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INTERPRETING RESIDUES OF PETROLEUM HYDROCARBONS IN WILDLIFE TISSUES

Fish and Wildlife Service
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Biological Report 88(15)
August 1988

Interpreting Residues of Petroleum Hydrocarbons in Wildlife Tissues

by

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Preface

This publication is the first of its kind in the field of evaluation of environmental contaminant effects on wildlife. Other publications have summarized the data generated by research or surveys and have attempted to do so in a way that would make the information more accessible to field biologists. As the first "how to" product in the contaminant area, this paper goes one step further in providing guidelines for the interpretation of analytical chemical results.

We are interested in getting reader reaction to this publication.

Acknowledgments

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Contents

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Introduction

Pollutant oils are frequent contaminants of wildlife habitats, and they are often ingested by wild birds and other higher vertebrates. Despite the frequency of exposure of wildlife to petroleum pollutants and a proven analytical technology (Gay et al. 1980), attempts are seldom made to detect these materials in tissues. Once ingested by animals, petroleum hydrocarbons are metabolized and tend to mix with the many similar compounds normally present in tissues. Chemical analysis of normal and contaminated tissue shows the presence of many of the same compounds and reports generated from such analyses seem to defy interpretation. A search of the literature during an investigation of an oil spill (Hall et al. 1983) revealed that meaningful conclusions can often be drawn from the seemingly incomprehensible analytical reports one receives when tissues of birds or other higher vertebrates are analyzed. Such conclusions have not often been drawn, however, because guidelines for the interpretation of residues have not been clearly stated and are not readily available. This report provides field biologists with a guide that will help them assess impacts on wildlife of oil pollution on the basis of chemical analyses.

In the late 1970's, Patuxent Wildlife Research Center scientists undertook a major effort to assess the effects of petroleum pollutants on wildlife (Stickel and Dieter 1979). Biological effects were investigated and a technology was developed to detect and quantify oil residues in tissues. It was learned that oils are often acutely toxic when applied to eggs and that ingested oil produces a variety of nonspecific, debilitating conditions in adult birds. Birds can be killed or brought to the brink of death by exposure to spilled oil, mostly from the effects of oiling of feathers. Animals consuming petroleum, however, show few characteristic signs that link their debilitation or death to oil consumption other than oil residues in tissues. Consequently, chemical analysis of tissues can be an important tool in determining if birds have consumed oil and in assessing the damage or risk of damage to wildlife consuming petroleum compounds in the environment.

Petroleum, either as crude oil or as refined products, consists of many compounds. Most analytical techniques that quantify oil in complex mixtures rely on analysis of only a small number of characteristic or important compounds. The Patuxent Analytical Control Facility (PACF), for example, routinely quantifies by gas chromatography or gas chromatography/mass spectrometry only 23 of many possible compounds. We have based our discussion on the compounds quantified by the PACF and its contractors though there are doubtless other combinations of compounds that might provide a useful basis of quantification.

Sampling and Handling

It is sometimes possible to draw conclusions on the basis of a single sample, but interpretation is easier and a better evaluation can be made if additional materials are collected.

Controls

As for most contaminant evaluations, possession of unexposed specimens from a clean area is important. Ideally, controls should be as nearly comparable to exposed animals as possible. Species, sex, age or size, time or collection, and condition should match closely to ensure that observed differences indeed result from contamination.

Pollutants

It is often helpful to analyze samples of the pollutant oils to compare with compounds found in tissues. Oil can be collected from the source and analyzed for this purpose, but the oil to which animals have actually been exposed is often altered by weathering. Weathering removes volatile compounds and reduces the abundance of those that are easily broken down by the elements or metabolized by microorganisms. Oil in or on food – aquatic vegetation, for example – can be analyzed in useful ways, as can oil removed by rinsings made of stomach contents or the external surface of an exposed animal.

Choice of Materials and Tissues

Petroleum hydrocarbons accumulate in a variety of animal tissues, including livers, kidneys, muscle, fat, and eggs. Whole carcasses should be skinned and digestive tracts removed to eliminate oil adhering to skin, hair, or feathers, as well as that present in the gut cavity. Fat and eggs normally contain more lipids than other tissues, and can be a storage site for ingested petroleum hydrocarbons. Nevertheless, the presence or absence of certain compounds, the relations of the amounts of others, and the relative concentrations of compounds in exposed and control animals are the basis for conclusions regarding contamination. Consequently, it cannot be said that any particular tissue is superior to others.

Handling

Samples should be treated the same as other materials intended for analysis of organic contaminants. Extreme care should be exercised to ensure that external oil or oil in the gut is not transferred to tissues during dissection. Samples should be placed separately in glass jars, frozen, and shipped on dry ice. If too large for jars, they should be wrapped completely in aluminum foil before being packaged in plastic – direct contact with plastics is to be avoided. Samples should be analyzed promptly. Albers et al. (1985) believed that there was a 25% loss of recoverable hydrocarbons in oil field wastewater samples stored for 6 months. Residues in tissue samples are believed to be much more stable.

Heavy oil ingestion by sea birds can result in characteristic blood changes that can be identified microscopically (Leighton et al. 1983; Leighton 1985). Therefore, when exposed animals are found alive, it might be helpful to have blood drawn for hematological work before euthanasia. Animals that have undergone rehabilitation treatment probably should not be analyzed.

Reports and Interpretation

Reports received from the analytical laboratories under contract to the PACF report analyses of 23 compounds; these reports should look much like Figures 1 and 2, which are reproductions of analytical reports issued on specimens from a Patuxent feeding study conducted some years ago. The left portion of each figure depicts a sheet reporting 14 aliphatic compounds and the right side depicts the sheet reporting 9 aromatic compounds. For each compound, the left column reports the total amount (μg) recorded in the sample analyzed, in these instances a 5-g aliquot or portion of a larger sample. The second column from the left converts this amount to a per gram basis (μg/g), effectively expressing it on a parts per million (ppm) basis. The two right columns (GC/MS and Comment) are reserved for additional confirmations of the analyses, as when analysis is repeated using a different methodology. Compounds not detected in a sample are represented by a blank reporting box or by a dash entered in the space. Occasionally, "total resolved hydrocarbons" is reported or calculated by the submitter; it consists of the sum of the amounts of all the analyzed hydrocarbons reported.

Figure 1 is the report of a control sample of two pooled mallard eggs, and Figure 2 is a pool of two eggs laid by a female mallard that consumed 25,000 ppm (2.5%) South Louisiana crude oil incorporated into the diet. Eggs tend to accumulate more petroleum hydrocarbons than some other tissues, but even so, these examples illustrate well some of the relations that permit interpretation of residues.

Presence of Aromatics

The aromatic compounds are not commonly found in clean tissues and, when they are, tend to be present in very small amounts. None were detected in the eggs of a control bird (Figure 1), but four compounds were found in the eggs from a hen fed oil (Figure 2).

Total Resolved Hydrocarbons

Less that 0.7 ppm of all analyzed hydrocarbons were found in the control tissues (Figure 1), whereas nearly 6 ppm of hydrocarbons were present in the exposed eggs (Figure 2). This nearly 10-fold difference might be greater than normally seen in field samples, but total resolved hydrocarbons can be a useful basis of comparison.

Dominance of High Molecular Weight Compounds

Low molecular weight hydrocarbons tend to be metabolized more readily than longer chain compounds, both by animals and by microorganisms in the environment. The analytic results from a mallard killed in an oil spill, probably reflect the effects of weathering on spilled oil (Figure 3). Microorganisms, evaporation and photooxidation first remove the lower molecular weight hydrocarbons. This episode is one in

Table 1. *Ratios of pristane and phytane to corresponding compounds in selected matrices.*

Sample	Ratios	
	Pristane/n-C^{17}	Phytane/n-C^{18}
(1) South Louisiana crude oil (SLCO)[a]	0.83	0.50
(2) Eggs from control mallard (Figure 1)	0.50	0.95
(3) Eggs from mallard fed SLCO (Figure 2)	3.3	6.5
(4) Fat from mallard killed by oil spill (Figure 3)	1.6	1.1

[a] From Belisle et al. 1981.

which collection and analysis of the pollutant oil would have aided evaluations.

Pristane and Phytane

These two compounds are frequently compared to the n-C17 and n-C18 compounds reported and have branched rather than straight-chain configurations. Pristane and phytane are often less abundant than the n-C17 and n-C18 compounds in tissue, but they tend to occur frequently in pollutant oils. They seem to be metabolized less readily than the straight-chain compounds and thus they tend to accumulate in tissues when exposure is chronic. Some of these relations are illustrated in Table 1. Ratios in the first three entries show relative amounts of pristane and phytane in a pollutant crude oil and in eggs of mallards from the experiment in which it was fed. A great magnification of pristane and phytane over levels in the oil (entry 1) and in control animals (entry 2) is seen in eggs of animals fed the oil for 4 months (entry 3). The mallard from the oil spill (entry 4) apparently shows some magnification of pristane and phytane, but the absence of control tissue or analysis of the pollutant makes it difficult to judge with certainty. Nevertheless, exposure was probably acute, rather than chronic, in this instance, and one would expect pristane and phytane to have accumulated less than in the 4-month feeding study. These relations are important ones for evaluating whether chronic low-level exposure to petroleum hydrocarbons has occurred. In acute exposure, external oiling is obvious and often fatal. Chronic low-level exposure to oil in food or ingestion through preening might be impossible to diagnose, however, without analytical evidence of pristane and phytane accumulation.

Dominance of Odd-numbered hydrocarbons

Hydrocarbons of recent biological origin are said to have aliphatic compounds with odd-numbers of carbons dominant (Farrington 1973; Giger et al. 1974), whereas petroleum compounds have approximately equal concentrations of compounds with odd- and even-numbered carbons. If present, this tendency has not been a strong one in the bird tissues we have examined (Figure 1); however, it might prove a useful basis for evaluating hydrocarbons in other types of biological materials.

In What Circumstances Should Analysis Be Requested?

Analysis of hydrocarbons in tissue is expensive and in some circumstances it might not yield meaningful results. For example, if exposure is acute and animals are clearly suffering from the physical effects of large amounts of oil stuck to feathers or skin, the question of whether oil is being consumed is probably moot. In general, analysis of tissues is most useful when the biologist has a clear question that demonstration of the presence of oil in tissues could answer. Such questions might include the following: Are wildlife in the vicinities of a potential source of oil pollution contaminated with petroleum hydrocarbons, relative to similar controls? Where dieoffs might have been caused by oil contamination, are the symptoms in specimens actually the result of such exposure? Is oil observed in the environment finding its way into wildlife tissues? Without well-drawn questions, productive interpretation of analytical results is unlikely. Routine screening of wildlife tissues for the presence of oil is costly and data are difficult to interpret.

How Much Oil in Tissues is Harmful to Wildlife?

Much work has been done on biological effects of oil ingestion. It indicates that adult birds tolerate oil in the diet reasonably well, but that a variety of biochemical, physiological, and other changes might occur. Studies relating these effects to residue levels in the tissues have not been performed. Even if performed, the results of such studies might not be particularly meaningful. Exposure to a large amount of oil for only a short period can produce death through external oiling, with little or no accumulation of residues. Lesser exposure over longer periods might produce significant residues without posing any immediate danger to the animal.

Nevertheless, the types of changes noted during feeding experiments (Holmes et al. 1978; Szaro et al. 1978; Leighton et al. 1983), including depression of growth, impaired avoidance behavior, liver hypertrophy, splenic atrophy, kidney degeneration, hyperphagia, biochemical lesions, hemolytic anemia, and depressed egg production (Coon and Dieter 1981), are the kinds of effects that could have serious consequences in a free-living population. Holmes et al. (1978) noted low mortality in ducks fed crude oil except when the nonspecific stress caused by oil contamination was combined with the stresses posed by simultaneous adaptation to sea water and cold. Because most animals in nature will be subjected to the stresses of reproduction, migration, and overwintering, to mention a few, it is reasonable to conclude that any decline in physiological fitness could result in corresponding effects on populations. It is likely that any animal with demonstrated petroleum hydrocarbon residues in the tissues has suffered effects of the pollutant, but studies conducted to date have not related such sublethal effects to the status of wildlife populations.

Petroleum is a natural product, and it occurs in the environment in some places independent of human activity. In such areas, local populations of wildlife might be expected to have adapted to chronic exposure.

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ENVIRONMENTAL RESIDUE CHEMISTRY - ANALYSIS DATA

PR# 1622 Date Completed 3-12-82 Yr. Collected 1978 Init. AB, DS

Sample # 82-0-19 Sample Group # 19-37 Submitter's # C-26A (1+2)

Specimen and Source 2 pooled mallard eggs; from mallards on control diet

GC Column & Special Instructions Long Limit of Sens. 0.02 ppm (0.4 mg)

Whole Wet Weight ______ g. Aliquot 5.0 g. Lipid ______ g.

GPC or Florisil Aliquot, ml ratio P/W 1/1 ______ g.

Silicic Acid Aliquot, ml ratio P/W 1/1 ______ g.

Compund	ug	ug/g	GC/MS	Comment
trans-Decalin	0.90	0.18		
n-C_{12}	0.25	0.050		
n-C_{13}	0.28	0.056		
n-C_{14}	0.25	0.050		
Octylcyclohexane	—	—		
n-C_{15}	0.24	0.048		
Nonylcyclohexane	—	—		
n-C_{16}	0.15	0.030		
n-C_{17}	0.30	0.060		
Pristane	0.15	0.030		
n-C_{18}	0.20	0.040		
Phytane	0.19	0.038		
n-C_{19}	0.22	0.044		
n-C_{20}	0.20	0.040		

ENVIRONMENTAL RESIDUE CHEMISTRY - ANALYSIS DATA

PR# 1622 Date Completed 3-12-82 Yr. Collected 1978 Init. D

Sample # 82-0-19 Sample Group # 19-37 Submitter's # C-2

Specimen and Source 2 pooled mallard eggs - controls

GC Column & Special Instructions 30 m OV-101 (J & W); saponification

Whole Wet Weight ______ g. Aliquot 5.0 g g. Lipid ______ g.

GPC or Florisil Aliquot, ml ratio P/W 1/1 ______ g.

Silicic Acid Aliquot, ml ratio P/W 1/1 ______ g.

Compund	ug	ug/g	GC/MS	Comment
Tetralin	—			
Naphthalene	—			
1-Methylnaphthalene	—			
2-Ethylnaphthalene	—			
1-5-Dimethyl-naphthalene	—			
4-Phenyltoluene	—			
1,3,5-Trimethyl-naphthalene	—			
Fluorene	—			
Phenanthrene	—			

Figure 1. *Analytical report on hydrocarbons in pooled mallard eggs from unexposed (control) ducks in a feeding study.*

ENVIRONMENTAL RESIDUE CHEMISTRY - ANALYSIS DATA

PR# 1622 Date Completed 3-12-82 Yr. Collected 1978 Init. DS, AB

Sample # 82-0-20 Sample Group # 19-37 Submitter's # H-3 (1+2)

Specimen and Source 2 pooled mallard eggs; 25,000 ppm S.L.C. diet

GC Column & Special Instructions OV-101

Whole Wet Weight ______ g. Aliquot 5.0 g. Lipid ______ g.

GPC or Florisil Aliquot, ml ratio P/W 1/1 ______ g.

Silicic Acid Aliquot, ml ratio P/W 1/1 ______ g.

Compund	ug	ug/g	GC/MS	Comment
trans-Decalin	0.28	0.056		
n-C_{12}	0.94	0.19		
n-C_{13}	1.4	0.28		
n-C_{14}	1.4	0.28		
Octylcyclohexane	0.43	0.086		
n-C_{15}	1.1	0.22		
Nonylcyclohexane	1.5	0.30		
n-C_{16}	1.0	0.20		
n-C_{17}	1.8	0.36		
Pristane	6.0	1.2		
n-C_{18}	1.0	0.20		
Phytane	6.3	1.3		
n-C_{19}	0.65	0.13		
n-C_{20}	2.8	0.56		

ENVIRONMENTAL RESIDUE CHEMISTRY - ANALYSIS DATA

PR# 1622 Date Completed 3-12-82 Yr. Collected 1978 Init. DS/AB

Sample # 82-0-20 Sample Group # 19-37 Submitter's # H-3 (1+2)

Specimen and Source 2 pooled mallard eggs, 25,000 ppm S.L.C. diet

GC Column & Special Instructions ______

Whole Wet Weight ______ g. Aliquot 5.0 g. Lipid ______ g.

GPC or Florisil Aliquot, ml ratio P/W 1/1 ______ g.

Silicic Acid Aliquot, ml ratio P/W 1/1 ______ g.

Compund	ug	ug/g	GC/MS	Comment
Tetralin				
Naphthalene	0.96	0.19		
1-Methylnaphthalene	0.59	0.12		
2-Ethylnaphthalene				
1-3-Dimethyl-naphthalene	0.31	0.062		
4-Phenyltoluene				
1,3,5-Trimethyl-naphthalene				
Fluorene	0.27	0.054		
Phenanthrene				

Figure 2. *Analytical report on hydrocarbons in pooled mallard eggs from ducks fed 25,000 parts per million South Louisiana crude oil.*

ENVIRONMENTAL RESIDUE CHEMISTRY - ANALYSIS DATA

PR# 1952 Date Completed 12-28-81 Yr. Collected 1980 Init. MG/AB

Sample # 81-0-271 Sample Group # 270-274 Submitter's # 10646F

Specimen and Source Fat from mallard, Wyoming oil spill

GC Column & Special Instructions OV-101, 30m coln

Whole Wet Weight 0.98 g. Aliquot 0.98 g. Lipid ______ g.

GPC or Florisil Aliquot, ml ratio P/W 1/1 0.98 g.

Silicic Acid Aliquot, ml ratio P/W 1/1 0.98 g.

Compund	ug	ug/g	GC/MS	Comment
trans-Decalin	—			
n-C_{12}	—			
n-C_{13}	—			
n-C_{14}	—			
Octylcyclohexane	—			
n-C_{15}	0.77	0.79		
Nonylcyclohexane	—			
n-C_{16}	0.90	0.92		
n-C_{17}	3.5	3.6		
Pristane	5.7	5.8		
n-C_{18}	5.7	5.8		
Phytane	6.4	6.5		
n-C_{19}	8.0	8.2		
n-C_{20}	6.5	6.6		

ENVIRONMENTAL RESIDUE CHEMISTRY - ANALYSIS DATA

PR# 1952 Date Completed 12-28-81 Yr. Collected 1980 Init. MG/AB

Sample # 81-0-271 Sample Group # 270-274 Submitter's # 10646F

Specimen and Source Fat from mallard, Wyoming oil spill

GC Column & Special Instructions OV-101, 30m column

Whole Wet Weight 0.98 g. Aliquot 0.98 g. Lipid ______ g.

GPC or Florisil Aliquot, ml ratio P/W 1/1 0.98 g.

Silicic Acid Aliquot, ml ratio P/W 1/1 0.98 g.

Compund	ug	ug/g	GC/MS	Comment
Tetralin	—			
Naphthalene	—			
1-Methylnaphthalene	—			
2-Ethylnaphthalene	—			
1-5-Dimethyl-naphthalene	—			
4-Phenyltoluene	—			
1,3,5-Trimethyl-naphthalene	0.55	0.56		
Fluorene	—			
Phenanthrene	0.61	0.62		

Figure 3. *Analytical report on hydrocarbons in the fat of a mallard killed in a Wyoming oil spill.*

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15. Supplementary Notes

16. Abstract (Limit: 200 words)

This is the first publication in the field of environmental contaminant to tell the reader how to interpret the results of analytical chemical results. Specifically, this publication describes how to interpret residues of petroleum hydrocarbons in wildlife tissues. Pollutant oil residues in avian species are emphasized.

17. Document Analysis a. Descriptors

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