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RESIDUES OF PETROLEUM HYDROCARBONS IN TISSUES OF SEA TURTLES EXPOSED TO THE IXTOC I OIL SPILL

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ABSTRACT: Sea turtles found dead when the Ixtoc I oil spill reached Texas waters were necropsied and tissues were analyzed for residues of petroleum hydrocarbons. Two of the three turtles were in poor flesh, but had no apparent oil-caused lesions. There was evidence of oil in all tissues examined and indications that the exposure had been chronic. Comparisons with results of studies done on birds indicate consumption of 50,000 ppm or more of oil in the diet. Some possible mechanisms of mortality are suggested.

INTRODUCTION

A massive blowout of the Ixtoc I oil well in Mexico's Bay of Campeche occurred on 2 June 1979. For a period of months the 10,000–15,000 barrels of oil released daily by the well drifted northward in the Gulf of Mexico. The first oiled bird found off U.S. shores was received by U.S. Fish and Wildlife Service personnel at South Padre Island, Texas on 11 August. Of the total of seven turtles (6 green turtles, *Chelonia mydas* and 1 Atlantic ridley, *Lepidochelys kempi*) collected during the spill episode, some were treated and one was released. Three turtles (2 green and 1 young ridley) were found dead in the Laguna Madre and were shipped frozen to Patuxent Wildlife Research Center for investigations to determine the cause of death. In summarizing these investigations it is intended to bring into focus the problems in determining the possible lethal effects of ingested oil. Also, the advances of our Center in detecting residues of petroleum hydrocarbons in tissues of wildlife found dead in the field are reported.

MATERIALS AND METHODS

The turtles were thawed, measured, weighed, and examined. External oil was carefully removed from the regions of incisions. Alternate sets of dissecting instruments were employed to prevent the transfer of external oil to internal organs or cross contamination between organs. Samples of kidney, liver, and pectoral muscle of the *Chelonia* were removed and placed in chemically clean jars. Selected tissues including lung, esophagus, intestine, liver, and kidney were fixed in 10% buffered neutral formalin. The tissues were submitted to a commercial laboratory (American Histolabs, Inc., Rockville, Maryland 20852, USA) for processing and staining by the hemotoxylin and eosin method for microscopic examination.

External oil from the whole young ridley was removed by ultrasonic extraction for 10 min with 200 ml of pentane followed by a similar extraction with 200 ml of 40% methylene chloride in pentane. The combined extracts were removed under a gentle stream of nitrogen and the oil residue was weighed and analyzed.

For chemical analysis, about 4 g of pooled kidneys, 11 g of whole ridley and 20 g each of two liver and two muscle specimens were digested with 15 ml KOH at 30 C for 24 hr. The hydrocarbons were extracted with 4 × 25 ml diethyl ether.

Each sample was reconcentrated into pentane and cleaned up on a Florisil Column as previously described (Gay et al., 1980). The Florisil eluate was reduced to 5 ml in a rotary evaporator, transferred to 10-ml Mills tubes, and reduced to final volume (≥0.5 ml) on a Kontes tube heater for instrumental analysis.

The samples were analyzed by gas chromatography–mass spectrometry (GC-MS) by using a Finnigan Model 3200 interfaced to a Finnigan model 6100 data system. Each sample was introduced via splitless injection onto a 30-m × 0.25-mm J & W glass capillary column coated with SP-2100. The column was held initially at room temperature for 2 min, after which the oven temperature was raised to 160 C without programming and then programmed from 160 C to 200 C at 2 C/min. Column flow rate was 8.5 ml/min of helium. Ionization was at 70 eV. Operation of the mass spectrometer in the selected ion monitoring mode was controlled by the data system. Internal standards were used for quantitation. Perdeuteromethylnaphthalene was used for aromatic compounds and perdeuterohexadecane was used for the aliphatics. The lower limits of detection were 0.04 μg for the aliphatic and aromatic hydrocarbons, corresponding to 0.01 ppm in a 4-g sample.

RESULTS AND DISCUSSION

External oil was present on all three turtles and large quantities were present on one. Even this amount of oil probably would not have prevented normal movement or have been otherwise fatal, however, and some of the external oil on the turtles may have accumulated following death.

Chelonia 10438 was a female with a 22-cm

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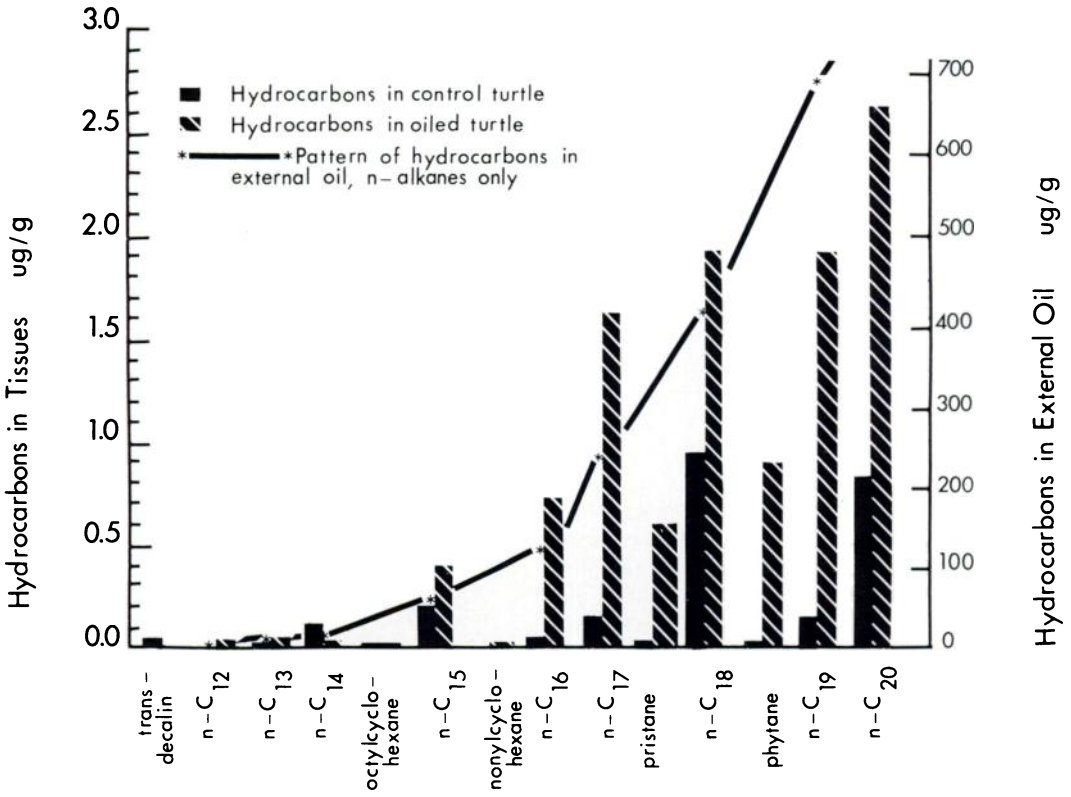

FIGURE 1. Concentrations of saturated hydrocarbons in control and oiled turtles, and in a sample of pollutant oil removed from the exterior of the oiled animal.

carapace and weighed 1,025 g. There was no mesenteric fat present; general body condition was judged to be poor. The surfaces of the oral and esophageal mucosa were brown and sticky, suggesting oil ingestion. The stomach was empty and the mucosa was sloughing due to autolysis. The terminal intestine contained about 10 cc of undigested plant material but no apparent oil. The kidneys were uniformly pale and the carcass had a uremic odor. The trachea and lungs were normal. Advanced decomposition precluded histopathological interpretation of liver and intestine. Lung and heart were histologically normal and there was no apparent oil-induced damage to the esophageal mucosa. There was mineralized debris in lumena of several renal collecting tubules suggesting mild renal dysfunction.

The *Lepidochelys* (10439) was small (carapace length 4.6 cm, 11 g) and was probably less than 1 yr old. There was oil in the mouth, and esophagus, and there was oily-appearing material in the duodenum. The liver was very pale. The trachea, lungs, and kidneys were unremarkable. Microscopic examination of the liver revealed several foci of autolysed polymorphonuclear cells with hypereosinophilic cytoplasm. Apparently autolysed aggregates of eosinophilic granulocytes were of unknown cause and pathological significance.

Chelonia 10440 was a 990-g individual of undetermined sex. There was no visceral fat present in the carcass. There was brown muddy material in the oral cavity and plant material in the stomach and intestine, but no oil was noted in the gastrointestinal tract. The trachea and lungs were normal. Microscopic examination was not done.

In summary, two of the turtles were in poor flesh and had petroleum in their upper alimentary systems; there was no evidence that the oil had caused alimentary lesions, and there was

TABLE 1. Total hydrocarbons in samples analyzed, with ratios of branch-chained hydrocarbons to corresponding normal alkanes.

Sample	Total resolved hydrocarbons (μg/g)	C_{17} pristane	C_{18} phytane
Control ridley	2.51	7.3	58.1
Oiled ridley	10.9	2.7	2.2
Chelonia kidneys	2.04	4.4	4.0
Chelonia muscle[a]	0.38	2.3	1.2
Chelonia muscle[b]	0.36	2.4	3.2
Chelonia liver[a]	0.58	1.5	2.8
Chelonia liver[b]	0.39	0.9	2.0
Pollutant oil[c]	2,932	4.0	1.8

[a] From specimen 10438.
[b] From specimen 10440.
[c] Removed from exterior of specimen 10439.

TABLE 2. Petroleum hydrocarbons in kidneys and livers of sea turtles and of ducklings fed crude oil.

Compound	Hydrocarbon residues (μg/g)			
	Livers of ducklings fed up to 50,000 ppm crude oil[a]	Livers of sea turtles found dead[b]	Kidneys of ducklings fed up to 50,000 ppm crude oil[a]	Kidneys of sea turtles found dead[c]
n-C_{12}	0.02	0.02	0.06	0.08
n-C_{13}	0.05	0.02	0.15	0.10
n-C_{14}	0.04	0.03	0.14	0.11
Octylcyclohexane	0.01	ND[d]	0.07	0.05
n-C_{15}	0.09	0.05	0.16	0.15
Nonylcyclohexane	0.01	ND	0.05	0.01
n-C_{16}	0.07	0.03	0.15	0.09
n-C_{17}	0.21	0.10	0.14	0.54
Pristane	0.93	0.08	0.22	0.12
n-C_{18}	0.09	0.05	0.09	0.29
Phytane	0.95	0.02	0.14	0.07
n-C_{19}	0.04	0.02	0.12	0.12
n-C_{20}	0.10	0.03	0.11	0.13
Naphthalene	0.02	0.02	0.02	0.09
1-methylnaphthalene	0.01	ND	0.01	0.01

[a] From data presented in graphical form by Lawler et al. (1979). Maximum levels in ducklings fed a variety of doses up to 50,000 ppm for 8 wk are given. Control ducklings averaged less than 0.01 ppm for all compounds.
[b] Mean of analyses from two *Chelonia mydas*.
[c] Kidneys from two *C. mydas* were analyzed as a single pool.
[d] ND, Not detected.

no evidence of pulmonary aspiration. Microscopic examination did not indicate the cause of death. Post mortem decomposition precluded detection of all but the most obvious of histopathological lesions and no lesions were apparent in any of the tissues except renal mineralization in 10439.

For chemical analyses it was necessary to obtain a control animal that had not been exposed to oil. We selected a young Atlantic ridley that died in hatching. Even in the unlikely event that it had been exposed to oil, its exposure would be neither as massive nor as direct as that of the turtles from the spill. Analysis of this animal revealed surprisingly high levels of hydrocarbons compared to avian tissues we had examined. High levels of these hydrocarbons may be characteristic of turtles but, more likely, they reflect the large amounts of lipids in the yolk of hatchlings. Whatever the source of the compounds, their presence is helpful in that it serves to illustrate the ways in which pollutant and naturally occurring hydrocarbons can be distinguished in tissues.

The concentrations of saturated hydrocarbons in the 10- to 20-carbon range are shown in Figure 1 for the control hatchling, the yearling ridley found dead during the spill and for the pollutant oil removed from the exterior of the exposed turtle. As indicated by the figure, the bulk of compounds in the oil had 15 carbons or more, with increasing amounts present as the 20-carbon compounds were approached. The control and oiled turtles seemed to vary randomly in the occurrence and concentrations of shorter-chain hydrocarbons. There were however, strikingly greater concentrations of longer-chain compounds in the oil-exposed animal. Of particular interest are pristane and phytane. These compounds are usually rare in living systems; their magnification in the oil-exposed animal was much greater than that of the normal alkanes. The normal alkanes were about four times as concentrated in the exposed turtles as in the control, but the more complex hydrocarbons (cyclohexanes, pristane, phytane) were 15 times control levels. Thus, the elevated levels of hydrocarbons in the oiled turtle can be concluded to be of pollutant origin.

Pristane and phytane are not only characteristic of petroleum, but once in living tissues, they tend to be more persistent than the corresponding (n-C_{17}, n-C_{18}) normal alkanes; their relative abundance is increased when oil is degraded by marine organisms (Blumer et al., 1973) and great enhancement of pristane is seen in feeding studies (Lawler et al., 1978; Lawler et al., 1979). Ratios of pristane and phytane to the normal alkanes are shown in Table 1. The great dominance of the normal alkanes in the control animal should be noted, as should the characteristic ratios in the pollutant oil. With

both n-C_{17}/pristane and n-C_{18}/phytane, all tissues examined from animals exposed to oil showed enhancement of pristane and phytane, indicating a pollutant source for the hydrocarbons detected. Further, in the case of the C_{17}/pristane ratios, all tissues except the kidneys had lower ratios than the pollutant oil, indicating selective accumulation of pristane over C_{17} alkanes. There was an apparent relationship between these ratios and the total resolved hydrocarbons; higher residues were somewhat correlated with numerically higher ratios, suggesting that tissues such as muscle and liver which store little oil can be expected to contain relatively more pristane and phytane than the tissues such as kidney where larger amounts are found. Elimination of residues and retention of more persistent components such as observed among these tissues would be consistent with the amount of metabolic processing the residues might have been expected to receive before reaching the organs in question.

It can be concluded that the three animals found dead had petroleum hydrocarbons in all tissues examined and that there was selective elimination of portions of this oil. Both presence of residues in various tissues and selective elimination indicate that exposure to the oil was chronic; the turtles evidently did not encounter the oil shortly before death, but had been exposed to it for some time.

The data of Table 2 provide for a comparison of the concentrations of selected hydrocarbons in livers and kidneys of *Chelonia mydas* and published residues (Lawler et al., 1979) in mallard ducklings dosed with up to 50,000 ppm of South Louisiana crude oil. Liver levels are greater in the ducklings than comparable levels in the turtles, but concentrations in the kidneys are more or less equal. There is not a consistent relationship between residue concentrations and dose in the ducklings analyzed by Lawler et al. (1979); intermediate dose levels often produced the highest hydrocarbon residues. As a result, it is not possible to estimate the levels of petroleum that might have been in the turtles' diets. Nevertheless, the turtles may have been consuming 50,000 ppm or more, if it can be assumed that the processes of tissue uptake and retention are similar in these two groups of animals.

Pattee and Franson (1982) fed 30,000 ppm Ixtoc I oil (wellhead sample) to American kestrels (*Falco sparverius*) for 28 days and noted no toxic signs. Birds on this dosage did, however, lose weight despite significant increases in food consumption. Prolonged exposure to oil may have caused the poor body condition observed in the turtles, perhaps disrupting feeding activity, as suggested by Gunkel and Gassman (1980). In such weakened condition, the turtles may have succumbed to some toxic component in the oil or some undiscovered agent.

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LITERATURE CITED

BLUMMER, M., M. EHRHARDT, AND J. H. JONES. 1973. The environmental fate of stranded crude oil. Deep-Sea Res. 20: 239–259.

GAY, M. L., A. A. BELISLE, AND J. F. PATTON. 1980. Quantification of petroleum-type hydrocarbons in avian tissue. J. Chromatogr. 187: 153–160.

GUNKEL, W., AND G. GASSMAN. 1980. Oil, oil dispersants and related substances in the marine environment. Helgol. Meeresunters. 33: 164–181.

LAWLER, G. C., J. P. HOLMES, B. J. FIORITO, AND J. L. LASETER. 1979. Quantification of petroleum hydrocarbons in selected tissues of male mallard ducklings chronically exposed to South Louisiana crude oil. Prof. Conf. of Assessment of Ecological Impacts of Oil Spill, C. C. Bates, Chairman, Arlington, Virginia, American Institute of Biological Sciences, 936 pp.

———, W. A. LOONG, AND J. L. LASETER. 1978. Accumulation of saturated hydrocarbons in tissues of petroleum-exposed mallard ducks. Environ. Sci. Technol. 12: 47–51.

PATTEE, O. H., AND J. C. FRANSON. 1982. Short-term effects of oil ingestion in American kestrels (*Falco sparverius*). J. Wildl. Dis. 18: 235–241.