinery (n = 7)	Reference (n = 5)
Total aromatics	0.01A[a]	0.02B	0.02B[b]	0.01A
Total aliphatics	7.1	7.1	6.4	8.3
Pristane:n-C_{17}	0.01A	0.12B	0.04	0.03
Phytane:n-C_{18}	0.13B	0.04A	0.09	0.05

[a] Species not sharing the same letter for each analyte are significantly different. Absence of a letter indicates no significant difference.
[b] Sites not sharing the same letter for each analyte are significantly different. Absence of a letter indicates no significant difference.

Monooxygenase activity

Mean EROD and BROD activities in tree swallow livers were significantly higher at the refinery than at the reference site (EROD p = 0.04, BROD p = 0.048; Table 4). Mean EROD and BROD activities were each more than nine times higher in swallow livers from the refinery than from the reference site.

DISCUSSION

Aromatic hydrocarbons

Analysis of our results suggests that tree swallow and house wren chicks were more exposed to PAHs near the refinery than upstream at the reference site. Twelve individual PAHs were detected in swallow and wren carcasses and diet near the refinery and only one PAH was detected in a wren carcass at

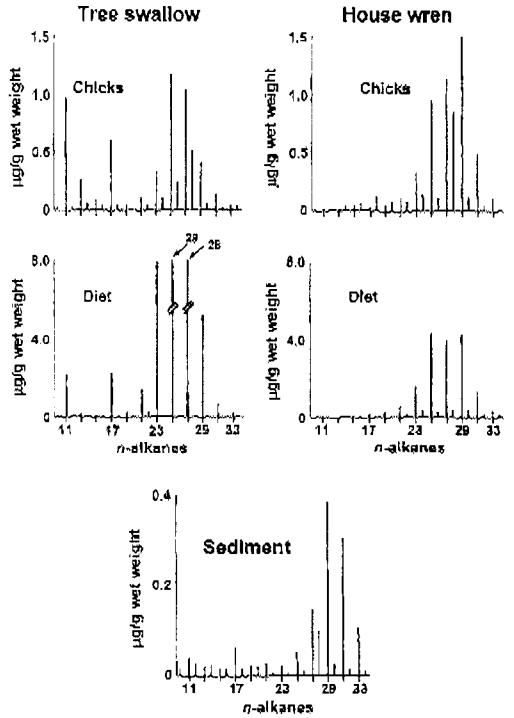

Fig. 2. Aliphatic hydrocarbon concentrations (mean µg/g wet weight) in tree swallow chicks (n = 4) and diet (n = 1), house wren chicks (n = 3) and diet (n = 1), and sediment (n = 1) collected near a refinery site on the North Platte River, Casper, Wyoming, USA, in 1998. Odd-numbered aliphatic hydrocarbons are identified with vertical tick marks on the x axis.

Table 4. Geometric mean ethoxyresorufin-*O*-dealkylase and benzyloxyresorufin-*O*-dealkylase activities (pmol product/min/mg microsomal protein) in livers of tree swallow chicks from two locations along the North Platte River, Casper, Wyoming, USA, in 1998

	Mean activity (range)	
	Reference (n = 2)	Refinery (n = 4)
Ethoxyresorufin-*O*-dealkylase	16.7A[a] (8.9–31.4)	151.4B (30.1–325)
Benzyloxyresorufin-*O*-dealkylase	5.7A (2.8–11.5)	54.0B (8.2–171)

[a] Means between sites that do not share a letter are significantly different.

the reference site. Additionally, total PAHs were significantly higher in swallow and wren carcasses at the refinery than at the reference site. Polycyclic aromatic hydrocarbons are not commonly found in the tissues of birds from noncontaminated sites and, when they are, tend to be present in very small amounts [3]. Once ingested by animals, PAHs are rapidly metabolized. For example, 94% of PAHs injected into chicken eggs on day 4 of incubation were metabolized within 14 d [4].

The general lack of methylated PAHs in all sediment, diet, and bird carcasses suggests combustion and not petroleum as the source of these PAHs [2]. Only two methylated PAHs were detected in samples and both of these were in the diet. The absence of methylated PAHs in herring gull (*Larus argentatus*) eggs and adults from the Canadian Great Lakes also suggested a combustion-related source rather than petroleum hydrocarbon contamination [6].

The higher concentration of PAHs in house wrens than in

Table 3. Trace element concentrations in eggs and livers of tree swallows, eggs and livers of house wrens, and sediment at two locations along the North Platte River, Casper, Wyoming, USA, in 1998. Except as noted, all trace elements were detected in all samples

	Geometric mean concentration (µg/g dry wt)				Concentration (µg/g dry wt) in sediment	
	Eggs		Livers			
Trace element[a]	Tree swallow (n = 7)	House wren (n = 6)	Tree swallow (n = 6)	House wren (n = 6)	Refinery (n = 1)	Reference (n = 1)
Aluminum	—[b]	ND[c]	—	—	8,440	7,870
Arsenic	ND	ND	ND	ND	5	5
Barium	4A[d]	7B	ND	ND	173	211
Boron	58	59	16A	36B	6	5
Cadmium	ND	ND	—	ND	1.1	1.1
Chromium	ND	ND	—	ND	13	10
Copper	2A	3B	26	23	12	10
Iron	140	140	990A	2,300B	12,430	11,530
Lead	ND	ND	ND	ND	16	8
Magnesium	330A	470B	800	880	6,700	6,220
Manganese	3	3	5A	6B	239	209
Mercury	0.3B	0.1A	0.2	—	ND	ND
Molybdenum	ND	ND	2	3	ND	ND
Nickel	ND	ND	—	ND	14	12
Selenium	7	6	21	19	1.0	1.0
Strontium	14	23	0.4	0.5	84	63
Vanadium	ND	ND	ND	ND	23	21
Zinc	57	60	88	106	51	43

[a] Beryllium was not detected in eggs, livers, and sediment. Mercury was detected in one wren egg and four swallow livers. Strontium was detected in five swallow livers.
[b] Dashed lines (—) indicate that one sample had a detectable concentration.
[c] The letters ND indicate that none of the samples had a detectable concentration.
[d] Means between species within eggs or livers that do not share the same letter are significantly different. Absence of a letter indicates no significant difference.

tree swallows may relate to the types of invertebrates fed to nestlings. Aquatic insects are typically a more important component of nestling tree swallow diets [18], whereas house wrens usually specialize on terrestrial insects [23]. These results suggest that terrestrial invertebrates near the refinery contain higher PAH concentrations than do aquatic invertebrates. This hypothesis needs to be further explored.

Total PAH concentrations in chick carcasses in this study (<0.01–0.06 μg/g wet weight) were intermediate to those reported in other birds. Total PAHs in lesser scaup (*Aythya affinis*) from Indiana Harbor Canal, USA, varied from 0.12 to 0.16 μg/g wet weight [5]. Redhead ducks (*Aythya americana*) collected in southern Texas, USA, and Chandeleur Sound, Louisiana, USA, had mean PAH concentrations of 0.03 μg/g wet weight [24]. Total PAH concentrations in wild juvenile common eider ducks (*Somateria mollissima*) from the Baltic Sea were 0.0045 μg/g wet weight [4].

The main route of exposure of PAHs to tree swallow and house wren chicks was probably through the diet. All five of the PAHs found in swallow and wren carcasses at the refinery site were present at high concentrations in the dietary samples. Additionally, the total PAHs in dietary samples at the refinery site were 28 and 38 times higher than found in swallow and wren carcasses. The high ratio of diet to carcass PAHs was expected because of the rapid rate of metabolism of these compounds by birds [4].

Aliphatic hydrocarbons

The preponderance of odd-numbered compared to even-numbered ALHs and the low (≤0.25) pristane:n-C_{17} and phytane:n-C_{18} ratios in tree swallow and house wren chick and diet samples suggest that chicks were not chronically exposed to oil. Aliphatic hydrocarbons derived from plants are mainly odd-numbered, whereas little or no difference is found in the odd to even ALHs ratios in petroleum [2]. An elevated ratio (>1.0) of pristane:n-C_{17} is indicative of chronic petroleum hydrocarbon exposure [2,25]. The pristane:n-C_{17} ratio in lesser scaup suggested exposure to petroleum hydrocarbons from the Indiana Harbor Canal; 8 of 17 scaup collected on the Indiana Harbor Canal had a pristane:n-C_{17} ratio > 1.0 [5]. A pristane:n-C_{17} ratio of 3.3 was reported in eggs of mallard ducks (*Anas platyrhynchos*) fed a diet including crude oil for four months [3]. The ratio was elevated in western sandpipers (*Calidris mauri*) collected at oilfield brine discharge sites (2.3 [26]) and double-crested cormorants (*Phalacrocorax auritus*) collected in the Houston Ship Channel, Houston, Texas, USA (1.6–3.6 [27]), locations contaminated with petroleum. The pristane:n-C_{17} ratio was not elevated (0.2) in redhead ducks collected in southern Texas and Louisiana [24].

The higher concentrations of lower-numbered ALHs (n-C_{11}, n-C_{13}, n-C_{15}, and n-C_{17}) in tree swallow than in house wren carcasses and the higher concentrations of higher-numbered ALHs (n-C_{20}, n-C_{29}, n-C_{31}, and n-C_{33}) in house wren than in tree swallow carcasses are consistent with their feeding habits. A preponderance of n-C_{15} and n-C_{17} is often attributed to hydrocarbons produced by aquatic plants such as algae [2], and tree swallows feed mainly on aquatic insects [18]. Higher-numbered ALHs (>n-C_{22}) are often observed in terrestrial plants [2], and house wrens feed mainly on terrestrial invertebrates [23].

Trace elements

Trace element concentrations in eggs or chicks were not higher at the refinery than at the reference site. In contrast, Ba concentrations were higher in reference eggs and Mo concentrations were higher in reference chicks.

Mercury concentrations in eggs (means = 0.1–0.4 μg/g dry weight, maximum concentration = 0.48 μg/g dry weight) and chicks (maximum concentration = 0.2 μg/g dry weight) in our study were at background levels. Impairment of reproductive success has been associated with egg concentrations of 2.5 to 10 μg/g dry weight (assuming 80% moisture [28]). Barn swallow (*Hirundo rustica*) eggs in Texas averaged 0.1 μg/g dry weight Hg (maximum value was 0.71 μg/g dry weight) and no reproductive effects were noted [13]. Mercury concentrations in livers of wild birds inhabiting areas of minimal Hg contamination ranged from 3.3 to 33 μg/g dry weight [29] (converted from 1 to 10 μg/g, wet weight, assuming 70% moisture).

Selenium was elevated in tree swallow and house wren eggs (means 6–7 μg/g dry weight, maximum 9 μg/g dry weight) but not to levels associated with lowered reproduction. Background concentrations in bird eggs are generally < 5 μg/g dry weight and an embryo viability threshold is estimated at 10 μg/g dry weight [30].

Selenium was also elevated in tree swallow and house wren livers (means 19–21 μg/g dry weight, maximum 28 μg/g dry weight) but not to a level considered toxic. Background hepatic concentrations are typically <10 μg/g dry weight and a threshold for juvenile and adult toxicity is estimated at 30 μg/g dry weight [30].

Strontium in tree swallow and house wren eggs (means 14–23 μg/g dry weight) and chicks (mean 0.2–0.6 μg/g dry weight, range not detectable to 0.8) was elevated. Background concentrations of Sr in chicken eggs (*Galus galus*) were 2 μg/g dry weight [31]. Strontium is rarely detected in bird livers, but concentrations in American coot (*Fulica americana*) livers from San Francisco Bay, California, USA, varied between 0.45 and 0.79 μg/g dry weight [32] and concentrations in lesser scaup livers varied from 0.4 to 1.0 μg/g dry weight in western Lake Erie, USA [33].

Boron concentrations in swallow and wren eggs (means 58–59 μg/g dry weight) were toxic based on pen studies of mallard ducks. Values for wren eggs were well above a level of concern for B in eggs (13–20 μg/g dry weight) and a level associated with an embryonic toxicity threshold (20 μg/g dry weight) [30].

The source of Se, Sr, and B in the North Platte River may be from irrigation return flow running into the river both above and below the reference site. Concentrations of Se, Sr, and B in biota collected from an upstream major irrigation project were elevated [34].

Zinc concentrations in livers of swallows and wrens varied from 70 to 130 μg/g dry weight. These values were well below levels associated with Zn toxicosis (1,200 μg/g dry weight [35]). Copper, Mg, Mn, and Mo in livers of swallows and wrens were within the normal range [31].

Monooxygenase activity

The ninefold induction of hepatic EROD and BROD activities in tree swallows near the refinery likely was related to PAH exposure. Three hepatic monooxygenase activities in lesser scaup, collected on the heavily petroleum-polluted Indiana Harbor Canal, East Chicago, Indiana, USA, were each significantly correlated with PAH concentrations in scaup carcasses [12]. Activity of EROD was induced in Barrow's goldeneye (*Bucephala islandica*) and harlequin ducks (*Histrion-*

icus histrionicus) in Prince William Sound, Alaska, USA, six years after the *Exxon Valdez* oil spill (Leslie Holland-Bartels, unpublished data). Numerous laboratory studies have documented the relationship between exposure to petroleum and cytochrome P450 induction. Activity of EROD was induced in adult Atlantic puffins (*Fratercula arctica*) after a single dose of Prudhoe Bay crude oil [36]. Hepatic naphthalene-metabolizing activities of mallard ducks increased almost four times after exposure to food dosed with South Louisiana crude oil [37].

Laboratory studies have also demonstrated a relationship between cytochrome P450 induction and PAHs. Cytochrome P450 induction was correlated with PAHs in the chicken embryo [8] and herring gull [9]. A variety of PAHs injected into chicken embryos induced cytochrome P450 activity [10]. Activity of EROD was induced in adult European starlings (*Sturnus vulgaris*) after being administered a synthetic PAH, 7,12-dimethylbenz[*a*]anthracene [11].

Also, EROD and BROD induction could be related to chemicals other than PAHs. For example, monooxygenase activity was correlated with dioxin [38] and total PCB [39,40] concentrations in bird embryos. Additionally, EROD and BROD activities in tree swallows chicks collected from PCB-contaminated Green Bay, Wisconsin, USA, were correlated with PCB concentrations in carcasses [15]. We did not measure either dioxin or PCBs in this study.

CONCLUSIONS

Analysis of our results suggests that oil seepage into the North Platte River is not contributing to significant PAH contamination of the aquatic food chain. Tree swallow and house wren chicks near a refinery site on the North Platte River near Casper, Wyoming, USA, were exposed to PAHs. However, the lack of methylated PAHs in carcasses, diet, and sediment suggests that the source of PAHs on the North Platte River was from combustion and not from petroleum. Additionally, the predominance of odd-numbered ALHs in swallow and wren carcasses suggests that they were not exposed to petroleum. Hepatic monooxygenase activities in swallows were induced nine-fold near the refinery and were probably a result of exposure to PAHs that originated from combustion. Based on the distribution and concentration of PAHs in dietary samples, the main route of exposure to PAHs was probably through the diet. None of the trace elements in tree swallow and house wren tissues were higher at the refinery than at an upstream reference site. Selenium, Sr, and B were elevated in eggs and chicks of both species at both sites.

Acknowledgement—We thank R. Jewett, L. Harms, S. Talbot, and P. Thompson for access to their properties; K. Tuggle for coordinating access to Texaco's property; L. Coppock, K. McGrath, and B. Rogers for their field assistance; P. Dummer for assistance with data analysis; and J. McCarty and an anonymous reviewer for comments on the manuscript.

REFERENCES

1. Schmelzer H. 1995. Sampling activities report: Texaco refinery site, Evansville, Natrona County, Wyoming. T08-9503-006. Technical Direction Document. U.S. Environmental Protection Agency, Denver, CO.
2. Voudrias EA, Smith CL. 1986. Hydrocarbon pollution from marinas in estuarine sediments. *Estuarine Coastal Shelf Sci* 22:271–284.
3. Hall RJ, Coon NC. 1988. Interpreting residues of petroleum hydrocarbons in wildlife tissues. Biological Report 88(15). U.S. Fish and Wildlife Service, Washington, DC.
4. Naf C, Broman D, Brunstrom B. 1992. Distribution and metabolism of polycyclic aromatic hydrocarbons (PAHs) injected into eggs of chicken (*Gallus domesticus*) and common eider duck (*Somateria mollissima*). *Environ Toxicol Chem* 11:1653–1660.
5. Custer TW, Custer CM, Hines RK, Sparks DW. 2000. Trace elements, organochlorines, polycyclic aromatic hydrocarbons, dioxins, and furans in lesser scaup wintering on the Indiana Harbor Canal. *Environ Pollut* 110:469–482.
6. Mineau P, Fox GA, Norstrom RJ, Weseloh DV, Hallett DJ, Ellenton JA. 1984. Using the herring gull to monitor levels and effects of organochlorine contamination in the Canadian Great Lakes. In Nriagu JO, Simmons MS, eds, *Toxic Contaminants in the Great Lakes*. John Wiley & Sons, New York, NY, USA, pp 425–452.
7. Schroder JL, Basta NT, Lochmiller RL, Rafferty DP, Payton M, Kim S, Qualls CW Jr. 2000. Soil contamination and bioaccumulation of inorganics on petrochemical sites. *Environ Toxicol Chem* 19:2066–2072.
8. Walters P, Kehn S, O'Brien PJ, Payne JF, Rahimtula AD. 1987. Effectiveness of Prudhoe Bay crude oil and its aliphatic, aromatic and heterocyclic fractions in inducing mortality and aryl hydrocarbon hydroxylase in chick embryo in ovo. *Arch Toxicol* 60: 454–459.
9. Peakall DB, Norstrom RJ, Jeffrey DA, Leighton FA. 1989. Induction of hepatic mixed function oxidases in the herring gull (*Larus argentatus*) by Prudoe Bay crude oil and its fractions. *Comp Biochem Physiol C* 94:461–463.
10. Jellinck PH, Smith G. 1973. Aryl hydrolase induction in the chick embryo by polycyclic hydrocarbons. *Biochim Biophys Acta* 304: 520–525.
11. Trust KA, Fairbrother A, Hooper MJ. 1994. Effects of 7,12-dimethylbenz[*a*]anthracene on immune function and mixed-function oxygenase activity in the European starling. *Environ Toxicol Chem* 13:821–830.
12. Custer TW, Custer CM, Hines RK, Sparks DW, Melancon MJ, Hoffman DJ, Bickham JW, Wickliffe JK. 2000. Mixed-function oxygenases, oxidative stress, and chromosomal damage measured in lesser scaup wintering on the Indiana Harbor Canal. *Arch Environ Contam Toxicol* 38:522–529.
13. King KA, Custer TW, Weaver DA. 1994. Reproductive success of barn swallows nesting near a selenium-contaminated lake in east Texas, USA. *Environ Pollut* 84:53–58.
14. Bishop CA, Koster MD, Chek AA, Hussell DJT, Jock K. 1995. Chlorinated hydrocarbons and mercury in sediments, red-winged blackbirds (*Agelaius phoeniceus*) and tree swallows (*Tachycineta bicolor*) from wetlands in the Great Lakes–St. Lawrence River basin. *Environ Toxicol Chem* 14:491–501.
15. Custer CM, Custer TW, Allen PD, Stromborg KL, Melancon MJ. 1998. Reproduction and environmental contamination in tree swallows nesting in the Fox River and Green Bay, Wisconsin, USA. *Environ Toxicol Chem* 17:1786–1798.
16. Custer CM, Custer TW, Coffey M. 2000. Organochlorine chemicals in tree swallows nesting in Pool 15 of the Upper Mississippi River. *Bull Environ Contam Toxicol* 64:341–346.
17. Quinney TE, Ankney CD. 1985. Prey size selection by tree swallows. *Auk* 102:245–250.
18. Blancher PJ, McNicol DK. 1991. Tree swallow diet in relation to wetland acidity. *Can J Zool* 69:2629–2637.
19. Fairchild WL, Muir DCG, Currie RS, Yarechewski AL. 1992. Emerging insects as a biotic pathway for movement of 2,3,7,8-tetrachlorodibenzofuran from lake sediments. *Environ Toxicol Chem* 11:867–872.
20. Burke MD, Mayer RT. 1983. Differential effects of phenobarbitone and 3-methylcholanthrene induction on the hepatic microsomal metabolism and cytochrome P-450-binding of phenoxazone and a homologous series of its *n*-alkyl ethers (alkoxyresorufins). *Chem-Biol Interact* 45:243–258.
21. Melancon MJ. 1996. Development of cytochromes P450 in avian species as a biomarker for environmental contaminant exposure and effect. Procedures and baseline values. In Bengston DA, Henshel DS, eds, *Environmental Toxicology and Risk Assessment: Biomarkers and Risk Assessment*, Vol 5. STP 1306. American Society for Testing and Materials, Philadelphia, PA, pp 95–108.
22. Lowry OH, Rosebrough NJ, Farr AL, Randall RJ. 1951. Protein measurement with the Folin phenol reagent. *J Biol Chem* 193: 265–275.
23. Johnson LS. 1998. House wren (*Troglodytes aedon*). In Poole A,

Gill F, eds, *The Birds of North America*, Vol 380. Academy of Natural Sciences, Philadelphia, PA, USA.

24. Michot TC, Custer TW, Nault AJ, Mitchell CA. 1994. Environmental contaminants in redheads wintering in coastal Louisiana and Texas. *Arch Environ Contam Toxicol* 26:425–434.
25. Rattner BA, Flickinger EL, Hoffman DJ. 1993. Morphological, biochemical, and histopathological indices and contaminant burdens of cotton rats (*Sigmodon hispidus*) at three hazardous waste sites near Houston, Texas, USA. *Environ Pollut* 79:85–93.
26. Rattner BA, Capizzi JL, King KA, LeCaptain LJ, Melancon MJ. 1995. Exposure and effects of oilfield brine discharges on western sandpipers (*Calidris mauri*) in Nueces Bay, Texas. *Bull Environ Contam Toxicol* 54:683–689.
27. King KA, Stafford CJ, Cain BW, Mueller AJ, Hall HD. 1987. Industrial, agricultural and petroleum contaminants in cormorants wintering near the Houston ship channel, Texas, USA. *Colon Waterbirds* 10:93–99.
28. Thompson DR. 1996. Mercury in birds and terrestrial mammals. In Beyer WN, Heinz GH, Norwood AW, eds, *Environmental Contaminants in Wildlife. Interpreting Tissue Concentrations*. Lewis, Boca Raton, FL, USA, pp 341–356.
29. Fimreite N. 1974. Mercury contamination of aquatic birds in northwestern Ontario. *J Wildl Manag* 38:120–131.
30. U.S. Department of Interior. 1998. Guidelines for interpretation of the biological effects of selected contaminants in biota, water, and sediment. National Irrigation Water Quality Program Information Report 3. Denver, CO.
31. Puls R. 1994. *Mineral Levels in Animal Health. Diagnostic Data*, 2nd ed. Sherpa International, Clearbrook, BC, Canada.
32. Hui CA. 1998. Elemental contaminants in the livers and ingesta of four subpopulations of American coot (*Fulica americana*): An herbivorous winter migrant in San Francisco Bay. *Environ Pollut* 101:321–329.
33. Custer CM, Custer TW. 2000. Organochlorine and trace element contamination in Great Lakes waterfowl since the zebra mussel invasion. *Environ Toxicol Chem* 19:2821–2829.
34. See RB, Naftz DL, Peterson DA, Crock JG, Erdman JA, Severson RC, Ramirez P Jr, Armstrong JA. 1992. Detailed study of selenium in soil, representative plants, water, bottom sediment, and biota in the Kendrick reclamation project. Water-Resources Investigation Report 91-4131. U.S. Geological Survey, Cheyenne, WY.
35. Gasaway WD, Buss IO. 1972. Zinc toxicity in the mallard duck. *J Wildl Manag* 36:1107–1117.
36. Peakall DB, Jeffrey DA, Boersma D. 1987. Mixed-function oxidase activity in seabirds and its relationship to oil pollution. *Comp Biochem Physiol C* 88:151–154.
37. Gorsline J, Holmes WN. 1981. Effects of petroleum on adrenocortical activity and on hepatic naphthalene-metabolizing activity in mallard ducks. *Arch Environ Contam Toxicol* 10:765–777.
38. Bellward GD, et al. 1990. Comparison of polychlorinated dibenzodioxin levels with hepatic mixed-function oxidase induction in great blue herons. *J Toxicol Environ Health* 30:33–52.
39. Rattner BA, Hatfield JS, Melancon MJ, Custer TW, Tillitt DE. 1994. Relation among cytochrome P450, Ah-active PCB congeners and dioxin equivalents in pipping black-crowned night-heron embryos. *Environ Toxicol Chem* 13:1805–1812.
40. Rattner BA, et al. 1993. Biomonitoring environmental contamination with pipping black-crowned night heron embryos: Induction of cytochrome P450. *Environ Toxicol Chem* 12:1719–1732.