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Exposure and Effects of Oilfield Brine Discharges on Western Sandpipers (*Calidris mauri*) in Nueces Bay, Texas

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Discharge of oilfield brines into fresh and estuarine waters is a common disposal practice in Texas. Petroleum crude oil (PCO) extraction from underground stores includes the removal of a significant amount of water along with the oil. Several methods may be used to separate the oil and water fractions, including tank batteries, heat separation, and skimming ponds. Disposal of the resultant produced water (oilfield brine) may be accomplished by deep-well injection or discharge to surface waters. In Texas, an estimated 766,000 barrels of oilfield brine were discharged daily into tidal waters in 1979 (Liebow et al. 1980). The maximum concentration for oil and grease in these discharges permitted by the Texas Railroad Commission is 25 ppm. Several studies have shown that oilfield brines are toxic to a wide range of marine life (Simmons *Texas Railroad Comm. Oil and Gas Docket No. 2 and 4-60, Nov. 4, 1970*; Spears *Texas Railroad Comm., Oil and Gas Docket 62099, July 26, 1972*; Andreasen and Spears 1983; Boelter et al. 1992), yet little is known about their effects on birds and mammals.

Exposure to petroleum in oilfield wastes could evoke toxicological effects in some waterbird species. Avian responses to PCO exposure are highly variable, including cessation of growth, osmoregulatory impairment, endocrine dysfunction, hemolytic anemia, altered blood chemistry, cytochrome P450 induction, reduced reproductive success, and mortality (Holmes 1984; Albers 1991; Leighton 1991). Oilfield brine discharges may soon be the largest and most pervasive source of contaminants entering Texas estuaries. Migratory and resident birds feeding in the vicinity of discharge sites may be ingesting food items contaminated with petroleum hydrocarbons, heavy metals and salts in sufficient quantities to evoke toxicity. The present study of wintering western sandpipers (*Calidris mauri*) that feed and roost near discharge sites sought to examine oilfield brine exposure and effects through quantification of contaminant burdens, morphological characteristics, and cytochrome P450-associated monooxygenase activities.

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MATERIALS AND METHODS

Whites Point ($27^{o}47'N$, $97^{o}35'W$), on the north shore of Nueces Bay, near Corpus Christi, Texas, is a multiple oilfield brine discharge area consisting of nine wastewater disposal sites located along a 3 km stretch of marsh, mudflat, and brackish water ponds. Bolivar Flats ($29^{o}19'N$, $94^{o}55'W$), located on the Bolivar peninsula east of Galveston Island, was selected as a presumably uncontaminated reference site. Whites Point is located 190 km southwest of Bolivar Flats.

Western sandpipers, a winter resident at both sites, were collected by shotgun in March 1990, several months after their migratory arrival. Immediately after collection, whole body and liver weight were determined. Livers were blotted free of blood, minced, placed in storage vials containing glycerol, frozen in liquid nitrogen, and stored at $-70^{o}C$ for subsequent biochemical analysis. Bill length was measured, and carcasses were prepared for contaminant analysis by removing the feathers, bill, feet, wing tips and gastrointestinal tract. When stomach contents were available, they were pooled to obtain sufficient mass for a composite sample from each site.

Pooled stomach contents and carcasses were analyzed for 13 aliphatic and 14 aromatic hydrocarbons (Belisle et al. 1981). The lower limit of detection for aliphatic and aromatic hydrocarbons was 0.01 ppm. In addition, half of the carcasses from each site were analyzed for 21 organochlorine pesticides and metabolites, and total polychlorinated biphenyls (PCBs) concentrations (Cromartie et al. 1975). The lower limit of detection was 0.01 ppm for organochlorine pesticides and 0.05 ppm for PCBs.

Cytochrome P450-associated monooxygenase activity of liver samples was determined as recently described in detail by Rattner et al. (1993). Samples were thawed, homogenized in phosphate buffer, and a microsomal pellet was prepared by differential centrifugation. The pellet was resuspended and assayed for protein concentration. Arylhydrocarbon hydroxylase (AHH) activity was measured by radioenzymatic determination of total hydroxylation products formed by the metabolism of ^{3}H-benzo[*a*]pyrene. Activity of AHH is expressed as pmol of total metabolites formed/min/mg microsomal protein. The activities of benzyloxyresorufin-*O*-dealkylase (BROD), ethoxycoumarin-*O*-dealkylase (ECOD), and ethoxyresorufin-*O*-dealkylase (EROD) and pentoxyresorufin-*O*-dealkylase (PROD) were determined by the rate of formation of fluorescent product. Dealkylase enzyme activity is expressed as pmol or nmol product/min/mg microsomal protein.

Contaminant burdens, morphological characteristics, and activities of monooxygenases were tested for homogeneity of variance using the F_{max} test, and some variables were $\log_{10}$ transformed to stabilize variances. One-half the lower limit of detection was used for statistical analyses for samples without detectable pristane, phytane, n-heptadecane and octadecane, and PCB burdens. In all cases, at least one half of the samples had detectable contaminant

levels. Sites were compared using Student's t-test (two-tailed). Linear relationships among contaminant burdens and biological parameters were examined using Pearson product-moment correlation.

RESULTS AND DISCUSSION

The concentration of total aliphatic petroleum hydrocarbons in pooled stomach contents of sandpipers was relatively similar at reference and discharge sites (1.05 and 0.71 ppm, respectively), but total aromatic petroleum hydrocarbon concentration in stomach contents of birds collected at the discharge site (1.42 ppm) was over tenfold greater than at the reference site (0.10 ppm). Total detectable aliphatics in carcasses did not differ between sites (Table 1), although the concentration of pristane and the ratio of pristane:n-heptadecane were at least fivefold greater ($p < 0.01$) at the discharge site. Pristane (a branch chain hydrocarbon often abundant in pollutant oils) and its ratio to n-heptadecane (predominantly of biological origin) were greater at the discharge site. The apparent accumulation of pristane and its elevated ratio to n-heptadecane are indicative of chronic petroleum hydrocarbon exposure (Giger et al. 1974; Hall and Coon 1988). Aromatic petroleum hydrocarbons were rarely detected in carcasses, and when present, concentrations were 0.02 ppm or less. Based on a six to seven month winter residency at this location (King *unpub. data*), the moderate concentration of petroleum hydrocarbons in stomach contents, and the limited accumulation of select hydrocarbons in carcasses, overall exposure appears to be modest compared to that encountered following an oil spill (Albers 1991).

Concentrations of organochlorine contaminants were relatively low at both sites (Table 1). Of the 10 carcasses analyzed from the discharge site, all contained detectable levels of p,p'-DDE ($\leq$ 0.25 ppm) and PCBs ($\leq$ 0.98 ppm), 7 carcasses contained both oxychlordane (0.01) and heptachlor ($\leq$ 0.05 ppm), 3 contained *trans*-nonachlor (0.01 ppm), and 1 contained dieldrin (0.01 ppm). Of the 5 carcasses analyzed from the reference site, all contained detectable levels of p,p'-DDE ($\leq$ 0.06 ppm), 4 contained PCBs ($\leq$ 0.80 ppm), 2 contained both oxychlordane (0.01) and heptachlor (0.01 ppm), and 1 contained dieldrin (0.01 ppm). No other organochlorine compounds were detected in the samples. Organochlorine concentrations were below those known to cause avian mortality or reproductive problems and comparable to background levels found in previous studies (White et al. 1980; White et al. 1983).

Body weight and bill length were similar between sites, although liver weight and liver:body weight ratio were significantly lower (>25% reduction) at the discharge site ($p < 0.01$; Table 2). Exposure to PCO is often accompanied by hepatic hypertrophy (Miller et al. 1978; Gorsline and Holmes 1981), although reduced liver weight has been observed in mallards (*Anas platyrhynchos*) following chronic low level dietary exposure (0.5% South Louisiana crude oil)(Gorsline and Holmes 1981). In the present study, liver weight was inversely correlated with the pristane:n-heptadecane ratio (r^2=-0.37; $P<0.05$), consistent with chronic low level exposure.

Table 1. Carcass contaminant burdens of western sandpipers collected at reference and oilfield brine discharge sites[a].

	Bolivar Flats Reference Site N = 11[b]	Whites Point Discharge Site N = 19[b]
Total aliphatics (ppm)	1.7 (1.0 - 4.4)	1.8 (0.02 - 2.0)
Pristane (ppm)	0.07 (ND[c] - 0.44)	0.40* (0.02 - 2.0)
Pristane:n-heptadecane ratio	0.312 (0.003 - 3.1)	2.33* (0.087 - 18.9)
Total organochlorine pesticides and metabolites (ppm)	0.05 (0.03 - 0.07) N = 5	0.09 (0.02 - 0.27) N = 10
Total PCBs (ppm)	0.36 (ND - 0.80) N = 5	0.53 (0.29 - 0.98) N = 10

[a]Values presented are geometric mean and (range); concentrations are reported on wet-weight basis.
[b]Unless otherwise noted.
[c]ND = not detected.
*Significantly different ($p < 0.01$) by Student's t-test.

Activities of AHH, BROD (log transformed), ECOD, EROD, and PROD (expressed per mg of microsomal protein) were similar ($p > 0.05$) in birds collected at both locations (Table 2). Inspection of monooxygenase activities of individual birds from the discharge site revealed that activity rarely exceeded two standard deviations of the reference site mean (1 of 19 observations for AHH, BROD, ECOD and PROD; 2 of 19 observations for EROD). Monooxygenases were also examined in terms of activity per gram liver, total activity per liver, and activity per gram body weight. No statistical differences between sites were detected, with the exception of slightly greater PROD activity per liver and per g body weight at the reference site (mean units/liver: 720.8 versus 415.3; mean units/g body weight: 29.1 versus 17.1). These data strongly suggest that hepatic cytochrome P450 is not induced in birds residing at or near the oilfield brine discharge site.

Induction of cytochrome P450 and associated monooxygenase activity has been used extensively as a biomarker of exposure to some organic pollutants in wildlife (Rattner et al. 1989, 1993). PCO induces monooxygenase activity in a variety of avian species (Miller et al. 1978; Gorsline and Holmes 1981; Peakall et al. 1987, 1989), and

Table 2. Morphological indices and hepatic microsomal monooxygenase activities of western sandpipers collected from reference and oilfield brine discharge sites[a].

	Bolivar Flats Reference Site	Whites Point Discharge Site
	N = 11	N = 19
Body weight (g)	24.7 ± 0.4 (22.6 - 26.4)	23.9 ± 0.5 (20.9 - 27.5)
Bill length (mm)[b]	23.6 ± 0.6 (21.0 - 26.0)	23.4 ± 0.5 (21.5 - 28.0)
Liver weight (g)	1.23 ± 0.07 (0.9 - 1.8)	0.87 ± 0.06* (0.5 - 1.7)
Liver:body weight ratio (g/100 g)	4.99 ± 0.34 (3.41 - 7.89)	3.63 ± 0.23* (2.39 - 7.20)
AHH (pmol/min/mg)	1041 ± 81 (435 - 1332)	970 ± 97 (524 - 2347)
BROD (pmol/min/mg)	75.3 ± 16.0 (0.4 - 149)	134.5 ± 35.4 (35 - 730)
ECOD (nmol/min/mg)	1.7 ± 0.1 (0.8 - 2.5)	1.8 ± 0.1 (0.9 - 3.1)
EROD (pmol/min/mg)	1119.8 ± 222.3 (394.4 - 2760)	719.0 ± 105.2 (196.3 - 1802)
PROD (pmol/min/mg)	72.1 ± 11.2 (2.5 - 114)	49.9 ± 5.5 (24.4 - 106)

[a]Values presented are mean ± S.E and (range).
[b]N=8 for reference site and N=18 for discharge site.
*Significantly different ($p < 0.01$) by Student's t-test.

induction seems to be preferentially linked to the aromatic fraction (Walters et al., 1987; Peakall et al. 1989). The absence of elevated hepatic microsomal monooxygenase activity at the Whites Point discharge site suggests that despite a tenfold difference in petroleum aromatic hydrocarbon content of foods between sites, the concentrations of organic contaminants were insufficient to induce cytochrome P450. The decreases in total hepatic PROD activity and PROD activity per g body weight in sandpipers collected at the Whites Point discharge site could be simply the result of the decreased liver size. Alternatively, oilfield brine discharges near Corpus Christi contained cadmium, lead and mercury at concentrations exceeding the U.S. EPA marine chronic water quality criteria by 700, 8 and 4000 times (Pedroy Ramirez, U.S. Fish and Wildlife Service, 1983 *pers. comm.*). These metals are capable of stimulating heme oxygenase activity and thus decreasing cytochrome P450 (Maines and

Kappas 1977). Monooxygenase activities were not correlated with contaminant burdens in the present study.

In conclusion, petroleum aromatic hydrocarbon concentrations in food items, carcass aliphatic contaminant burdens and reduced liver weight of sandpipers collected at the oilfield discharge site suggest chronic exposure to petroleum hydrocarbons. Some avian species are sensitive to PCO, whereas others can ingest substantial quantities without deleterious effect (Holmes 1984). Ingested contaminants were not of sufficient quantity or potency to induce cytochrome P450-associated monooxygenase activity. These data suggest that adult sandpipers may be relatively tolerant to petroleum hydrocarbons typically contained in oilfield brine discharges. Furthermore, because early avian life stages are particularly sensitive to these pollutants, additional studies may be warranted in waterbird species that nest and feed near these discharge sites. Future wildlife hazard assessments at oilfield brine discharge sites should also include biomarkers of metal exposure and effects.

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