

POLYCYCLIC AROMATIC HYDROCARBONS IN THE AQUATIC ENVIRONMENT

Sources, Fates and Biological Effects

JERRY M. NEFF, Ph.D.

*Department of Biology, Texas A & M University,
College Station, Texas 77843, USA*

APPLIED SCIENCE PUBLISHERS LTD
LONDON

Acknowledgements

The preparation of this review was supported in part by a contract from the American Petroleum Institute, Washington, D.C. I should like to express my appreciation to Margaret Weirich, Lawrence A. Reitsema, and William J. Rue, Jr., for their assistance in the literature search and to Becky Schult, Kay Barker, and Gail Snook for their assistance in typing the manuscript. Michelle M. Michot provided invaluable assistance in preparing the figures and in checking the manuscript. I should also like to express my appreciation to Dr Geraldine V. Cox, Dr L. W. Pollock, Dr Winston K. Robbins, and Dr Fred T. Weiss for reviewing a preliminary draft of the review and for offering many valuable suggestions for its improvement.

Preface

It has been known for many years that some polycyclic aromatic hydrocarbons (PAH) can cause cancer in laboratory mammals. In addition, the incidence of certain types of human cancer has been correlated with occupational or other exposure to these compounds. Concern over the hazards to human and animal health from exposure to carcinogenic PAH has led to an accumulation over the last twenty years of a very large body of scientific literature dealing with the sources, distribution in the environment, and biological effects of PAH. With improvements in chemical methods for analysis of complex PAH assemblages in environmental samples has come the realization that PAH are nearly ubiquitous trace contaminants of marine and freshwater ecosystems. Considerable discussion has arisen in recent years about the possible sources of these aquatic PAH, their effects on aquatic organisms, and the possible health hazards they pose to man. In this volume, I have attempted to critically review and bring together the available scientific literature on the subject with the objective of drawing some general conclusions about the relative rates of entry of PAH from different sources into the aquatic environment, the rates and pathways of degradation of aquatic PAH by abiotic and biotic processes, the biological effects, including cancer, of PAH on aquatic organisms, and the potential health hazards to man of aquatic PAH. The major emphasis is on the biological aspects of the aquatic PAH problem.

It is my sincere hope that this review will be of use to scientists, technologists, and students in several environmental disciplines because it not only provides a broad summary and synthesis of our current state of knowledge of the aquatic PAH problem but also reveals important areas where information is scanty or completely lacking. It is hoped that this review will encourage environmental scientists to conduct the research necessary to fill in these gaps in our knowledge.

Information on the sources and distribution of PAH in the aquatic environment has come from many countries. Russian scientists have provided much of the available data on PAH concentrations in industrial wastewaters, while information on emissions of airborne PAH from industrial, domestic, and internal combustion engine sources has come mainly from the United States and Canada. British and German scientists have published much of the data on PAH in fresh waters, while much of the research on PAH in marine sediments and organisms has been conducted by French, American, and Canadian scientists. To gain a better understanding of the cycle of PAH in the environment, a single study should be conducted using the most modern analytical techniques in which selected PAH from a single point source are monitored simultaneously in the wastewater, gaseous, and solid effluents from the source, and in the air, water, and aquatic biota at different distances downstream from the source.

The question of the possible biosynthesis of PAH by bacteria, fungi and higher plants has not been adequately resolved. Much of the research in this area was performed before the advent of modern high-resolution techniques for PAH analysis and without full realization of the magnitude of the problem of sample contamination from exogenous PAH sources. Carefully designed experiments utilizing modern analytical methodologies could perhaps resolve this important question.

Much of our concern about environmental PAH stems from the knowledge that a few of them are carcinogenic. However, the vast majority of PAH detected in the environment have not been demonstrated to be carcinogenic or mutagenic to laboratory mammals or aquatic organisms. Relatively little is known about the toxicity to and sublethal biological effects on aquatic organisms of those PAH which are not known carcinogens. More research is needed on the carcinogenicity of known or suspect PAH carcinogens and on the chronic toxicity of non-carcinogenic PAH to aquatic organisms.

Although the scientific literature on the metabolism of PAH by aquatic microorganisms and animals is growing rapidly, more information is needed on the chemical identity of the PAH metabolites (are they the same as or different from those produced by well-characterized mammalian systems?) and on the influence of physical environmental variables such as temperature, salinity, and dissolved oxygen on the rate of uptake, metabolism, and release of PAH by aquatic organisms.

Finally, there are several other important topics which warrant further careful investigation. These include: rates of photochemical oxidation of PAH in the water column and surface sediments under different conditions

of salinity, temperature, and oxygen concentration; bioavailability of sediment-adsorbed and food-adsorbed PAH to aquatic organisms; biomagnification of PAH in aquatic food chains; induction of cancer in aquatic organisms by exposure to realistic levels of PAH in the water, sediment and food; and induction of cancer in laboratory mammals by consumption of PAH-contaminated water and aquatic foods.

January, 1979

JERRY M. NEFF
College Station, Texas

APPLIED SCIENCE PUBLISHERS LTD
RIPPLE ROAD, BARKING, ESSEX, ENGLAND

British Library Cataloguing in Publication Data

Neff, Jerry M

Polycyclic aromatic hydrocarbons in the aquatic environment.

1. Aquatic ecology
2. Water—Pollution
3. Hydrocarbons—Environmental aspects
4. Aromatic compounds—Environmental aspects
5. Polycyclic compounds—Environmental aspects

I. Title

574.5'263

QH541.5.W3

ISBN 0-85334-832-4

WITH 89 TABLES AND 30 ILLUSTRATIONS

© APPLIED SCIENCE PUBLISHERS LTD 1979

All rights reserved. No part of this publication may be reproduced, stored in a retrieval system, or transmitted in any form or by any means, electronic, mechanical, photocopying, recording, or otherwise, without the prior written permission of the publishers, Applied Science Publishers Ltd, Ripple Road, Barking, Essex, England

Printed in Great Britain by Galliard (Printers) Ltd, Great Yarmouth

Contents

<i>Acknowledgements</i>	v
<i>Preface</i>	vii
1 INTRODUCTION	1
2 SOURCES OF PAH IN THE AQUATIC ENVIRONMENT	7
2.1 Biosynthesis of PAH	8
2.1.1 Indirect Biosynthesis	9
2.1.2 Complete Biosynthesis by Bacteria	12
2.1.3 Complete Biosynthesis by Plants	16
2.2 PAH in Fossil Fuels	22
2.2.1 PAH in Coal	22
2.2.2 PAH in Petroleum	24
2.3 Formation of PAH by Pyrolysis of Organic Matter	29
2.3.1 Sources of Pyrolytic PAH	33
3 RATES AND ROUTES OF PAH ENTRY INTO THE AQUATIC ENVIRONMENT	44
3.1 PAH from Biosynthesis	44
3.2 PAH from Fossil Fuels	45
3.3 PAH from Industrial and Domestic Wastewater	46
3.4 PAH from Runoff from Land	50
3.5 Atmospheric Fallout of PAH	50
3.5.1 Photooxidation of Airborne Particulate PAH	53
4 DISTRIBUTION OF PAH IN THE AQUATIC ENVIRONMENT	61
4.1 PAH in water	63
4.1.1 Solubility of PAH	63
4.1.2 Solubilization of PAH	65
4.1.3 Particulate PAH	65
4.1.4 PAH Concentrations in Natural Waters	66
4.2 PAH in Sediments	71
4.2.1 Sedimentation of PAH	71
4.2.2 PAH Concentrations in Sediments	73
4.2.3 PAH Composition in Sediments	81

4.3 PAH in Aquatic Organisms	85
4.3.1 Benzo[<i>a</i>]pyrene	86
4.3.2 Other PAH	98
5 DEGRADATION OF PAH IN THE AQUATIC ENVIRONMENT	102
5.1 Photooxidation of PAH	102
5.2 Chemical Oxidation of PAH in Water	108
5.3 Metabolic Degradation of PAH by Aquatic Bacteria and Fungi	115
5.4 Biotransformation of PAH by Aquatic Animals	122
5.4.1 Hepatic Microsomal MFO Activity	122
5.4.2 Tissue Distribution of MFO Activity	137
5.4.3 Effect of Endogenous and Exogenous Factors on MFO Activity	139
5.4.4 Induction of MFO Activity	142
5.5 Interaction of Degradative Processes in the Removal of PAH from the Aquatic Environment	149
6 ACCUMULATION AND RELEASE OF PAH BY AQUATIC ORGANISMS	152
6.1 Accumulation and Release of PAH from Water	152
6.2 Accumulation of PAH from Food	164
6.3 Accumulation of PAH from Petroleum	169
6.3.1 Distribution of PAH in Tissues of Aquatic Animals	180
6.3.2 Effect of Dissolved Organic Matter on Uptake of PAH	183
6.4 Accumulation of PAH from Sediment	184
6.5 Effects of Endogenous and Exogenous Factors on the Accumulation and Release of PAH by Aquatic Organisms	189
6.5.1 Endogenous Factors	189
6.5.2 Exogenous Factors	190
7 BIOLOGICAL EFFECTS OF PAH IN THE AQUATIC ENVIRONMENT	196
7.1 Mechanisms of PAH Toxicity	196
7.2 The Acute Toxicity of PAH to Aquatic Animals	198
7.3 Sublethal Effects of PAH in Aquatic Organisms	205
7.3.1 Effects of PAH on Bacteria	205
7.3.2 Effects of PAH on Aquatic Plants	206
7.3.3 Effects of PAH on Aquatic Animals	208
7.4 PAH-induced Cancer in Aquatic Organisms	215
7.4.1 Laboratory Studies	217
7.4.2 Field Studies	221
7.4.3 Aquatic PAH and Human Cancer Risk	223
8 SUMMARY—CYCLE OF PAH IN THE AQUATIC ENVIRONMENT	226
BIBLIOGRAPHY	229
GLOSSARY OF ABBREVIATIONS	256
<i>Index</i>	259

CHAPTER 1

Introduction

There has been a growing concern in recent years regarding possible harmful effects to man and other living organisms of pollutant organic chemicals released into the environment as a consequence of man's activities. Polycyclic aromatic hydrocarbons (PAH) are one of the more significant classes of pollutant chemicals which give rise to this concern. This concern arises primarily from the fact that a small fraction of the PAH generated and released to the environment by human activities has been shown to be carcinogenic to mammals. The environmental impact of the majority of PAH which are not carcinogenic is largely unknown. The purpose of this review is to summarize and synthesize recent data on the sources, fates, and effects of PAH in the aquatic environment with special emphasis on the biological aspects of this problem.

PAH are composed of two or more fused aromatic (benzene) rings. Two aromatic rings are said to be fused when a pair of carbon atoms is shared. The resulting structure is a molecule with all carbon and hydrogen atoms lying in a single plane. Naphthalene ($C_{10}H_8$), which consists of two fused aromatic rings, is the lowest molecular weight member. The ultimate fused-ring aromatic system is graphite, an allotropic form of elemental carbon. Of primary environmental concern are mobile compounds ranging in molecular weight from naphthalene ($C_{10}H_8$, MW 128.16) to coronene ($C_{24}H_{12}$, MW 300.36). Within this range is an extremely large number of PAH differing in the number and positions of aromatic rings and in the number, chemistry, and position of substituents on the basic ring system.

Physical and chemical characteristics of PAH vary in a more or less regular fashion with molecular weight. Resistance to oxidation and reduction tends to decrease with increasing molecular weight. Vapor pressure and aqueous solubility decrease almost logarithmically with increasing molecular weight. As a consequence of these differences, PAH of

different molecular weight vary substantially in their behavior and distribution in the environment and their effects on biological systems. Two molecular weight classes of PAH can be distinguished on the basis of physical, chemical, and biological properties. These are the lower molecular weight 2–3 ring aromatics (naphthalenes, fluorenes, phenanthrenes, and anthracenes) and the higher molecular weight 4–7 ring aromatics (chrysene–coronene). The low molecular weight PAH have significant acute toxicity to aquatic organisms, whereas the high molecular weight PAH do not. On the other hand, all of the 20–30 proven PAH carcinogens are in the high molecular weight PAH group.

Most methods designed for the collection, extraction, and analysis of PAH in environmental samples do not efficiently handle the low molecular weight PAH. Thus, the majority of published values for 'total PAH' in environmental samples do not include the low molecular weight PAH.

Several systems of nomenclature have been used to describe PAH ring structures. Nomenclature used in this review is that adopted by the International Union of Pure and Applied Chemistry (IUPAC) and described in detail in *The Ring Index* (Patterson *et al.*, 1960). The most important rules used in this system are the following:

1. The structural diagram is written to present the greatest possible number of rings in a horizontal row.
2. Horizontal and vertical axes are drawn through the center of the horizontal row and the molecule is oriented in such a way as to place the maximal number of rings in the upper right quadrant and the minimal number of rings in the lower left quadrant.
3. Carbon atoms are numbered in a clockwise direction starting with the carbon atom that is not part of another ring and is in the most counterclockwise position of the uppermost ring farthest to the right; carbon atoms common to two or more rings are not numbered.
4. Ring faces (except those common to two rings) are lettered in alphabetical order with the side between carbon atoms 1 and 2 designated 'a'. Alphabetical order is continued clockwise around the molecule.
5. In naming a compound formed by the addition of a component, numbers and letters are enclosed in square brackets and placed immediately after the name of the added component to describe where a substituent group is attached or where a ring is fused to the face of the molecule. If a ring is fused to more than one face of the

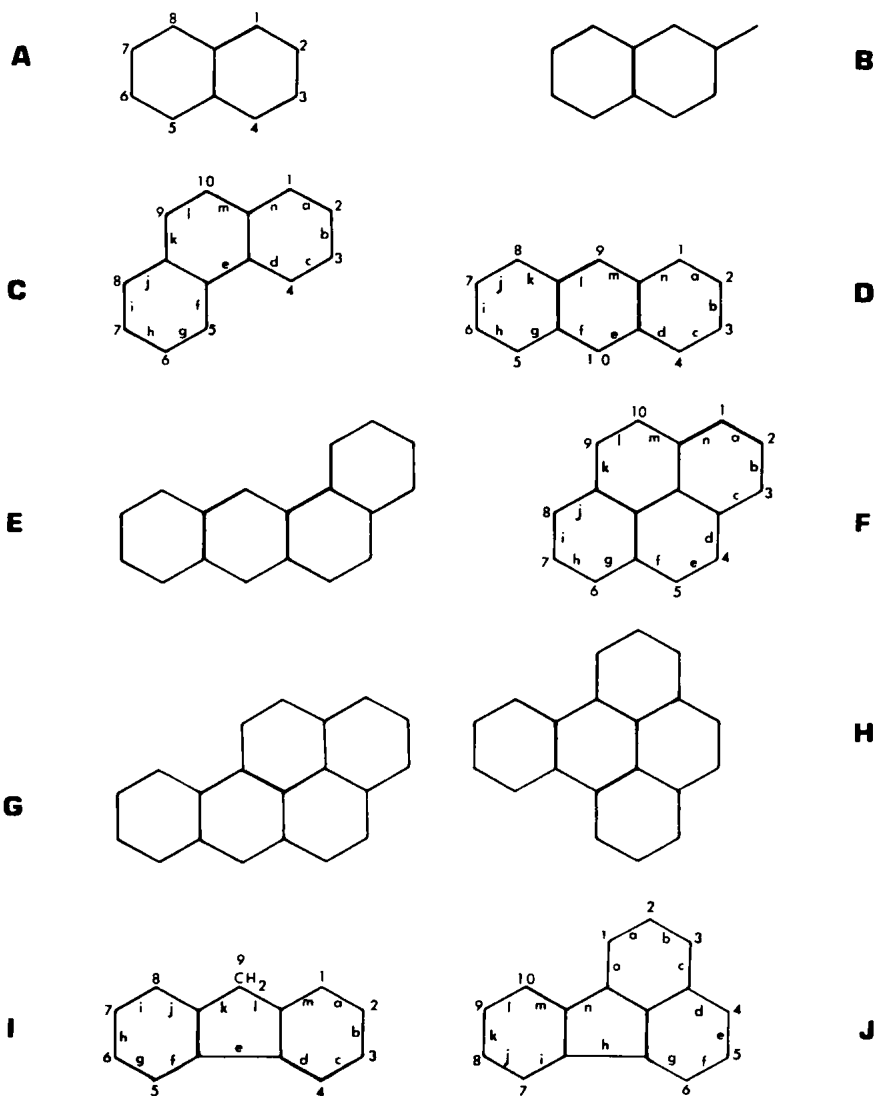


FIG. 1. Ring structures of representative polycyclic aromatic hydrocarbons. The numbering and lettering system for several PAH is also given. A, naphthalene; B, 2-methylnaphthalene; C, phenanthrene; D, anthracene; E, benz[a]anthracene; F, pyrene; G, benzo[a]pyrene; H, benzo[e]pyrene; I, fluorene; J, fluoranthene.

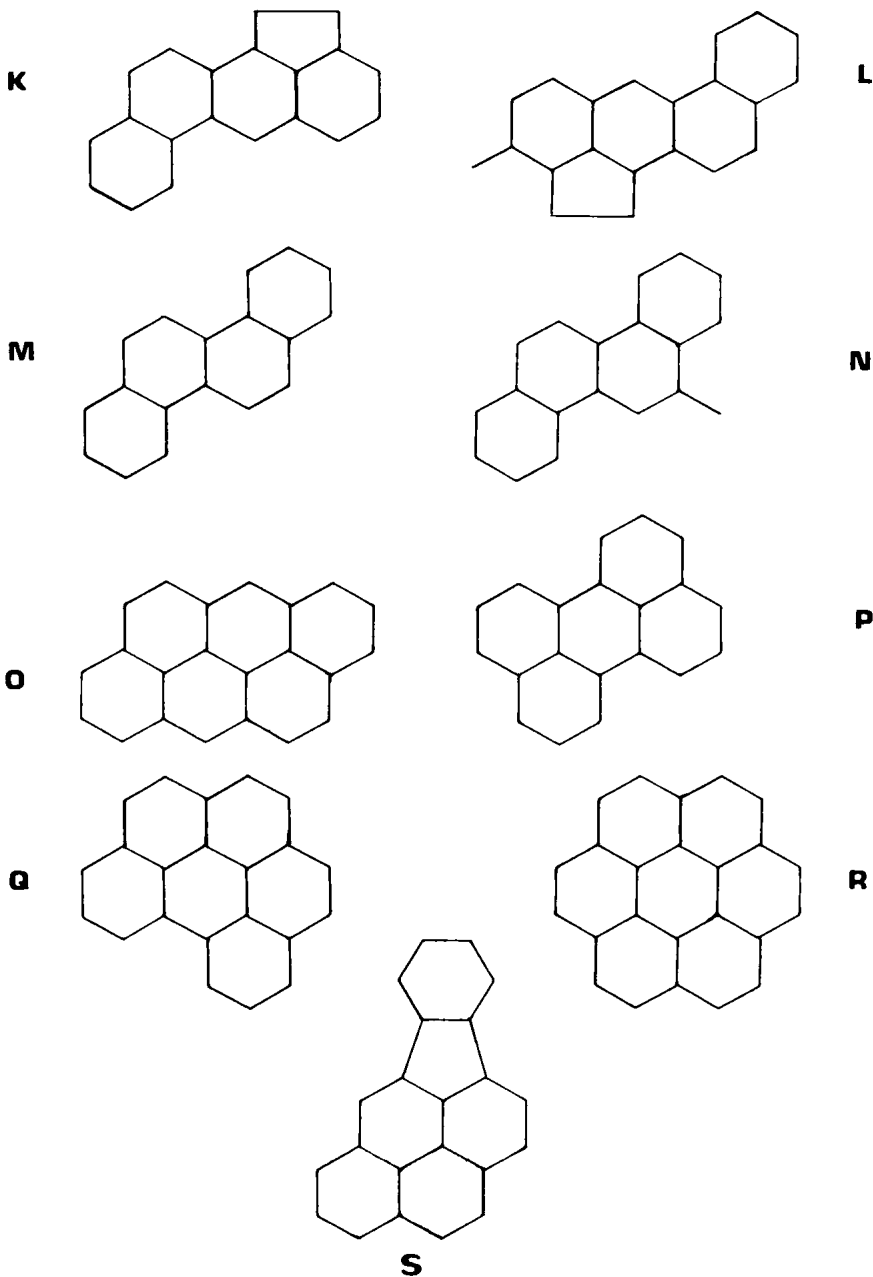


FIG. 1—*contd.* K, benz[*j*]aceanthrylene = cholanthrene; L, 3-methylcholanthrene; M, chrysene; N, 5-methylchrysene; O, dibenzo[*cd,jk*]pyrene = anthranthrene; P, perylene; Q, benzo[*ghi*]perylene; R, coronene; S, indeno[1,2,3-*cd*]pyrene.

molecule simultaneously, this is indicated by using the appropriate letters to denote the faces so involved.

- The structural formulas used show aromatic rings as plain hexagons and a methylene group as CH_2 .

Nomenclature for a few PAH, such as phenanthrene and anthracene, differs from these rules. Structural formulas of several PAH typical of those found in environmental samples are shown in Fig. 1. Examples of the numbering system for carbon atoms and lettering systems for ring faces are also included.

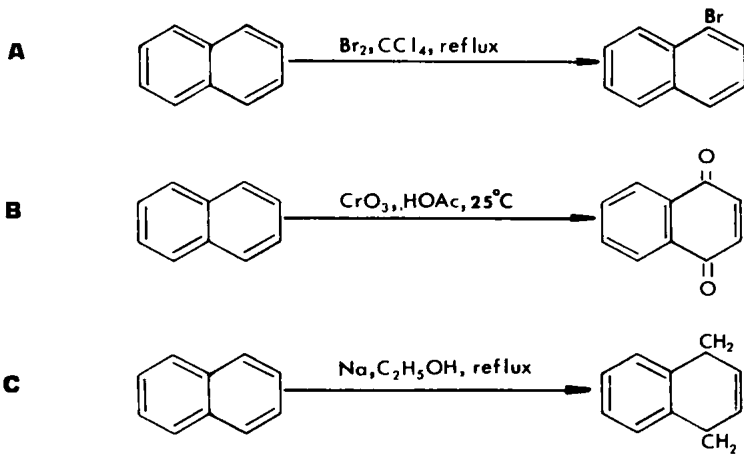


FIG. 2. Typical reactions of PAH. A, Electrophilic substitution; B, oxidation; C, reduction.

PAH undergo three types of chemical reactions characteristic of aromatic hydrocarbons—electrophilic substitution, oxidation, and reduction. Examples of these reactions are shown in Fig. 2. Oxidation and reduction reactions destroy the aromatic character of the affected benzene ring but electrophilic substitution does not.

The presence of PAH in water, aquatic sediments, and organisms has been recognized for more than 20 years. With the development of sensitive high resolution techniques for the analysis of PAH in environmental samples, it has become apparent that extremely complex mixtures of PAH, including the carcinogenic forms such as benzo[*a*]pyrene, are nearly ubiquitous trace contaminants of aquatic ecosystems. A considerable amount of research has been conducted in recent years on the sources, fates, and biological effects of these PAH mixtures in aquatic ecosystems. Much

of the earlier literature has been reviewed by Andelman and Suess (1970) and Andelman and Snodgrass (1972). Special aspects of the problem relating to drinking water were reviewed by Harrison *et al.* (1975). Much of the recent literature up to May 1975 on the environmental fate of PAH was reviewed by Radding *et al.* (1976).

The data presented in the above reviews and in this review on the sources and distribution of PAH in the environment were collected over a period of about 20 years. Sample collection, extraction, and analysis techniques have varied widely (Harrison *et al.*, 1975) and the analytical results obtained by different techniques are often not strictly comparable. Earlier investigators often were not fully aware of the extreme compositional complexity of environmental PAH assemblages. Thus, estimates of total PAH based on the quantitative analysis of a relatively few PAH in a sample (often only 6–12 individual PAH in earlier studies) gave low and variable results. In addition, the extreme difficulty in isolating a really pure PAH fraction from environmental samples, particularly those with a complex organic matrix such as aquatic sediments and biological tissues, has not been appreciated fully by some investigators. This could result in high and variable estimates of total or individual PAH. Giger and Blumer (1974) have reviewed some of the difficulties in characterizing the PAH composition and concentration of complex environmental samples. Much of the data discussed in the sections which follow on the sources and distribution of PAH in the environment should be viewed as semiquantitative estimates of PAH distributions in nature.

CHAPTER 2

Sources of PAH in the Aquatic Environment

Until relatively recently it was generally considered that PAH were formed only during the high temperature (e.g., 700°C) pyrolysis of organic materials. The discovery of complex mixtures of PAH spanning a wide molecular weight range in fossil fuels such as coal and crude petroleum has led to the conclusion that, given sufficient time, pyrolysis of organic materials at temperatures as low as 100–150°C can lead to the production of PAH (Blumer, 1976). In addition, there has been considerable speculation in recent years, and some experimental evidence in its favor, that PAH are synthesized by bacteria and plants. Thus, PAH may be formed in three ways: high temperature pyrolysis of organic materials, low to moderate temperature diagenesis of sedimentary organic material to form fossil fuels, and direct biosynthesis by microbes and plants.

Although PAH are produced by natural processes, a wide variety of human activities increases the environmental load of these substances. Industrial activities resulting in the production of PAH include: preparation of acetylene from natural gas; pyrolysis of kerosene to form benzene, toluene and other organic solvents; pyrolysis of wood to form charcoal, wood tars and carbon blacks; manufacture of electrolytic aluminium using graphite electrodes; coke production; gas production from petroleum; coal gasification; production of synthetic alcohol; and oil refinery operations (Andelman and Snodgrass, 1972). Incineration of industrial and domestic wastes, forest and grass fires, power generation from fossil fuels, and the combustion of fuels in internal combustion engines also produce emissions rich in PAH. These anthropogenic PAH may reach the aquatic environment in industrial and domestic sewage effluents, surface runoff from land, deposition of airborne particulates, and spillage of petroleum and petroleum products into water bodies. Cycling of PAH in aquatic environments is very poorly understood.

In the sections that follow, the available scientific literature on the possible biosynthesis of PAH, PAH in fossil fuels and related materials, and high temperature pyrolytic sources of PAH will be briefly reviewed.

2.1 BIOSYNTHESIS OF PAH

The extensive studies of Professor Borneff and associates in the German Federal Republic (GFR) and Professor Mallet and associates in France during the 1960s led to the conclusion that PAH were nearly ubiquitous microcomponents of freshwater, marine, and terrestrial environments, even in regions far removed from industrial activities. PAH, including the carcinogen benzo[*a*]pyrene, were detected in the sediments, water, and marine biota from the west coast of Greenland (Mallet *et al.*, 1963) and the Clipperton Lagoon in the eastern equatorial Pacific Ocean (Niaussat and Auger, 1970). In the past 15 years, a large number of publications have appeared providing circumstantial or direct evidence both for and against the biosynthesis of PAH.

It is well known that a wide variety of organic molecules containing fused-ring polyaromatic systems are synthesized by organisms, particularly bacteria, fungi, and higher plants (Gerarde and Gerarde, 1962). Many of these compounds are not true PAH since they contain oxygen, nitrogen, or sulfur substituents. Examples of these biogenic polycyclic compounds are shown in Fig. 3. Numerous reports, especially in older chemical literature, concern the isolation of PAH from plant material. However, many of these findings are questionable in that isolation of the PAH-containing oils and tars was accomplished by methods that might lead to the pyrolytic formation of PAH from suitable precursors (Guddal, 1959). For instance, Jonsson (1968) isolated and identified a group of alkylphenanthrenes from pine wood tar. The tar contained about 20 per cent retene, 1-methyl-7-isopropylphenanthrene. Small amounts of 1-methylphenanthrene, 2-ethylphenanthrene, 1,7-dimethylphenanthrene, 1-methyl-7-ethylphenanthrene, 9,10-dihydroretene and possibly 1,3-dimethyl-7-ethylphenanthrene, and 1,3-dimethyl-7-isopropylphenanthrene were isolated from the crude retene. Although Jonsson considered this phenanthrene assemblage natural, wood tars are produced by destructive distillation of wood under conditions that would favor PAH pyrosynthesis (Youngblood and Blumer, 1975). The high concentration of this unusual assemblage of PAH suggests instead a pyrosynthesis from natural products related to phenanthrene which are common in plant materials (Thomson, 1965).

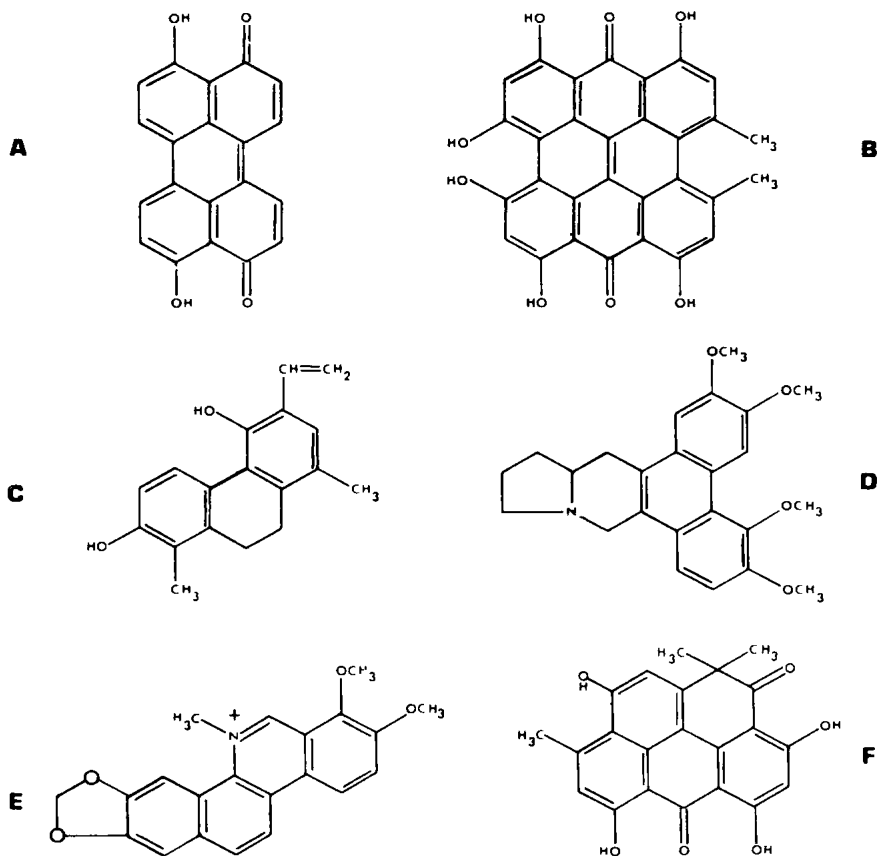


FIG. 3. Examples of natural products with a PAH-like structure. A, 4,9-dihydroperylene-3,10-quinone from the fungus *Daldinia concentrica*. Closely related pigments are found in the insect family Aphididae. B, hypercin from St. John's wort *Hypericum perforatum*; C, juncusol from the marine marsh grass *Juncus roemerianus*; D, tylophorine, the major alkaloid from *Tylophora asthamatica*; E, cherylthrine from roots of *Chelidonium majus*; F, resistomycin from *Streptomyces resistomycificus*.

2.1.1 Indirect Biosynthesis

Bacteria, fungi, higher plants, and some animals synthesize a wide variety of polycyclic quinone pigments (Thomson, 1971). Naphthoquinones are common in flowering plants. One example of a naphthoquinone is vitamin K₂. The largest group of naturally occurring quinones is the anthraquinones. Naphthacenequinones and phenanthraquinones are less