CONTAMINANT SURVEY OF THE SAN BERNARD NATIONAL WILDLIFE REFUGE BRAZORIA COUNTY, TEXAS

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INTRODUCTION

The San Bernard National Wildlife Refuge (Fig. 1) is located on the Texas coast in the southeast corner of Brazoria County. It is bordered on the south by Cedar Lake Creek and to the north by Farm to Market Road 2918, which parallels the San Bernard River. The lower section of the refuge is bisected by the Gulf Intracoastal Waterway (GIWW), a shipping lane for barge traffic. Both Cedar Lake Creek and the San Bernard River drain pasture land, bottomland hardwood forest, and some urban development areas. Farming occurs in both watersheds but is not extensive, whereas ranching is the predominant land use in both watersheds.

Contamination that may occur in the two watersheds that drain along the refuge boundaries would be due to urban runoff, some farm chemicals, and waste water from petroleum production facilities. Flooding, due to heavy rainfall in these watersheds, would bring sediment and its associated contaminants onto the refuge land.

There are no major industrial complexes in the immediate area of the refuge, or up either of the two watersheds. However, a large industrial complex exists at Freeport, Texas, approximately 10 miles to the northeast of the refuge (Fig. 1). This industrial complex refines crude oil into several products that are then used to manufacture plastics, pesticides, and other raw materials. The GIWW is the shipping lane for many of these products that are sent to other cities on the lower Texas coast, and allows for surface water connection between this industrial complex and the tidal marshes on the refuge. During periods of high river flow down the Brazos River, which is north of the refuge near Freeport, Texas, the GIWW flows southward through the refuge carrying a large sediment load down the GIWW.

Cedar Lake and Cow Trap Lake are two major estuarine systems located on the San Bernard National Wildlife Refuge. These systems are separated by the GIWW (Fig. 1) and receive their tidal exchange via this waterway. If contaminants are being transported southward from Freeport, Texas, via the GIWW, or by the San Bernard River, these two tidal estuarine systems will be impacted by the tidal distribution of these sediments and contaminants.

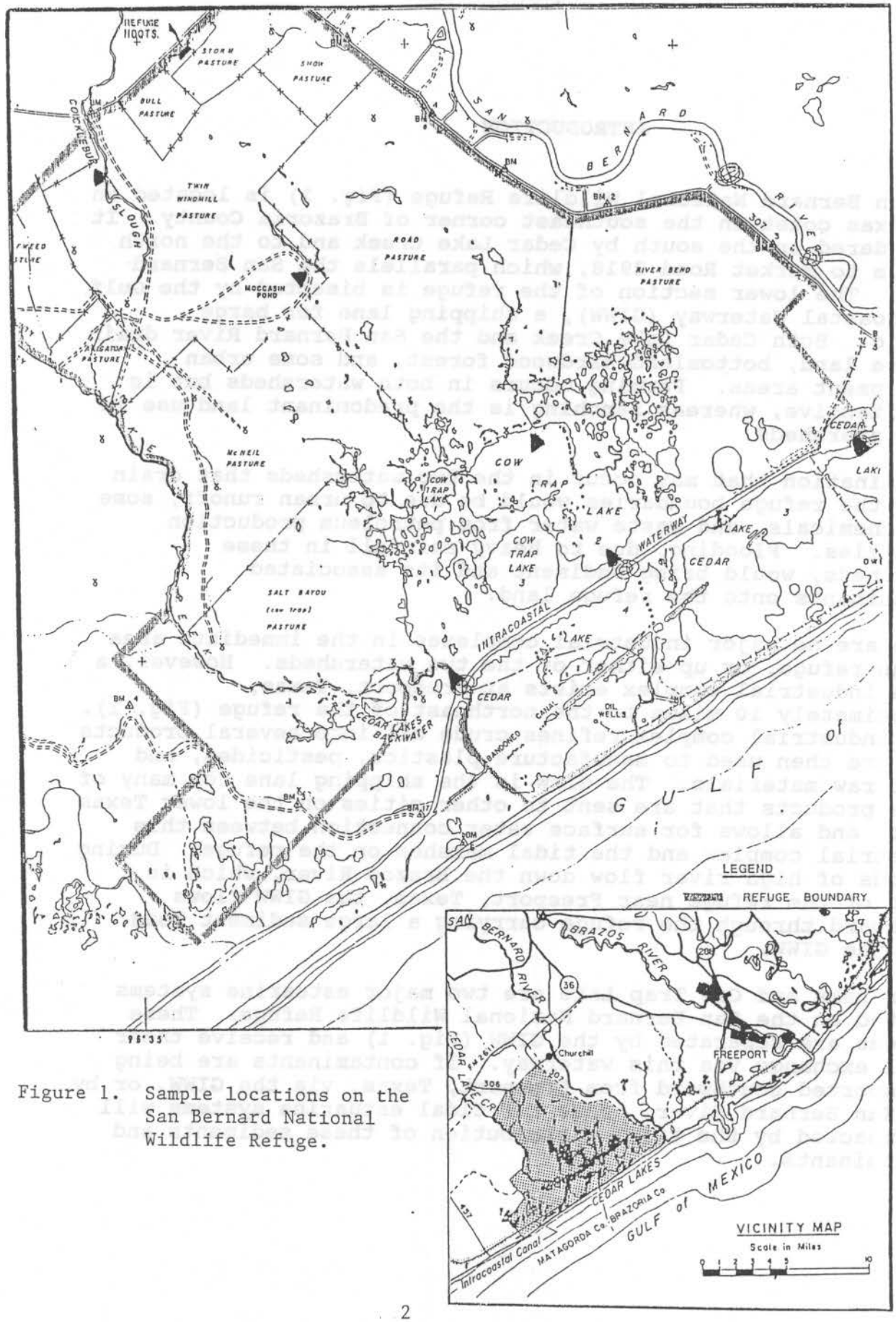

Figure 1. Sample locations on the San Bernard National Wildlife Refuge.

The Cow Trap Lake and Cedar Lake systems are shallow, and during periods of low tide several acres of oyster reefs are emergent above the water. Thousands of shorebirds are attracted to the exposed reefs and mud substrate during low tides where they feed on the small organisms that are left exposed. In the Cedar Lake system, there are also many small islands that are used as nesting grounds for colonial waterbirds. Contamination potential of these habitats is the focus of this report.

MATERIALS AND METHODS

SAMPLE COLLECTIONS

Sediment samples were collected with a stainless steel petite ponar sampler, placed in a chemically cleaned glass jar, and chilled in an ice chest as they were transported to the laboratory. Samples were stored at a minus 20°C and shipped to a contract laboratory for chemical analysis. Sediment samples were taken from Cow Trap Lake, Cedar Lake, Cedar Lake Creek, Cocklebur Slough, and Moccasin Pond within the refuge (Fig. 1). One sediment sample was taken from an abandoned pit at a gas well site on Big Boggy. Big Boggy is a separate section of the refuge and not illustrated in Figure 1.

Oysters were collected from reefs in Cow Trap Lake, Cedar Lake, and Cedar Lake Creek in the same area as the sediment samples. Each oyster was placed in a plastic bag and chilled with ice as they were transported to the laboratory. In the laboratory, the oysters were pried open with a stainless steel blade and the fleshy part along with any liquor was put in a chemically cleaned glass jar and handled in the same fashion as the sediment samples.

Aquatic invertebrates were collected with a nylon dip net or from a 20-foot seine dragged through aquatic vegetation in Cocklebur Slough, Moccasin Pond, and the gas well pit on Big Boggy. Any invertebrate caught in the net was picked up with stainless steel forceps and dropped into a chemically cleaned jar. These samples were thus a composite of water beetles, aquatic larvae, worms, snails, glass shrimp, crayfish, spiders, and frog larvae. No attempt was made to classify these organisms.

LABORATORY METHODS

All samples were sent to laboratories under contract to the U.S. Fish and Wildlife Service and analyzed for heavy metals, organochlorine pesticides, and petroleum hydrocarbons (Table 1). All residue concentrations are reported as parts per million (ppm) wet weight. Samples were homogenized at the laboratory before analysis. Analytical methodologies are not described in this text but are available upon request from the Patuxent Analytical Control Facility, U.S. Fish and Wildlife

Service, Laurel, Maryland. A brief description of the analytical techniques is provided in Appendix I. Acceptable performance (a recovery variation of <20%) of all chemical analyses in spikes, blanks, and duplicates was documented in quality control reports from the analytical laboratory.

Table 1. Contaminants surveyed on the San Bernard National Wildlife Refuge.

Heavy Metals	Organochlorine Pesticide	Petroleum Hydrocarbons	
		Aromatics	Aliphatics
Aluminum	Aldrin	Antracene	Dodecane
Antimony	Benzene hexachloride	Acenaphthene	Tridecane
Barium	Chlordane	Acenaphthylene	Tetradecane
Beryllium	Dieldrin	Benzo(a)anthracene	Pentadecane
Boron	DDE	Benzo(a)pyrene	Hexadecane
Cadmium	DDD	Benzo(b)fluoranthrene	Pristine
Chromium	DDT	Benzo(ghi)perylene	Octadecane
Copper	Endrin	Benzo(k)fluoranthrene	Phytane
Iron	Heptachlor	Biphenyl	Nonadecane
Lead	Heptachlor epoxide	Chrysene	Eicosane
Magnesium	Hexachlorobenzene	Dibenzanthracene	
Manganese	Lindane	2,6-Dimenthylnaphthlene	
Molybdenum	Mirex	Fluoranthrene	
Nickel	Nonachlor	Fluorene	
Strontium	PCB's	Indenopyrene	
Thallium	Toxaphene	1-Methylnaphthalene	
Tin		2-Methylnaphthalene	
Vanadium		1-Methylphenanthrene	
Zinc		Naphthalene	
		Perylene	
		Phenanthrene	
		Pyrene	
		2,3,4-Trimethylnaphthalene	

RESULTS AND DISCUSSION

ESTUARINE HABITAT ASSESSMENT

There were no organochlorine pesticides listed in Table 1 detected at the 0.05 ppm level in either the sediment or oyster samples from Cow Trap Lake or Cedar Lake Creek. Residues of DDE (0.45 ppm) and toxaphene (0.5 ppm) were reported by Schmidt et al. (1990) from striped mullet collected from the Brazos River at Richmond, Texas, which is several miles upstream. The Brazos River crosses the GIWW north of the San Bernard National Wildlife Refuge, but apparently is not transporting organochlorine residues into the study area. South of the refuge the Colorado River also crosses the GIWW, and it also had low levels of organochlorine pesticide residues in the sediment (Hinson 1990). It appears as if the sediment load distributed by the GIWW into the refuge's estuarine habitat is not contaminating this area with organochlorine pesticides.

Heavy metals were detected in all samples collected from the estuarine habitat (Table 2). A few metals such as antimony, molybdenum, silver, thallium, and tin were not detected in either the sediment or tissue samples and will not be discussed further. These elements have also not been detected in sediment samples collected from other areas of the Texas coast (Gamble et al. 1988 and Mauer et al. 1989). Three metals were detected at low levels in the sediment but not in the oyster tissue (i.e. beryllium, lead and vanadium). Beryllium and lead are on the EPA's list of 129 priority pollutants, and vanadium is considered to be one of the 14 most noxious heavy metals (Irwin 1988, 1989). Each of these metals have a high bioaccumulation factor for mussels and oysters, but in this study they did not appear to be of concern because of the low levels detected (Table 2).

Several heavy metals were detected at high levels in sediment samples (Table 2) while others, that are known to be harmful to animals at low levels, were detected only in the oyster tissue. These metals will be discussed below in order to make an assessment of their contaminant potential.

Aluminum

Aluminum is the most abundant metal in the earth's crust (Haug 1984) averaging 81,000 ppm (Brooke and Stephan 1988). It is more soluble in acidic and basic solutions than in

Table 2. Heavy metals detected in estuarine samples from the San Bernard National Wildlife Refuge.

	Sediment		Oysters		
	Cow Trap	Cedar Lake Creek	Cow Trap[1]	Cow Trap[2]	Cedar Lake Creek
Aluminum	8510.00	11600.00	102.00	79.20	105.00
Antimony	<5.00	<5.00	<1.00	<1.00	<1.00
Barium	75.40	74.50	2.68	2.14	1.84
Beryllium	0.35	0.50	<0.05	<0.05	<0.05
Boron	13.70	10.80	4.17	3.78	4.10
Cadmium	<0.25	<0.25	0.49	0.34	0.53
Chromium	9.70	11.30	5.05	4.00	1.05
Copper	4.65	7.25	28.90	16.80	20.60
Iron	8240.00	8670.00	113.00	94.60	89.70
Lead	7.60	11.20	<0.30	<0.30	<0.30
Magnesium	4040.00	3080.00	890.00	887.00	897.00
Manganese	166.00	204.00	7.32	5.71	5.30
Molybdenum	<2.50	<2.50	<0.50	<0.50	<0.50
Nickel	6.85	7.75	2.44	2.17	0.69
Silver	<2.50	<2.50	<0.50	<0.50	<0.50
Strontium	48.70	110.00	12.80	7.85	10.00
Thallium	<10.00	<10.00	<2.00	<2.00	<2.00
Tin	<2.50	<2.50	<0.50	<0.50	<0.50
Vanadium	14.60	15.60	<0.50	<0.50	<0.50
Zinc	22.00	114.00	295.00	271.00	247.00

[1] Mouth of Cow Trap Lakes and the Gulf Intracoastal Waterway (Fig. 1).

[2] Interior of Cow Trap Lakes.

circumneutral (pH 7) solutions. Aluminum forms soluble complexes with ions such as chloride, fluoride, nitrate, phosphate, and sulfate (Brooke and Stephan 1988) which allows for easy mobility in the environment. Aluminum toxicity is increased with a decrease in the pH of water. The ambient water quality criteria for aluminum recommends that a four-day average concentration in freshwater not exceed 87 ppb more than once every three years when the ambient pH is between 6.5 and 9.0 (Brooke and Stephan 1988). There is no saltwater criteria or sediment criteria for aluminum, and it was not considered as a soil contaminant by Beyer (1990).

Aluminum is known to cause root growth retardation in plants grown in acidic soil (Haug 1984). Several human and animal studies in recent years link aluminum uptake with several disorders such as osteomalacis dialysis osteodystrophy, encephalopathy (King et al. 1981), runted fetuses, microcardia, gonad agenesis, fused ribs and vertebrae, and absence of leg bones (McCormack et al. 1979, Gilani and Chatzinoff 1981). A primary mechanism for aluminum toxicity is the free-ion ($Al^{3}+$) substitution for magnesium at critical enzyme sites in some cellular functions (Macdonald and Martin 1988).

Aluminum detected in the estuarine habitat of the San Bernard National Wildlife Refuge (Table 2) was similar to the levels reported in sediments from the lower Rio Grande Valley of Texas (Gamble et al. 1988). These authors reported that oyster tissue did have higher levels of aluminum than did fish tissue (geometric mean of 15.7 ppm) or bird tissue (8.7-11.4 ppm). However, the aluminum residue in oysters (103 ppm) reported by Gamble et al. (1988) was essentially identical to the aluminum level in oyster tissue in this study (Table 2). Without a better understanding of aluminum kinetics in estuarine habitat, there apparently is no need to assume that aluminum is a contaminant problem at this time on the San Bernard National Wildlife Refuge.

Barium

Barium exists naturally as barite which is used as a weighting agent in drilling muds. Energy exploration in aquatic habitats may increase the barium content of water and sediment (Brannon and Rao 1979). These authors also reported that the exoskeleton, hepatopancreas, and abdominal muscle tissues discriminated in favor of barium uptake relative to calcium. The effects of this are unknown. Other authors (Tagatz and Tobia 1978, and Cantelmo et al. 1979) reported no toxic effect of barium to a meiofaunal community or to oysters, however, the experimental meiofaunal community decreased in abundance apparently as a result of the physical structure of the barite mud.

Barium residues in the sediment (Table 2) were very similar to that detected in 95 samples from the Lower Rio Grande Valley, reported by Gamble et al (1988), and from the Aransas National Wildlife Refuge (Mauer et al. 1989), and much lower than residues reported by Hoffman et al. (1990) from irrigation drainwater areas. Oysters collected from Cow Trap Lake and Cedar Lake Creek (Table 2) also had barium residues at levels not suspected of causing any adverse effects. Barium as a soil contaminant received little attention from Beyer (1990) and should not be a contaminant of concern for the refuge.

Boron

Boron is ubiquitous in the environment averaging about 34 ppm in soils from the United States (Beyer 1990). Human activities such as mining, coal burning, drainwater, and use of borax in detergents has resulted in elevated levels in some aquatic areas (Eisler 1990). Many species of aquatic plants, fish, invertebrates, and amphibians may tolerate up to 10 ppm boron in the water (Eisler 1990). The current boron criteria to protect sensitive species is 30 ppm in waterfowl diets and 1.0 ppm for aquatic species in the water (Eisler 1990).

Boron was detected in the sediments at 10.8 and 13.7 ppm (Table 2), and up to 4.17 ppm in oysters collected in the Cow Trap Lake area. It does not appear that boron is at a level to cause concern for natural resources utilizing the estuarine habitat, or that oysters are bioaccumulating boron to a high level. Boron was not listed as an element for assessment by Gamble et al. (1988), Irwin (1988, 1989) or Maurer et al. (1989), and is not considered as a toxic element in the published literature.

Cadmium

Cadmium is not considered as a biologically essential or beneficial element for animal metabolism. Background levels of cadmium in crops and other plants are usually less than 1.0 ppm (Eisler 1985). Many States have established a criteria that cadmium may not exceed 5 kg/ha in municipal sludge to be disposed on agricultural lands (Beyer 1990) because of the biotransfer potential of cadmium. Cadmium is known to interfere with calcium mobilization and is responsible for osteoporosis (EPA 1980a) in postreproductive mammals.

Cadmium is toxic to a variety of fish and wildlife species and causes behavior, growth, and physiological problems in aquatic organisms at sublethal concentrations (Rompala et al. 1984). Crustaceans, crabs, fish, mammals, and birds bioconcentrate cadmium from food and water. Earthworms concentrated cadmium to 100 ppm from soil containing only 2 ppm cadmium (Beyer et al. 1982).

Sediment cadmium residue (Table 2) was less than 0.25 ppm from Cow Trap and Cedar Lake Creek samples. Three oyster samples from these areas had cadmium residues of 0.34, 0.49, and 0.53 ppm. Oysters are known to bioconcentrate cadmium more that 2000 times the ambient water concentration (Eisler 1985). The level of cadmium detected in oysters from Cow Trap and Cedar Lake Creek may indicate the ambient water has a cadmium level of 0.2 ppb, which is below most reported sublethal effects concentration of 0.5 ppb for sensitive aquatic species.

Cadmium, at this time, does not appear to be a contaminant of concern for the San Bernard National Wildlife Refuge, but its presence should be monitored in the future. Residues in sediment and oysters were similar to those reported by Gamble et al. (1988) from oysters collected in estuaries in the Lower Rio Grande Valley.

Chromium

Chromium is listed as one of the 14 noxious heavy metals and is on the EPA priority pollutant list (Keith and Telliard 1979). Chromium appears in several valence states (+1 to +6) but the +6 valence form is the most toxic to aquatic organisms. The potential for accumulation of chromium is high in mollusks and crustaceans (Jenkins 1981). Little is known however, about the relation between concentrations of total chromium in a particular environment and biological effects on the organisms living there (Eisler 1986). The most sensitive saltwater organism tested was a polychaete worm that had a maximum acceptable toxicant concentration (MATC) range of 0.017 to 0.038 ppm (Eisler 1986). Chromium can be scavenged by colloidal iron and readily moved from an estuary to the ocean.

Chromium residue averaged 10.5 ppm from the two sediment samples taken from the estuary (Table 2). In the oyster tissue, the chromium residue averaged 3.36 ppm which is very near the 4.0 ppm tissue level suggested by Eisler (1986) as presumptive evidence of chromium contamination. Tissue residue of chromium in two oyster samples from the Lower Rio Grande Valley study averaged only 0.24 ppm (Gamble et al. 1988).

The high chromium residue in oysters (3.36 ppm) suggests that further monitoring should be considered in the estuarine portion of the San Bernard National Wildlife Refuge to determine if chromium is on the rise or if residues are stable. Chromium may enter the aquatic system from municipal discharges, metal finishing industries, and scrap metal works. Chromium may also be distributed to the Refuge from the Freeport, Texas area via the GIWW.

Iron, Magnesium, Manganese

These three heavy metals are discussed as a group because each is an essential element in biological processes and are also considered as macronutrients. The role of iron in hemoglobin for oxygen transport is well known. Magnesium is a known activator of many enzyme systems, and manganese is also needed for several other biological functions. Manganese is also absorbed more readily from the gastrointestinal tract than iron. Levels of iron and manganese (Table 2) were less than 50 percent of the levels reported by Beyer (1990) to represent polluted sediment from the Great Lakes harbor. Magnesium levels were also below any threshold that implies pollution (Beyer 1990, Hoffman et al. 1990, Long and Morgan 1990). There is no toxicological literature that would indicate the levels of iron, magnesium, or manganese detected in the sediments of oyster tissue in this study are at a level of concern. It is not necessary to recommend further monitoring for these metals in the estuarine portion of the Refuge.

Nickel

Environmental contamination by nickel occurs in local areas as a result of mining, smelting, combustion of fossil fuels, and industrial activities such as nickel plating and alloy manufacturing (Cain and Pafford 1981). Near the Copper Cliff smelter in Sudbury, Ontario, nickel concentrations in the Wanapitei River water averaged 42 ppb, and 826 ppm in the algal periphyton, a 2000 fold magnification (Hutchinson et al. 1975). Cain and Pafford (1981) suggested that nickel at 800 ppm in the diet of mallard ducklings caused severe paresis and tremors, and the concentration of nickel in the liver and kidney tissues would not exceed 1.0 ppm. Thus, severe contamination by nickel may go undetected if certain tissue is used as a monitoring matrix.

Nickel occurs naturally in rivers due to soil erosion and will usually be elevated in sediments that receive urban and industrial runoff. Soil concentrations of nickel average 40 ppm and river water concentrations average 0.3 ppb. Bioaccumulation of nickel in birds and mammals is low but will readily accumulate in mollusks, crustacea, and algae (Jenkins 1981). The National Status and Trends Program of the National Oceanic and Atmospheric Administration (NOAA), concluded that the potential for biological effects of nickel contamination is low in estuaries if sediments have less than 30 ppm nickel (Long and Nelson 1990). Ingersoll and Nelson (1989) classified sediments in the Great Lakes as "non polluted" if nickel residues were less than 20 ppm.

Sediment samples collected from the estuarine habitat on the refuge averaged 7.3 ppm nickel (Table 2). Nickel residues in

oysters from the same area averaged 1.7 ppm. These levels are not indicative of a contamination problem on the refuge but are more a reflection of background nickel. No further monitoring of nickel as a potential contaminant is necessary at this time.

Strontium

Strontium has many of the chemical and physical properties of calcium (Anonymous 1988) and contributes to the water quality parameter of "hardness". Pure strontium is not toxic, however several of the strontium compounds are hazardous to fish and wildlife. Several strontium compounds are used as explosives in pyrotechnics (Sax and Lewis 1987).

Strontium is poorly absorbed from food but is stored in the skeletal system of animals. There appears to be very little information on strontium effects on fish and wildlife species, ambient concentrations in water, or uptake by animals from their habitat. A sediment survey conducted along the Upper Texas Coast (U. S. Fish and Wildlife Service, unpublished data) detected strontium up to 10 times higher in sediments that receive produced water from petroleum wells than from sediments not receiving discharged production water. Sediments receiving produced water had over 800 ppm strontium.

Levels of strontium detected in the sediments from the estuarine portion of the San Bernard National Wildlife Refuge (Table 2) are not in the elevated range. No recommendation concerning strontium monitoring is proposed at this time.

Zinc

Zinc is an essential element in both plant and animal life (Keller 1988) but if residues are elevated beyond acceptable levels, signs of toxicity are seen in most life forms. Beyer et al. (1984) and Beyer et al. (1985) documented zinc toxicosis in insects as well as vertebrates in an area near a zinc smelter. The average level of zinc in U. S. soils is 300 ppm (Davies 1986), and if soil erosion is high then zinc will be elevated in the receiving water. Zinc is generally high in urban runoff because zinc oxide is used in rubber tires; the chloride form is used in dry cell batteries; and the sulfide form is used in fluorescent lamps (Keller 1988). Zinc is also used to galvanize metal.

Zinc in water acts synergistically with copper and ammonia to produce an increase in the toxic effect on fish (Schneider 1971). A review of sediment data allowed NOAA to suggest that the potential for biological effects for zinc sorbed to sediments was highest if the concentration exceeded 270 ppm and was low if zinc residues were less than 120 ppm (Long and Nelson 1990). Oysters bioconcentrate zinc up to 16,000 times

the ambient concentration in water (EPA 1980b). If zinc is present in sediments and is mobilized into the water column, oysters in the area may acquire a high concentration of zinc in their tissue.

Zinc residues in sediment samples collected from the estuarine portion of the San Bernard National Wildlife Refuge averaged only 68 ppm (Table 2). Oysters collected from the same location averaged 271 ppm zinc, a four fold increase. These levels in oysters are high relative to the 25 ppm normally detected in meal, fish and poultry (EPA 1980b), however, they are similar to the zinc detected in oysters collected from the Lower Rio Grande Valley (Gamble et al. 1988). At this time, there is no need to initiate monitoring for zinc as a contaminant on the refuge.

The third class of contaminants evaluated in this study was petroleum hydrocarbons (Table 1). Petroleum hydrocarbons are subdivided into aliphatics and polycyclic aromatics (PAH). Aliphatic compounds are carbon based chain structures, whereas PAH compounds are carbon based ringed compounds (i.e. benzene). Aliphatics are grouped into alkanes and paraffins. Small carbon chains with less than five carbon atoms are gases. Pentane (C_5) is the first liquid member of the alkanes (Sandmeyer 1981). Most of the aliphatics and paraffins are insoluble in water and toxicity to aquatic life is low at environmental levels commonly found in sediment and water samples. Published literature on biological effects of aliphatic compounds on estuarine species is lacking and prevents an assessment at this time. This survey did not detect aliphatics above the 0.01 ppm detection level in the estuarine samples, which suggests that no further monitoring for these contaminants is necessary at this time.

PAH compounds are known carcinogens to fish (Baumann et al. 1987) and are associated with fish tumors (Black 1982) and reduced scope for growth in mussels (Widdows et al. 1990). Aromatic compounds may be absorbed from the water and stored in fat tissue of oysters.

Several PAH compounds were detected in the sediment sample and the oyster sample collected in Cow Trap Lake near the GIWW (Fig. 1). There were 11 compounds detected in the sediment sample and 19 PAHs from the oyster sample (Table 3). The oyster sample was highly contaminated (8.44 ppm). The oyster sample collected from the upper reach of Cow Trap Lake (Fig. 1) had no detectable level of the 24 PAH compounds in the analytical screen. The high number of compounds detected in the sediment sample and oyster sample collected near the opening of Cow Trap Lake with the GIWW may indicate a contaminant problem from the GIWW. Oil spills, marine motors

Table 3. Aromatic hydrocarbons in estuarine samples from the San Bernard National Wildlife Refuge.

Polycyclic Aromatic Hydrocarbons	Sediment		Oysters		
	Cow Trap	Cedar Lake Creek	Cow Trap	Cow Trap	Cedar Lake Creek
Anthracene	BDL	BDL	BDL	BDL	BDL
Acenaphthene	0.02	BDL	0.06	BDL	BDL
Acenaphthylene	0.03	BDL	0.03	BDL	BDL
Benzo(a)anthracene	0.01	BDL	0.46	BDL	BDL
Benzo(a)pyrene	0.01	BDL	0.38	BDL	BDL
Benzo(b)fluoranthrene	0.01	BDL	0.79	BDL	BDL
Benzo(e)pyrene	BDL	BDL	0.30	BDL	BDL
Benzo(ghi)perylene	0.02	BDL	0.15	BDL	BDL
Benzo(j)fluoranthrene	BDL	BDL	0.31	BDL	BDL
Biphenyl	0.03	BDL	0.03	BDL	BDL
Cyrysene	0.02	BDL	0.77	BDL	BDL
Dibenzanthracene	BDL	BDL	0.05	BDL	BDL
2,6-Dimethylnaphthlene	0.02	BDL	0.03	BDL	BDL
Fluoranthrene	BDL	BDL	2.46	BDL	0.03
Fluorene	BDL	BDL	0.01	BDL	BDL
Indenopyrene	0.02	BDL	0.21	BDL	BDL
1-Methylaphthalene	BDL	BDL	BDL	BDL	BDL
2-Methylnaphthalene	BDL	BDL	BDL	BDL	BDL
1-Methylphenanthrene	BDL	BDL	0.05	BDL	BDL
Naphthalene	BDL	BDL	BDL	BDL	BDL
Perylene	BDL	BDL	0.17	BDL	BDL
Phenanthrene	0.02	BDL	0.34	BDL	BDL
Pyrene	0.03	BDL	1.84	BDL	0.06
2,3,4-Trimethylnaphthalene	BDL	BDL	BDL	BDL	BDL
Total Residue	0.23	BDL	8.44	BDL	0.09
Total Compounds	11	0	19	BDL	2

on tugboats, and a large number of small outboard motor boats which use the GIWW, all are sources of PAH compounds. The levels detected in the sediment, however, do not suggest the GIWW is a major source of contaminants, but may be a chronic source. Levels of PAH compounds in the oyster sample do suggest further monitoring should be planned for the area.

FRESHWATER HABITAT ASSESSMENT

The freshwater samples collected from Moccasin Pond and Cocklebur Slough (Fig.1) in this evaluation were sediments, aquatic invertebrates, and small fish. Only one sediment and one aquatic invertebrate sample was collected from the small pit on the Big Boggy unit of the refuge. There was no detectable organochlorine pesticide residues in any of these samples. These data suggest that no further monitoring of organochlorine pesticides is needed for the San Bernard National Wildlife Refuge. These compounds are banned from use in the United States and should not be a future contaminant problem in this area.

Most of the heavy metals listed in Table 1 were detected in the samples collected from the freshwater areas of the refuge. However, there was no elevated level of most heavy metals in the sediment or aquatic biota collected from Moccasin Pond or Cocklebur Slough (Table 4). Barium was the only metal that exceeded normal levels seen in published literature (Beyer 1990). The elevated level of barium is probably from previous drilling activity and is not at an environmentally degrading level. It does not appear that runoff from surrounding land is transporting heavy metal contamination to Moccasin Pond or Cocklebur Slough.

Four heavy metals were highly elevated in the sediment sample collected from the gas well pit on Big Boggy (Table 4). Barium and boron are elements that are used in drilling muds and are dumped in these reserve pits at the time the well is drilled. Strontium is usually present in areas that receive produced water from gas and oil production wells. Discussion earlier in this report on barium, boron, and strontium indicate that these elements are not at levels to be of concern for the refuge. Lead, however, was at a much higher level than all other samples collected in the freshwater habitat (Table 4) or in the estuarine habitat (Table 2).

Lead is a very toxic metal to aquatic organisms and all measured effects of lead on living organisms are adverse (Eisler 1988). Lead shot poisoning of waterfowl has been widely publicized and studied for many years. Lead is known to leach from municipal landfills (Lu et al. 1982) and is a common contaminant in used motor oil (Hoffman et al. 1982). The

Table 4. Heavy metals detected in freshwater areas on the San Bernard National Wildlife Refuge.

	Moccasin Pond			Cocklebur Slough		Big Boggy[2]	
	Sediment	Aquatic[1] Biota	Fish	Sediment	Aquatic Biota	Sediment	Aquatic Biota
Aluminum	8040.00	356.00	53.00	9610.00	44.50	6440.00	456.00
Antimony	<0.50	<1.00	<1.00	<5.00	<1.00	<5.00	<0.10
Barium	52.00	72.00	2.90	92.40	9.59	5900.00	134.00
Beryllium	0.30	<0.05	<0.50	0.45	<0.05	0.30	<0.05
Boron	8.00	0.95	<0.05	10.70	<0.05	19.20	3.37
Cadmium	<0.25	<0.05	<0.25	<0.05	<0.05	0.35	<0.05
Chromium	8.00	1.40	1.32	11.00	1.42	10.00	3.65
Copper	5.85	23.00	1.42	6.10	3.11	10.80	6.73
Iron	7580.00	374.00	63.70	9210.00	45.60	7760.00	307.00
Lead	7.05	<0.30	<0.30	7.50	<0.30	115.00	<0.50
Magnesium	2030.00	570.00	507.00	4180.00	618.00	2520.00	745.00
Manganese	159.00	249.00	23.60	128.00	31.70	116.00	37.20
Molybdenum	<2.50	<0.50	<0.50	<2.50	1.03	<2.50	<0.50
Nickel	5.95	2.24	0.86	8.55	0.74	6.55	1.90
Silver	<2.50	<0.50	<0.50	<2.50	<0.50	<2.50	<0.50
Strontium	15.50	71.80	17.50	24.80	46.40	193.00	58.90
Thallium	<10.00	<2.00	<2.00	<10.00	<2.00	<10.00	<2.00
Tin	<2.50	0.54	0.93	<2.50	<0.50	<2.50	0.64
Vanadium	13.40	0.70	<0.50	17.70	<0.50	13.20	0.91
Zinc	42.70	20.50	27.90	22.70	32.80	97.10	20.40

[1] A combination of several species of invertebrates.

[2] Samples from a well site pit on the Big Boggy section of the refuge complex.

source of lead in the pit is unknown at this time, but it may have been deposited there during drilling or well servicing operations in the past. Since lead was not elevated in the aquatic biota from this pit (Table 4), it is possible the lead is not in a bioavailable form and may not pose a significant contamination problem for the refuge. Once the well ceases operations the pit should be closed and the land surface restored to its original contour. This will also dilute the lead concentration; bury it below the oxidation zone in the soil; and make it even less bioavailable than it is currently.

Petroleum hydrocarbons were essentially absent from the sediment and aquatic biota collected from Moccasin Pond and Cocklebur Slough. The sediment sample from the gas well pit on Big Boggy had elevated levels of methylated naphthylenes (total concentration of 0.46 ppm wet weight) and elevated levels of aliphatic hydrocarbons (total concentration of 9.61 ppm wet weight). These levels are indicative of petroleum contamination in the sediment but not at levels that require any cleanup action. In order to remove all potential for petroleum hydrocarbon contamination of migratory birds or their food items, this pit should be drained, dried, and the soil plowed or turned to a depth of eight inches. This will allow the soil bacteria to biodegrade the remaining hydrocarbons within a two to three month period to a level that will have no residual potential to contaminate natural resources that utilize the pit.

CONCLUSIONS

The San Bernard National Wildlife Refuge is located approximately 10 miles south of a major industrial complex at Freeport, Texas, and is connected to this complex by the Gulf Intracoastal Waterway (GIWW), a canal used primarily for barge traffic. The potential for spills of oil and chemicals is high in this waterway, as is contamination from urban runoff from Freeport, Texas, and waste discharges from the industrial complex. Data from this survey does not indicate any contamination by organochlorine or organophosphate pesticides is occurring at this time on the San Bernard National Wildlife Refuge.

Two heavy metals, cadmium and chromium, had elevated residue levels in the sediment and oyster samples collected from Cow Trap Lake, the estuarine portion of the refuge. The presence of these two metals may indicate some contamination from the Freeport, Texas area is being transported south along the GIWW. These metals were not at high enough levels to evoke action at this time, but future monitoring for heavy metals is recommended to detect if a trend toward increased contamination is evident.

Petroleum hydrocarbons were also detected at elevated levels in sediment and oyster samples collected in Cow Trap Lake. These residues probably reflect the petroleum spills that occur in the GIWW as well as the minor oiling that occurs from small outboard motors on the numerous fishing boats that utilize the GIWW and Cow Trap Lake.

The freshwater portion of the refuge is apparently free from contamination. The small reserve pit at the gas well on the Big Boggy unit is contaminated with lead and petroleum hydrocarbons. The lead does not appear to be in a bioavailable form presently. This pit should, however, be drained, dried, and plowed to a depth of eight inches in order to bioremediate the hydrocarbons. This would also make the lead even less available to natural resources.

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APPENDIX I

METAL ANALYSIS

Samples were lyophilized prior to sample digestion. If necessary, the dried sample was then passed through a 2 mm plastic sieve and a split was then ground using a mortar and pestle. Percent moisture was determined using Standards Methods for the Examination of Water and Wastes, 14th ed. (Section 208A).

Digestions for ICP analysis were performed in accordance with "Procedures for Handling and Chemical Analysis of Sediment and Water Samples", US EPA/COE, Technical Report EPA/CE-81-1, May 1981. One gram aliquots of the dried samples were digested in a vigorous nitric acid-hydrogen peroxide procedure with a final aqueous matrix dilution of 100 mm after filtration. The sample results are reported in mg/kg dry weight. No extraordinary reactions or color changes were noted for the ICP digestion.

One sample was spiked and duplicated. Summaries of the ICP QC pages follow:

1. **Digestion Blanks** - Two blanks were digested with the samples. Normal contamination levels for several analytes were found in the blanks.

2. **Initial Calibration Checks** - The ICP spectrometer was calibrated properly as indicated by the percent recoveries of the elements analyzed (within ten percent windows) in the initial check solutions.

3. **Initial Interference Check** - Background correction factors for selected analytes were properly determined as indicated by percent recoveries for the interference check solutions (within twenty percent windows).

4. **Duplicate Analysis** - The duplicate precision, as indicated by the Relative Percent Differences (RPD), was acceptable (inside the 20% windows) for all elements with the exception of Al and Pb. Al is only slightly high (21%). The high Pb RPD, at 30%, is probably due to the variability normally found when concentrations are near the IDL.

5. **Spike Analysis** - Spike recoveries in the sample were within 75 to 125% for most elements. Sb, B, Ag, and Sn were all low. Low recoveries are typically seen for these elements. As a result, the sample results are probably biased low.

6. **Reference Materials** - A solid EPA laboratory control sample (0287) was used as a reference material. Recoveries

for certified analyte values which could be quantitated at a level above the reporting limit were all within +/- 25% with the exceptions of Ag. Ag recoveries are typically low with this type of digestion.

ORGANOCHLORINE AND AROCHLOR ANALYSIS

Twenty-four sediment samples were analyzed by Patuxent methods.

A subsample of each well-mixed sediment (5.0 g to 7.3 g), and sodium sulfate (heat treated at 550°C) were blended in a one-half-pint food blender. This mixture was added to a fiber extraction thimble (pre-extracted with petroleum ether). Internal standard solution from a syringe was placed on the sample in the thimble. The sample was extracted with petroleum ether (B&J distilled in glass) for at least 20 hours. The extract was concentrated to 10 mL with a Kuderna-Danish on a steam bath. During the concentration stages, the extract was never allowed to go to dryness.

The 9 mL of extract was exchanged into methylene chloride (Omnisolve distilled in glass) and brought to a 10 mL volume. A volume of extract equivalent to approximately 1 g of sample was loaded into a loop on the GPC unit (ABC model No. 1002A) and injected. The GPC unit transfers the eluted fraction containing the chlorinated organics to an autoconcentrator that concentrates during elution and exchanges the solvent to hexane for a final volume of 10 mL.

The sample was concentrated to 1 mL by nitrogen blowdown and subjected to alumina micro column cleanup. The alumina (Biorad neutral alumina AG7, 100 to 200 mesh) was ignited and then deactivated with distilled water (7% by weight). The analytes were eluted with 10 mL of 4:1 hexane/methylene chloride. The eluent was concentrated to 1 mL for GC capillary analysis.

Percent moisture was determined by placing 2 g of the homogenate into a tared aluminum pan and placed in a drying oven (105°C) for at least 48 hours. The weight was recorded after cooling in a desiccator overnight.

For organochlorine analysis, six chlorinated biphenyl congeners were added before extraction of the sample and served the following purposes:

1. Monitoring sample extract losses due to extraction efficiency, GPC cleanup, or extract transfer.

2. Estimating detection limits.

3. Increasing accuracy of predicted retention times (±0.005 min) for the analytes.

4. Providing backup internal standards in the event of sample matrix interference with the normal quantification internal standard.

Before organochlorine GC analysis, two additional internal standards were added to the sample. These were used for monitoring the instrument's health; e.g., to indicate if there were any problems with the injection of each sample.

A Hewlett-Packard 5880A GC equipped with dual capillary column/dual ECD detectors was used for the organochlorine and arochlor analysis. The analysis was a single splitless (Grob) injection onto two 30-meter columns (DB-1 and DB-1701) of different polarities. The dual column analysis, besides providing confirmation of the pesticides, checks for coelution of unknowns with each individual pesticide. Because of the high resolving power of the capillary columns, coelution by an unknown on both columns is improbable. Except as explained below, the amount and variance shown on the sample report pages was calculated from the values given by the two GC columns for each compound detected. If the variance was greater than 15% of the mean, it was assumed that coelution was occurring on the column showing the higher amount and only the lower amount was reported. In that case, a variance indicator NA (Not Applicable) was printed in the "Variance" list. Also, if near coelution occurs, where a positive identification on one of the GC columns was not possible, then only the amount given by the GC column that allows positive identification was reported. In this case, the variance indicator NA also was printed. The indicator NA also was used in the "Variance" list in cases where nothing was found above the detection limits on either column where the indicator ND was printed in the "Amount" list.

The temperature program was 50°C for two minutes to 280°C at 3°C/minute and a post-run temperature of 290°C for five minutes. Linear flow rate was at 30 cm helium/second.

Quantitation was done on the Hewlett-Packard 5880A GC. Due to the narrowness of the capillary peaks, all data were based on peak height, resulting in less biasing due to tailing, near coelution and baseline drift ("Assessment of the Results from Data Processing Systems using a Digital Chromatogram Simulator", R.J. Hunt, Journal of High Resolution Chromatography Communications, Vol. 8, July 1985, pp. 347-355). All data were collected directly from the GC into databases in an Amiga computer. The databases, besides providing report 8generation, allow the monitoring of the standard curves and internal standards over time. The data on the Amiga also was

used for pattern recognition in arochlor analysis and to develop the organochlorine pesticide "unknowns" report. Appendices A and B contain the results of the organochlorine-arochlor and "unknowns" analyses, respectively.

The batch size for soxhlet extraction was 12 (11 samples and 1 blank). Two batches went onto the GPC at a time. No analytes were detected in the blank at concentrations greater than 0.5 ppb.

No GC/MS confirmation was done since no analytes were detected.

ALKANE AND AROMATIC ANALYSIS

Sample preparation for the alkanes and aromatics was as follows. Five micrograms deuterium labeled surrogate spikes were added to 5-15 g of the sample homogenate. There were labeled analogs for each of the polyaromatic hydrocarbons to be analyzed except benzo(e)pyrene and perylene. Aqueous potassium hydroxide (4 N) was added to each of the mixtures and the sample saponified in a steam bath for two hours. The centrifuge tubes were vortex mixed every 40 minutes. The hydrolysates were acetified with hydrochloric acid, the mixture transferred to a separatory funnel and extracted three times with 25 mL methylene chloride each time. The aqueous layer was discarded. Soil and sediment samples were not hydrolyzed. The samples were mixed with sodium sulfate and soxhlet-extracted overnight with methylene chloride. The combined organic extract filtered through muffled NA_2SO_4 and rotary-evaporated to several millimeters. One hundred mL petroleum ether and 0.7 mL iso-octane was added prior to initial evaporation and the extract again reduced to several millimeters.

The alkanes and aromatics were fractionated on a column of 20 g 2.0% water-deactivated silica gel. Alkanes were eluted with 100 mL 40% methylene chloride in petroleum ether and an additional 60 mL methylene chloride. Each fraction was concentrated by rotary evaporation followed by nitrogen evaporation. The alkane fraction was evaporated to 1 mL, internal standards added and the extract transferred to a vial in preparation for GC analysis.

The aromatic fraction was concentrated to 10 mL and cleaned by gel permeation chromatography on Bio-Beads SX-3. The collected gel permeation fraction was first rotary-evaporated, then nitrogen-evaporated to 1 mL and finally shaken with aqueous sodium hydroxide. This step removed residual fatty acids. An injection internal standard was added to each extract and it was transferred to a vial in preparation for GC analysis.

Three compounds, n-undecane, n-docosane, and n-triacontane were added to each of the final alkane extracts before GC analysis to serve as quantitation internal standards.

Gas chromatography was done using a 30 M DB-5 capillary column with splitless injection on a Hewlett-Packard 5880A GC with flame ionization. The temperature program was 60°C for three minutes to 310°C at 6°/minute for alkanes and a post run temperature of 320°C for two minutes. Linear flow rate was 30 cm helium/second.

Internal standards for the polyaromatic hydrocarbons were the deuterium labeled compounds added at the saponification stage. The deuterium labeled fluorene has been found to deuterium/hydrogen exchange during base hydrolysis. Thus, D_{10} phenanthrene was used as the internal standard for fluorene.

Use of these internal standards automatically compensates for any losses during sample preparation. An injection internal standard was added to each extract before analysis on the GC/MS and was used to determine if recovery of labeled compounds were within the normal expected range.

Gas chromatography was done using a 30 M DB-5 capillary column with splitless injection on a Hewlett-Packard 5890 GC in conjunction with a Finnigan-MAT INCOS 50 mass spectrometer. The temperature program was 50°C for two minutes to 320°C at 8°/minute. The mass spectrometer scanned from 35 to 450 m/z in 0.56 seconds at 70 eV.

The target polyaromatic hydrocarbons were purchased from Supelco (Supelpreme) and mixtures of isotope labeled compounds were purchased from MSD Isotopes. Responses of the labeled compounds to 2,2'-difluorobiphenyl internal standard and of the target to the labeled compounds was used to create a polyaromatic hydrocarbon library response list. The response curves for the target polyaromatic hydrocarbons were generated from 1 to 50 ng on column and were linear in this range.

The mass spectrometer was calibrated and an on-going calibration verification standard at either 1 or 2 ng on column injected daily. Compounds were searched for and quantified with "TCA", a program available from Finnigan-MAT for the analysis of target compounds. Mass spectra were examined manually to verify identification.