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POLYCYCLIC AROMATIC HYDROCARBONS IN THE TAMAR ESTUARY

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University of Plymouth

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POLYCYCLIC AROMATIC HYDROCARBONS IN THE TAMAR ESTUARY

J.W. READMAN
Ph.D. 1982
POLYCYCLIC AROMATIC HYDROCARBONS

IN THE TAMAR ESTUARY

James William Readman, B.Sc.

A Thesis submitted to the Council for National Academic Awards
in part fulfilment of the requirements for admittance to the
Degree of:

DOCTOR OF PHILOSOPHY

Plymouth Polytechnic,
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In collaboration with:

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Plymouth,
Devon. PL1 3DH.

Submitted July 1982.
I dedicate this Thesis to my dear Mother, the late

Elizabeth Mary Readman.
Declaration

I, James William Readman, hereby declare that the following Thesis is based on the results of experiments performed by myself and that the Thesis is of my own composition and has not been previously presented, in part or in whole, for a higher degree. The research was conducted in the Department of Environmental Sciences, Plymouth Polytechnic in collaboration with The Institute for Marine Environmental Research under the supervision of Dr. M.M. Rhead and Dr. R.F.C. Mantoura.

[Signature]

J.W. Readman, B.Sc.

Statement

I certify that James William Readman has complied with the requirements specified by the Council for National Academic Awards and is qualified to submit the accompanying Thesis in application for admission to the Degree of Doctor of Philosophy.

[Signature]


[Signature]

R.F.C. Mantoura, B.Sc.(Joint Hons), Ph.D.
Papers published


Papers presented

'Polycyclic Aromatic Hydrocarbons in the Tamar Estuary'. Presented to a meeting of the Marine Chemistry Discussion Group, 9th-10th April 1981, at the Institute for Marine Environmental Research, Plymouth.

'Distribution, composition and sources of Polycyclic Aromatic Hydrocarbons in surface sediments of the River Tamar Catchment and Estuary'. Presented to a meeting of the Estuarine and Brackish Water Sciences Association (EBSA), 14th-17th September 1981, at the Fresh Water Biological Association, Windermere.

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Course in Estuarine Chemistry - Part of the Marine Chemistry Option of the B.Sc.(Hons). Environmental Sciences degree course (final year) at Plymouth Polytechnic.
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My wife, Maria, deserves a special mention, I thank her for her boundless caring and encouragement.

Finally, I wish to thank my Father for his encouragement and guidance.
POLYCYCLIC AROMATIC HYDROCARBONS (PAH) IN THE TAMAR ESTUARY

James W. Readman

ABSTRACT

The high particulate association of PAH in aquatic systems has often been attributed to the hydrophobic nature of the compounds leading to adsorption. Results presented in this thesis strongly suggest that a significant proportion of PAH in the Tamar are particle-incorporated and unavailable for dynamic partition and physical, chemical and biological fates.

The generally uniform PAH composition identified throughout the estuary is highly indicative of a combustion or road and urban runoff source. PAH distribution in a dated Tamar Estuary sediment core showed an exponential decrease with depth and identified dramatic increases in PAH flux during the last 20 years. This suggests an association with motor vehicles probably via road runoff. No significant trends of change in PAH composition with depth were apparent, indicating uniformity of source and the unimportance of PAH degradation mechanisms in the anoxic sediment. Perylene displayed an anomalous distribution that could be attributed to its biogenic origin.

A survey of PAH distribution in the estuarine water column identified two areas exhibiting high concentrations. The first was observed at the turbidity maximum at the head of the estuary (by virtue of particle-associated PAH) and the second as a result of an emission in the urban region during the sampling period. A similar distribution was identified in the estuarine surface sediments which are shown to act as a sink for both riverine and urban PAH. Suspended particulates in the water column, in general, contain similar levels and compositionally reflect the higher molecular weight PAH (MW > 200) in the sediments. This underlines the importance of particulate transport and indicates that degradation of these PAH in the water column is of minor significance, with sediment incorporation as their primary fate. Lower MW PAH (MW < 200) were enriched in soluble forms in the water column. Microbial heterotrophic degradation and volatilisation are proposed as important environmental fates of these low MW compounds.
**CONTENTS**

<table>
<thead>
<tr>
<th>CHAPTER</th>
<th>PAH IN THE ENVIRONMENT</th>
<th>Page</th>
</tr>
</thead>
<tbody>
<tr>
<td>I.1.</td>
<td>Introduction</td>
<td>1</td>
</tr>
<tr>
<td>I.1.1.</td>
<td>Structures of PAH</td>
<td>2</td>
</tr>
<tr>
<td>I.1.2.</td>
<td>Physical properties</td>
<td>5</td>
</tr>
<tr>
<td>I.1.3.</td>
<td>Chemical properties</td>
<td>7</td>
</tr>
<tr>
<td>I.2.</td>
<td>Sources of PAH in the environment</td>
<td>8</td>
</tr>
<tr>
<td>I.2.1.</td>
<td>Natural production</td>
<td>8</td>
</tr>
<tr>
<td>a.</td>
<td>Biosynthesis of PAH</td>
<td>8</td>
</tr>
<tr>
<td>b.</td>
<td>Thermal diagenesis of organic material in sediments</td>
<td>12</td>
</tr>
<tr>
<td>c.</td>
<td>Natural combustion</td>
<td>14</td>
</tr>
<tr>
<td>I.2.2.</td>
<td>Anthropogenic sources</td>
<td>14</td>
</tr>
<tr>
<td>a.</td>
<td>Formation of PAH during combustion of organic material</td>
<td>14</td>
</tr>
<tr>
<td>b.</td>
<td>Individual sources</td>
<td>17</td>
</tr>
<tr>
<td>c.</td>
<td>Recent increases in environmental PAH concentrations</td>
<td>17</td>
</tr>
<tr>
<td>I.2.3.</td>
<td>Routes of entry into the aquatic environment</td>
<td>18</td>
</tr>
<tr>
<td>I.3.</td>
<td>Environmental distribution and transport of PAH</td>
<td>19</td>
</tr>
<tr>
<td>I.3.1.</td>
<td>The ubiquity of PAH</td>
<td>21</td>
</tr>
<tr>
<td>I.3.2.</td>
<td>PAH in natural waters</td>
<td>22</td>
</tr>
<tr>
<td>a.</td>
<td>Particulate associated PAH</td>
<td>23</td>
</tr>
<tr>
<td>I.3.3.</td>
<td>PAH in surface sediments</td>
<td>25</td>
</tr>
<tr>
<td>I.3.4.</td>
<td>PAH distribution in sediment cores</td>
<td>30</td>
</tr>
<tr>
<td>I.3.5.</td>
<td>PAH in aquatic organisms</td>
<td>31</td>
</tr>
<tr>
<td>I.4.</td>
<td>Environmental fates of PAH</td>
<td>32</td>
</tr>
<tr>
<td>I.4.1.</td>
<td>Photo-oxidation</td>
<td>34</td>
</tr>
<tr>
<td>I.4.2.</td>
<td>Biological degradation of PAH</td>
<td>36</td>
</tr>
<tr>
<td>I.4.3.</td>
<td>The relative contributions of individual processes to the environmental fate of PAH</td>
<td>40</td>
</tr>
<tr>
<td>I.5.</td>
<td>The Environmental Cycle of PAH</td>
<td>42</td>
</tr>
<tr>
<td>I.6.</td>
<td>The Tamar Estuary</td>
<td>44</td>
</tr>
<tr>
<td>I.7.</td>
<td>Aims of this Research</td>
<td>48</td>
</tr>
<tr>
<td>References</td>
<td></td>
<td>50</td>
</tr>
</tbody>
</table>
CHAPTER II. EXPERIMENTAL

II.1. Environmental Analyses

II.1.1. Water Chemistry
   a. Salinity, Temperature, pH, Turbidity, Dissolved Oxygen, Ammonia
   b. Particle characterisation

II.1.2. Elemental Analyses
   a. Carbon and Nitrogen
   b. X-ray fluorescence
   c. Atomic Absorption Spectroscopy

II.1.3. Analysis of Polycyclic Aromatic Hydrocarbons
   a. A Review of current methods
   b. Techniques applied and developed for the analysis of PAH in estuarine samples
      (i) Extraction and Clean-up
      (ii) High-performance Liquid Chromatography
      (iii) Work-up efficiencies

II.1.4. 210Po activity

II.1.5. Statistical treatment of results

II.2. Experimental Analyses

II.2.1. Radiochemical experiments
   a. Microbial heterotrophic degradation studies
   b. Adsorption of PAH onto estuarine suspended particulates

II.2.2. Direct analysis of PAH in experimental waters by High-performance Liquid Chromatography
   a. Analytical design
   b. Experimental design

References

CHAPTER III. THE ASSOCIATION BETWEEN PAH AND ESTUARINE PARTICULATES

III.1. Introduction

III.2. Sample collection

III.3. Experimental

III.4. Results and Discussion

III.4.1. Environmental Variables

III.4.2. Sorption of spiked PAH onto estuarine particulates
   a. The relationship between adsorption of spiked PAH and suspended particulate loading
b. The influence of organic carbon on adsorption 117
c. The affect of salinity on adsorption 119
d. Other factors potentially important in controlling sorption 124
e. Comparison of the adsorption experiment results with data reported in the literature 125

III. 4.3. Fractionation of Environmental Samples 127

References 129

CHAPTER IV  THE DISTRIBUTION OF PAH IN A DATED SEDIMENT CORE SAMMED FROM THE TAMAR ESTUARY

IV.1. Introduction 131

IV.2. Sample collection 131

IV.3. Experimental 134

IV.4. Results and Discussion 135

IV.4.1. Variability of the parameters monitored with depth 135

IV.4.2. Dating of the core 139

IV.4.3. Composition of the PAH assemblage 142

IV.4.4. Distribution of PAH and heavy metals with date 145

IV.4.5. Sources of pollution 147

a. PAH composition 147

b. Distribution with date 150

c. Distribution of combustion particulates 151

d. Methylene 155

e. Heavy metals 155

IV.4.6. Statistical results 157

References 160

CHAPTER V  DISTRIBUTION, TRANSPORT MECHANISMS AND FATES OF PAH IN THE TAMAR

V.1. Introduction 162

V.1.1. Study area and sample collection 162

V.2. Experimental 165

V.2.1. Water column survey 165
V.2.2. Surface sediment survey 165
V.2.3. PAH associated with particle size fractions 166
V.2.4. Microbial heterotrophic degradation 166

V.3. Results and Discussion 167

V.3.1. Aquatic distribution 167
  a. Environmental variables 167
  b. PAH concentrations in the water column 167
  c. Group 1 PAH (naphthalene, anthracene, phenanthrene) 170
  d. Group 2 PAH (higher molecular weight homologues) 173
  e. Statistical results 175

V.3.2. Sedimentary PAH in the Tamar Estuary and River Catchment 180
  a. Concentrations 180
  b. Distribution and transport mechanisms 180
  c. Compositional structure 184
  d. Sources 184

V.3.3. Comparison of PAH in estuarine surface sediments and suspended particulates in the water column 190

V.3.4. PAH associated with suspended particulate size fractions 192

V.3.5. Microbial heterotrophic degradation 195

References 199

CHAPTER VI SUMMARY 202

Appendix
CHAPTER I

PAH IN THE ENVIRONMENT
Polycyclic Aromatic Hydrocarbons (PAH) are an important class of environmental carcinogens. The first recorded observation linking increased incidence in human cancer with exposure to environmental contaminants was that of Rott in 1775. He observed a higher incidence of scrotal cancer in chimney sweeps and linked this finding with exposure to coal soot. Although not appreciated at that time, coal tar and soot contain exceptionally high levels of PAH. Following his discovery, biological studies of the carcinogenic properties of coal tar were performed without success. It was not until 1930 that the carcinogenicity of an individual PAH (dibenzo(a)anthracene) was demonstrated by Kennaway. Cook et al. isolated benzo(a)pyrene from coal tar in 1933 and it is this compound that has been adopted by many researchers as a representative PAH in environmental material. Since this early work many other PAH have been shown to be carcinogenic to laboratory mammals and probably to man. Proven carcinogenic homologues in the complex PAH assemblages associated with environmental samples represent only a minor proportion of the total PAH. The action of the remainder, whether synergistic or antagonistic to cancer induction is generally unknown. Other carcinogenic polycyclic aromatic 'compounds' are frequently associated with the PAH, adding to the environmental hazard.

Concern has been expressed over increased anthropogenic production of PAH owing to fossil fuel usage, particularly via combustion processes (Section I.2.2.). This fact together with evidence that no level of carcinogen can be deemed 'safe' (ie, no
threshold response has been demonstrated) has resulted in extensive research being directed towards this group of compounds in both medical and environmental disciplines. The abundance of environmental research has been summarised in many review articles 6-15 which are supplemented with published proceedings from annual symposia on PAH 16-20.

Although estuaries are regions of steep hydrodynamic, chemical and biological gradients and high biological productivity, there have been no systematic investigations of the effects of environmental variables in estuaries on the distribution, transport mechanisms and fates of PAH in estuaries. In addition, the proximity of large urban and industrial conurbations render estuaries of particular interest.

This introduction describes the chemistry and environmental data that are applicable to the assessment of distribution, behaviour and fate of PAH in estuarine systems.

I.1.1. Structures of PAH

PAH are composed of two or more fused aromatic (benzene) rings. The term 'fused' refers to the sharing of a pair of carbon atoms between two adjoining rings. This results in planar molecular structures. Naphthalene (C10H8) is the lowest molecular weight member, and the ultimate fused ring aromatic system is graphite (an allotropic form of elemental carbon). The compounds most frequently assessed in environmental studies of PAH are comprised of between 2 and 6 aromatic rings often with alkyl groups in various positions on the rings. Examples of unsubstituted PAH frequently
Several systems of nomenclature have been used to describe PAH. The most recent, and the system adopted in this study is that of the International Union of Pure and Applied Chemistry (IUPAC) as detailed in "The Ring Index". Neff has summarised the most important rules:

1. The structural diagram is written to present the greatest number of rings in a horizontal row.
2. Horizontal and vertical axes are drawn through the centre of the horizontal row and the molecule is orientated 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 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 a molecule. If a ring is fused to more than one face of the molecule simultaneously, this is indicated by using the appropriate letters to denote the faces so involved.

Examples of the numbering and lettering systems are shown below:

```
naphthalene
```

```
fluoranthenne
```
Figure 1.1.
Structures of unsubstituted PAH frequently identified in extracts of environmental samples.
IUPAC exceptions to these general rules do occur, most notably in
the numbering of phenanthrene and anthracene:

\[
\begin{align*}
\text{phenanthrene} & \quad \text{anthracene}
\end{align*}
\]

1.1.2. Physical Properties

PAH are solids at room temperature and are the least volatile
of the hydrocarbons. Their boiling points are markedly higher than
those of equivalent molecular weight alkanes. Aqueous solubilities
of PAH are very low (Table 1.1.) owing to their high molecular
weights and absence of polar functional groups. Solubilities
decrease with increasing molecular weight and linearly fused
homologues are less soluble than their angular isomers. Solubilities
of PAH have been discussed most recently by May. The author
investigates effects of changes in temperature and salinity (the
latter being of particular significance in the estuarine environment)
and records calculated values for Setschenow constants. Only
limited data is available on solubilities of PAH measured in sea
water and distilled water owing to 'salting out'. The presence of
detergents and soluble organic compounds can increase solubilities
of PAH, for example by micelle formation. Concentrations of
detergents in sewage and industrial effluents, however, are normally
insufficient to produce micelle formation. The hydrophobic nature
<table>
<thead>
<tr>
<th>Compound</th>
<th>Number of aromatic rings</th>
<th>Solubility (μg dm⁻³)</th>
<th>Distilled water (25-30°C)</th>
<th>Sea water (25°C)</th>
<th>Sea water (35°C)</th>
</tr>
</thead>
<tbody>
<tr>
<td>Naphthalene</td>
<td>2</td>
<td>2200^d, 31200^d, 12500^d, 31300^d</td>
<td>2000^k</td>
<td>22000^d</td>
<td></td>
</tr>
<tr>
<td>1-Methylnaphthalene</td>
<td>2</td>
<td>25800^b</td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>1,5-Dimethylnaphthalene</td>
<td>2</td>
<td>2740^d</td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>2-Ethynaphthalene</td>
<td>2</td>
<td>8000^d</td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Acenaphthalene</td>
<td>(2)</td>
<td>3470^d</td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Fluorene</td>
<td>(2)</td>
<td>168^b</td>
<td></td>
<td>830^k</td>
<td></td>
</tr>
<tr>
<td>Phenanthrene</td>
<td>3</td>
<td>100^g, 1180^g, 1600^g, 1600^g, 1070^d</td>
<td>600^k</td>
<td>710^3</td>
<td></td>
</tr>
<tr>
<td>1-Methylphenanthrene</td>
<td>3</td>
<td>269^d</td>
<td></td>
<td>300^k</td>
<td></td>
</tr>
<tr>
<td>Anthracene</td>
<td>3</td>
<td>44.6^a, 30.0^a, 75.0^a, 75.0^a</td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>2-Methylanthracene</td>
<td>3</td>
<td>21.3^a</td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Fluoranthenne</td>
<td>(3)</td>
<td>2.06^b, 5.36^b, 125^b, 280^b</td>
<td>100^k</td>
<td></td>
<td></td>
</tr>
<tr>
<td>Pyrene</td>
<td>4</td>
<td>132^b, 172^b, 175^b, 165^b</td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Triphenylene</td>
<td>4</td>
<td>4.8^b, 3.8^b</td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Benz(a)anthracene</td>
<td>4</td>
<td>9.4^b, 10^b, 11^b</td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Chrysene</td>
<td>4</td>
<td>1.6^b, 1.5^b</td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Naphthacene</td>
<td>4</td>
<td>1.5^b, 1.0^b</td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Perylene</td>
<td>5</td>
<td>0.5^b</td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Benzo(a)pyrene</td>
<td>5</td>
<td>0^d</td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Benzo(a)pyrene</td>
<td>5</td>
<td>0.172^o, 0.5^o, 6.05^o, 4.0^o</td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Dibenz(ac)anthracene</td>
<td>5</td>
<td>0.6^o, 0.5^o</td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Picene</td>
<td>5</td>
<td>2.5^i</td>
<td></td>
<td></td>
<td></td>
</tr>
</tbody>
</table>

(a) May et al., 1976 (24)
(b) May et al., 1976 (25)
(c) Lu et al., 1977 (26)
(d) Schwartz and Wesik, 1976 (27)
(e) Mushope and Getzen, 1972 (28)
(f) Eisenbrand, 1971 (29)
(g) Klevens, 1950 (30)
(h) Well-Malherbe, 1946 (31)
(i) Davis et al., 1982 (32)
(j) Haghani and Calder, 1976 (33)
(k) Rubai and Neff, 1978 (34)
of the compounds leads to sorption of PAH onto particulates present in aqueous systems (see Chapter III).

The availability of high energy \( \pi \) -bonding orbitals and of relatively low energy \( \pi^* \) -antibonding orbitals in PAH leads to absorption of visible and ultra violet (UV) radiation by the transition of electrons from the \( \pi \) to \( \pi^* \)-orbitals. Subsequent release of the energy gained by the electrons results in fluorescent emissions. The electron transitions occurring during excitation and emission in relation to the characteristic UV and fluorescence spectra produced from PAH are summarised by Lee et al. and are discussed in further detail by Clar. These authors also discuss factors involved in production of characteristic NMR and mass spectra.

1.1.3. Chemical Properties

The conjugated \( \pi \)-electron systems of PAH together with the lack of reactive functional groups render PAH relatively unreactive. This results in their persistence in the environment. PAH do, however, undergo electrophilic substitution (retaining aromaticity) and addition reactions during which some aromatic character is destroyed. The most recent review of PAH chemistry is that by Clar.

Lee et al. identify two series of addition reactions (involving anthracene and phenanthrene respectively) as being of particular interest in the environmental chemistry of PAH. Anthracene in the presence of light and oxygen photo-oxidises to give an endoperoxide. When irradiated without oxygen the compound dimerizes. In contrast, phenanthrene is relatively inert to
photo-oxidation and does not form a photodimer. These differences between angular and linear arrangement of rings in PAH are maintained with increasing molecular weight. There is a greater tendency for the larger linear molecules to undergo addition reactions, whereas there is little change in reactivity of angular homologues with increasing molecular weight.

I.2. SOURCES OF PAH TO THE ENVIRONMENT

PAH can originate from both natural and anthropogenic sources. The majority of current research supports the view that the major contributor to the environmental burden is linked to anthropogenic sources.

I.2.1. Natural Production of PAH

Mechanisms for natural production of PAH include biosynthesis, thermal diagenesis of sedimentary organic material (to form fossil fuels) and 'natural' combustion.

(a) Biosynthesis of PAH

The nearly ubiquitous distribution of PAH throughout the environment led to the consideration of biosynthesis as a possible explanation. During the past 20 years circumstantial and direct evidence have appeared both for and against biosynthesis of PAH.

Direct complete biosynthesis of PAH by bacteria, algae, and plants have been reported in the literature and are
reviewed by Neff \(^9\). In contrast to these reports Hase and Hites \(^56\) found no evidence of biosynthesis of PAH in a mixed anaerobic bacterial culture and criticized previous data of experimental description, blank measurements and lack of consideration of bioaccumulation factors. Grimmer and Duvel \(^57\) have disputed direct biosynthesis of PAH in plants following careful experiments preventing external contamination. The distribution of PAH observed in sediment cores have also precluded biosynthesis of many homologues (see Chapter IV).

Polycyclic aromatic non-hydrocarbon compounds are synthesised in nature \(^39,40,41\). Many of these compounds contain oxygen, nitrogen or sulphur. Selected examples of these natural products are shown in Fig. 1.2. There is good evidence that certain PAH are produced in Recent sediments from compounds similar in structure to those shown in Fig. 1.2. Perylene in particular, is thought to be derived from biogenic precursors under reducing conditions during early diagenesis \(^38,42-45\). Extended quinones have been proposed as the precursors, although it is thought unlikely that sufficient would be present to produce the concentrations of perylene often encountered \(^41\). Fig. 1.3. shows structures of potential precursors suggested by Laflamme and Hites \(^38\). The structures and origins of the precursors of perylene, however, remain unproven. In addition, Wakeham et al. \(^44\) identify further natural PAH including an extended series of phenanthrene homologues, retene and pimanthrene (derived from diterpenes), a series of tetra-and penta-cyclic PAH (derived from pentacyclic triterpenes of the amyrin-type) and tetra-and penta-cyclic PAH formed from pentacyclic triterpenes with five-membered B-rings. These authors suggest that the transformation
Figure 1.2.
Examples of natural products with a PAH-like structure.
(a) juncusol from the marine marsh grass Juncus roemerianus;
(b) tylophorine, the major alkaloid from Tylophora asthmatica;
(c) cherylthrine from roots of Chelidonium majus;
(d) resistomycin from Streptomyces resistomycoicus;
(e) hypercin from St. John's wort Hypericum perforatum.
Structures of some extended quinone pigments which may be possible precursors of perylene in sediments. Stereochemistry is not given.

(a) 4,9-dihydroxyperylene-3,10-quinone.
(b) xanthoaphin - fb.
(c) erythroaphin - fb.
(d) rhodoaphin - bs.

reactions are microbially mediated. Tan and Heit\textsuperscript{45} confirm biogenic conversion of abiotic acid\textsuperscript{38} to retene and alkylated and partially hydrogenated phenanthrenes, and also suggest the formation of hydrochrysene and hydropicene from pentacyclic triterpenes. By incorporation of these naturally produced PAH into this section, which also discusses direct biosynthesis, would infer biological activity is involved. Wakeham et al.\textsuperscript{44} have suggested microbial mediation of the processes. Abiotic pyrolytic production, discussed in the following section, demonstrates the presence of increasing quantities of some PAH. The role (if any) of micro-organisms in such processes is unresolved.

(b) Thermal diagenesis of organic material in sediments

Coals\textsuperscript{58,59} and crude oils\textsuperscript{60-63} contain complex mixtures of PAH resulting from the low temperature (100 - 200\textdegree C) thermal alteration of organic material over geological time\textsuperscript{40}. PAH compositions tend to be enriched in lower molecular weight homologues (naphthalenes and phenanthrenes)\textsuperscript{59,62,63} and are scarce in unsubstituted species\textsuperscript{40} (Table 1.2.). Formation of alkylated naphthalenes has been proposed via dehydrogenation of sesquiterpenoids\textsuperscript{64} and from breakdown of \(\beta\)-carotene\textsuperscript{65}. Aromatic hydrocarbons (including naphthalenes) have been produced by reaction of elemental sulphur with steroids and terpenoids in experiments maintained at temperatures comparable to those associated with the genesis of petroleum\textsuperscript{66}.

Geochemical processes acting on organic material at higher temperatures than those associated with oil production but lower than that for production of a liquid silicate phase, followed by
<table>
<thead>
<tr>
<th>Compound</th>
<th>Concentration in mg/kg</th>
<th>South Louisiana crude</th>
<th>Kuwait crude</th>
</tr>
</thead>
<tbody>
<tr>
<td>Naphthalene</td>
<td>400</td>
<td></td>
<td>400</td>
</tr>
<tr>
<td>1-Methylnaphthalene</td>
<td>800</td>
<td></td>
<td>500</td>
</tr>
<tr>
<td>2-Methylnaphthalene</td>
<td>900</td>
<td></td>
<td>700</td>
</tr>
<tr>
<td>Dimethylnaphthalenes</td>
<td>3600</td>
<td></td>
<td>2000</td>
</tr>
<tr>
<td>Trimethylnaphthalenes</td>
<td>2400</td>
<td></td>
<td>1990</td>
</tr>
<tr>
<td>Fluorenes</td>
<td>200</td>
<td></td>
<td>&lt;100</td>
</tr>
<tr>
<td>Phenanthrene</td>
<td>70</td>
<td></td>
<td>26</td>
</tr>
<tr>
<td>1-Methylphenanthrene</td>
<td>111</td>
<td></td>
<td>-</td>
</tr>
<tr>
<td>2-Methylphenanthrene</td>
<td>144</td>
<td></td>
<td>89</td>
</tr>
<tr>
<td>Fluoranathene</td>
<td>5.0</td>
<td></td>
<td>2.9</td>
</tr>
<tr>
<td>Pyrene</td>
<td>3.5</td>
<td></td>
<td>4.5</td>
</tr>
<tr>
<td>Benz(a)anthracene</td>
<td>1.7</td>
<td></td>
<td>2.3</td>
</tr>
<tr>
<td>Chrysene</td>
<td>17.56</td>
<td></td>
<td>6.9</td>
</tr>
<tr>
<td>Triphenylene</td>
<td>10</td>
<td></td>
<td>2.8</td>
</tr>
<tr>
<td>Benzo(ghi)fluoranthene</td>
<td>1</td>
<td></td>
<td>&lt;1</td>
</tr>
<tr>
<td>Benzo(b)fluoranthene</td>
<td>&lt;0.5</td>
<td></td>
<td>&lt;1</td>
</tr>
<tr>
<td>Benzo(j)fluoranthene</td>
<td>&lt;0.9</td>
<td></td>
<td>&lt;1</td>
</tr>
<tr>
<td>Benzo(k)fluoranthene</td>
<td>&lt;1.3</td>
<td></td>
<td>&lt;1</td>
</tr>
<tr>
<td>Benzo(a)pyrene</td>
<td>0.75</td>
<td></td>
<td>2.8</td>
</tr>
<tr>
<td>Benzo(e)pyrene</td>
<td>2.5</td>
<td></td>
<td>0.5</td>
</tr>
<tr>
<td>Perylene</td>
<td>34.8</td>
<td></td>
<td>&lt;0.1</td>
</tr>
<tr>
<td>Benzo(ghi)perylene</td>
<td>1.6</td>
<td></td>
<td>&lt;1</td>
</tr>
</tbody>
</table>

Naphthalene - Fluorenes from Anderson et al., 61
Phenanthrene - Benzo(ghi)perylene from Pancirov and Brown 62
subsequent crystallisation and recrystallisation during hydrothermal transport are thought to have produced some rare PAH containing minerals \(^67,68\). 'Tendletonite' \(^67,68\) has been characterised as nearly pure coronene, 'curtisite' \(^67,69\) contains dibenzofluorene, picene, chrysene and their alkylated homologues and 'idrialite' \(^67\) is comprised predominantly of tribenzofluorones and their homologues.

(c) Natural Combustion

Pyrolytic processes result in the production of PAH (Section I.2.2.(a)). Youngblood and Blumer \(^70\) have suggested that forest and prairie fires represent an important source of PAH to the atmosphere. The relative importance has been further discussed by Farrington and Meyers \(^71\) and Suess \(^72\). Il'nytskii et al. \(^73,74\) propose volcanic activity as a significant source of PAH into the atmosphere.

I.2.2. Anthropogenic Sources

Anthropogenic combustion processes have been cited as the major source of PAH to the environment \(^38\).

(a) Formation of PAH during combustion of organic material

Combustion mechanisms involved in the formation of PAH from organic materials (in both anthropogenic and natural combustion) are hypotheses and are not completely understood. Two stages of reaction are believed to be involved. Pyrolysis describes the partial cracking (at high temperature) of organic molecules into unstable fragments followed by subsequent pyrosynthesis during which
these fragments (mostly radicals) recombine to form aromatic hydrocarbons. Badger et al. proposed the basis of current hypotheses. Their classical stepwise synthesis of benzo(a)pyrene is shown in Fig. 1.4.(a). The pyrosynthesis of PAH have been more recently and further discussed by Crittenden and Long and Schneltz and Hoffmann (Fig. 1.4.(b)). Factors affecting the yields and composition of PAH produced by combustion include temperature, duration of exposure to elevated temperature, availability of oxygen (atmosphere), the chemical composition of the precursor fuel, and the presence in the pyrolysis mixture of catalysts or C,H radical scavengers. Blumer has suggested that the relative abundance and distribution of alkyl-substituted PAH is highly dependent on the temperature at which the PAH are formed..... as pyrolysis temperature decreases the ratio of alkyl-substituted PAH to unsubstituted PAH increases. For example, at very high temperatures, as in the coking of coal, the products consist of a relatively simple mixture of unsubstituted hydrocarbons, presumably because of the rapid cleavage of less stable alkyl-bonds. At intermediate temperatures, as in the smouldering of wood, complex mixtures of alkylated rings survive. When the temperature of formation is lower, as might be exemplified by the formation of crude oil, alkylated homologues far exceed the unsubstituted hydrocarbons (although the action of catalysts in the production of PAH in crude oils might influence composition). Further aspects of factors controlling the synthesis of PAH during combustion have been reviewed. Environmental PAH assemblages associated with anthropogenic combustion origins show similarity in composition.
Typical pathways of PAH pyrosynthesis.

(a) Benzo(a)pyrene pyrosynthesis as proposed by Badger et al. (reference 75).

(b) Pyrosynthesis of naphthalene from benzene as postulated by Schmeltz and Hoffmann, (reference 78). At high temperatures in the presence of air, benzene is converted to phenol, which upon loss of water would be converted to 'benzyne'. The latter, through a 1,4- addition across benzene, gives rise to benzosicyclo(2,2,2)octatriene, which spontaneously decomposes to acetylene and naphthalene.
(b) **Individual Sources**

Virtually any process in which organic carbon is subjected to high temperature will result in the production of some PAH. This results in tremendous diversity of sources of PAH to the environment. Incineration of industrial and domestic wastes, heat and power generation from fossil fuels, and the combustion of fuels in internal combustion engines, all produce emissions rich in PAH. 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 electrolyte aluminium using graphite electrodes; coke production; gas production from petroleum; coal gasification; production of synthetic alcohol; and oil refinery operations.

Contamination of the environment also occurs via spillage and leaching of materials containing PAH, for example, oil spillage during shipment and from motor vehicle crank-case leakage, vehicle tyre wear and leaching of asphalt road surfaces and from wood treated with creosote preservative.

Several reviews are available that describe sources in detail 9,10,7,6,79-82.

Sources of particular significance localised to the Tamar region are discussed in Chapters IV and V.

(c) **Recent increases in environmental PAH concentrations**

The PAH burden in the environment has increased dramatically during the past century. The evidence for this increase has been derived from sediment core data (discussed in Chapter IV) and has
been attributed directly to man's activities, primarily owing to combustion of fossil fuels.

1.2.3. Routes of entry into the aquatic environment

From the origins discussed in the previous sections, PAH may reach the aquatic environment from dry or wet deposition from the atmosphere; surface runoff from land; industrial and domestic effluents; spillage of petroleum and associated products; leaching of naturally occurring deposits containing PAH (e.g. crude oil seepage, oil-bearing shales, coal, PAH-rich minerals and peat); and from in situ synthesis.

The majority of PAH from combustion sources are emitted into the atmosphere. It is generally assumed that most atmospheric PAH are associated with aerosols and particulate matter. Low molecular weight homologues with sufficiently high vapour pressures (e.g. naphthalene) might, however, exist in significant concentrations in the vapour phase. PAH residence times are dependent on size and density of the particulates with which the PAH are associated, and the prevailing climatic conditions. Atmospheric conditions resulting in deposition onto land and water include rain (and snow), dry fallout and vapour phase deposition onto surfaces.

Land runoff incorporates contributions from all of the origins listed in the first paragraph of this section. Of localised significance is urban runoff and particularly that associated with road surfaces.

Many industrial and domestic waste waters contain significant quantities of PAH. Industrial PAH contributions are highly
dependent on the industries involved, selected examples of which have been reviewed 6-8. Sewage primarily from domestic sources generally contains increased quantities of PAH as the industrial contribution to the sewage is increased 85 (Table 1.3.). Following heavy rain, PAH in sewage increases substantially owing to increased storm sewer runoff from urban areas and roadways 83-85 (Table 1.3.). The amounts of PAH entering aquatic environments by this route is large 6-8,83-85.

Direct spillage of oils and petroleum products increase PAH concentrations in the aqueous environment 86. This source would be of particular significance in certain areas, for example dockyards. Creosoted pilings of shoreline structures have been cited as important localised sources of PAH 87,88 (creosote contains up to 92g PAH/kg 89). Natural seepage and leeching of oils, shales, coals, peat and (to a much lower extent) PAH minerals will also contribute to the PAH burden.

PAH derived from natural synthesis, for example perylene and retene, appear to represent only a minor proportion of the total PAH in polluted environments, and only contribute significantly in areas remote from man's activity.

1.3. ENVIRONMENTAL DISTRIBUTION AND TRANSPORT OF PAH

If the PAH associated with the aquatic environment were evenly distributed throughout the world's oceans and fresh water bodies, resulting concentrations would be negligible and inconsequential 9. This is not the case and anthropogenic PAH remain relatively near
Table 1.3.

PAH in municipal wastewater effluents in West Germany

<table>
<thead>
<tr>
<th>Compound</th>
<th>PAH concentrations in effluents to the Rotach River (µg.dm(^{-3}))</th>
<th>PAH concentrations in domestic sewage from Hegne (µg.dm(^{-3}))</th>
<th>Domestic</th>
<th>Industrial</th>
<th>Dry weather</th>
<th>During heavy rain</th>
</tr>
</thead>
<tbody>
<tr>
<td>Fluoranthene</td>
<td>0.273</td>
<td>2.198</td>
<td></td>
<td></td>
<td>0.352</td>
<td>16.350</td>
</tr>
<tr>
<td>Pyrene</td>
<td>-</td>
<td>1.957</td>
<td></td>
<td></td>
<td>0.254</td>
<td>16.050</td>
</tr>
<tr>
<td>Benz(a)anthracene</td>
<td>0.191</td>
<td>0.167</td>
<td></td>
<td></td>
<td>0.025</td>
<td>10.360</td>
</tr>
<tr>
<td>Benzo(b)fluoranthene</td>
<td>0.036</td>
<td>0.114</td>
<td></td>
<td></td>
<td>0.039</td>
<td>10.790</td>
</tr>
<tr>
<td>Benzo(j)fluoranthene</td>
<td>0.037</td>
<td>0.045</td>
<td></td>
<td></td>
<td>0.057</td>
<td>9.910</td>
</tr>
<tr>
<td>Benzo(k)fluoranthene</td>
<td>0.031</td>
<td>0.032</td>
<td></td>
<td></td>
<td>0.022</td>
<td>1.840</td>
</tr>
<tr>
<td>Benzo(a)pyrene</td>
<td>0.038</td>
<td>0.100</td>
<td></td>
<td></td>
<td>0.001</td>
<td>3.840</td>
</tr>
<tr>
<td>Benzo(ghi)perylene</td>
<td>0.040</td>
<td>0.073</td>
<td></td>
<td></td>
<td>0.004</td>
<td>4.180</td>
</tr>
<tr>
<td>Indeno(1,2,3-cd)pyrene</td>
<td>0.022</td>
<td>0.057</td>
<td></td>
<td></td>
<td>0.017</td>
<td>4.980</td>
</tr>
<tr>
<td>Total identified PAH</td>
<td>0.668</td>
<td>4.743</td>
<td></td>
<td></td>
<td>0.771</td>
<td>78.300</td>
</tr>
<tr>
<td>Total unidentified PAH</td>
<td>0.130</td>
<td>0.390</td>
<td></td>
<td></td>
<td>0.075</td>
<td>9.200</td>
</tr>
<tr>
<td>Total PAH</td>
<td>0.798</td>
<td>5.133</td>
<td></td>
<td></td>
<td>0.846</td>
<td>87.500</td>
</tr>
</tbody>
</table>

From Borneff and Kunte.\(^85\)
their respective sources and decrease approximately logarithmically with distance from the source. PAH entering the aquatic environment from the land are thus concentrated in rivers, estuaries and coastal marine waters.

I.3.1. The ubiquity of PAH

Although PAH are mainly concentrated in areas influenced by man, low concentrations are nearly ubiquitous throughout the environment. This was initially attributed to direct biosynthesis of PAH (Section I.2.1.(a)). With increased power in analysis of PAH, it now seems that their ubiquity is a function of long range airborne transport of combustion particulates. Exceptions to this general rule occur for selected homologues that can be ascribed products of natural syntheses (Section I.2.1.(a)), eg. perylene, retene and alkylated phenanthrenes.

Numerous studies over the last 30 years have been performed to characterise PAH associated with airborne particulate matter with respect to geographical location, point sources, seasonal variation and meteorological conditions. These are listed by Lee et al. who summarise the findings as follows:-

1. The range of benzo(a)pyrene in unpolluted non-urban air varied between 0.1 and 0.5 ng.m$^{-3}$, while the concentration in polluted air samples has reached 74 ng.m$^{-3}$.
2. There was considerable variation in the concentrations of PAH in air particulate matter collected at different sampling sites which were representative of different point sources.
3. Inconsistent variations in the concentrations of PAH in airborne particulate matter sampled for short periods at the same sampling site were dependent on variations in the
4. Air at commercial sites was more polluted during the day than at night corresponding to automobile traffic and industrial emissions as major sources.

5. Pollution was more pronounced in the autumn and winter than in spring or summer, indicating the higher use of combustion sources in the winter, presumably for heating purposes, or seasonal meteorological variations such as lower than average wind speed and more frequent thermal inversions during the winter months.

The effects of atmospheric physics, chemical degradation and meteorological conditions on airborne PAH have been reviewed. 81

I.3.2. PAH in natural waters

No published data is presently available on concentrations of individual PAH in estuarine or coastal waters. Estimates of total aromatics by fluorescence spectroscopy (which is subject to interference from non-PAH compounds 9) has, however, been applied to U.K. marine waters 92. Results (expressed as Ekofisk crude oil equivalents) demonstrate that highest levels are encountered in estuaries and inshore areas and particularly in areas exposed to industrial and shipping activity. Similar approaches have been applied to other coastal and oceanic waters. Barbier et al. 92 reported a concentration of 137 μg.dm⁻³ for total hydrocarbons in surface waters off the French Coast at Brest of which 2.5% were PAH (ie. 3.4 μg.dm⁻³). Concentrations of PAH in Atlantic Ocean surface waters can be calculated from the data of Brown and Huffman 93 as approximately 0.4 μg.dm⁻³ with a range of 0.13 to 1.3 μg.dm⁻³.
Marty et al. working in the Eastern tropical Atlantic Ocean reported hydrocarbon concentrations in sea water of 10 \( \mu g dm^{-3} \) of which 33% were aromatics. PAH in these water samples included phenanthrene, alkyl-phenanthrenes, perylene, fluoranthene, pyrene and traces of benzo[b]fluoranthene and benzo[ghi]pyrene.

The majority of research into PAH in aqueous systems has been directed towards fresh-waters. Most of this data has been reviewed. Table 1.4. gives typical values of benzo(a)pyrene and total PAH concentrations in selected fresh-water systems. Although benzo(a)pyrene is cited as an exemplary PAH, many of the authors also report values for other individual homologues. There is a large range in riverine PAH concentrations owing to differential exposure to anthropogenic PAH sources. Industrialisation and other human activities are reflected by increases in PAH. Rivers remote from man's influence are relatively uncontaminated. Ground and well water contain considerably less PAH (usually by a factor of 10 or more). Tap and reservoir water generally contains slightly higher concentrations of PAH than ground water. Although subject to considerable temporal variation, rain water is shown to contain significant amounts of PAH owing to airborne particulate contamination.

(a) **Particulate associated PAH**

PAH in natural waters tend to be greatly enriched in the suspended particulate fraction in comparison to PAH in solution. Herrmann has recently reported the importance of particulates in PAH transport through a partly urbanised river basin. The relationship with particulates has been attributed to the
### Table 1.4. Benzo(a)pyrene and total PAH concentrations in selected fresh waters.

<table>
<thead>
<tr>
<th>Source</th>
<th>Concentration (ng dm⁻²)</th>
<th>Reference</th>
</tr>
</thead>
<tbody>
<tr>
<td>River Rhine at Mainz, GFR</td>
<td>50-110, 730-1500</td>
<td>Borneff &amp; Kunte 95</td>
</tr>
<tr>
<td>River Rhine at Koblenz, GFR</td>
<td>10-60, 500-3000</td>
<td>Hellmann 96</td>
</tr>
<tr>
<td>River Aach at Stockach, GFR</td>
<td>4-13, 1440-3100</td>
<td>Borneff &amp; Kunte 85</td>
</tr>
<tr>
<td>Other GFR rivers</td>
<td>0.6-10, 200-1000</td>
<td>Borneff &amp; Kunte 85,95</td>
</tr>
<tr>
<td>Raykov region, USSR, remote from human activity</td>
<td>0.01-0.1, -</td>
<td>Il'ntskii et al. 97</td>
</tr>
<tr>
<td>Sunzha River, USSR, below discharge of an oil refinery</td>
<td>50-3500, -</td>
<td>Samoilovich &amp; Red'kin 98</td>
</tr>
<tr>
<td>Thames River, England</td>
<td>170-280, 800-2350</td>
<td>Acheson et al. 84</td>
</tr>
<tr>
<td>Trent River, England</td>
<td>5.3-504, 25-3790</td>
<td>Lewis 99</td>
</tr>
<tr>
<td>Severn River, England</td>
<td>1.5-48, 20-266</td>
<td>Lewis 99</td>
</tr>
<tr>
<td>Oyster River, Connecticut, USA</td>
<td>78-150, -</td>
<td>Keegan 100</td>
</tr>
<tr>
<td>Monongahela River at Pittsburgh, Pennsylvania, USA</td>
<td>42-77, 600-663</td>
<td>Basu &amp; Saxena 101</td>
</tr>
<tr>
<td>Ohio River at Huntington, West Virginia, USA</td>
<td>5.6, 57.9</td>
<td>Basu &amp; Saxena 101</td>
</tr>
<tr>
<td>Delaware River at Philadelphia, Pennsylvania, USA</td>
<td>41.1, 351.8</td>
<td>Basu &amp; Saxena 101</td>
</tr>
<tr>
<td>Ground water (GFR)</td>
<td>0.4-7.0, 10.9-123.5</td>
<td>Borneff &amp; Kunte 102</td>
</tr>
<tr>
<td>Ground water (GFR)</td>
<td>2-4, 100-200</td>
<td>Hellmann 96</td>
</tr>
<tr>
<td>Ground water (USA)</td>
<td>0.2, 8.3</td>
<td>Basu &amp; Saxena 101</td>
</tr>
<tr>
<td>Well water (GFR)</td>
<td>2-15, 100-750</td>
<td>Hellmann 96</td>
</tr>
<tr>
<td>Well water (England)</td>
<td>0.2-0.6, 3.6-5.8</td>
<td>Lewis 99</td>
</tr>
<tr>
<td>Tap water (GFR)</td>
<td>0.5-9.0, 29.2-125.5</td>
<td>Borneff &amp; Kunte 102</td>
</tr>
<tr>
<td>Tap water (9 US cities)</td>
<td>0.2-1.6, 0.9-14.9</td>
<td>Basu &amp; Saxena 101</td>
</tr>
<tr>
<td>Reservoirs (Moscow)</td>
<td>4-13, -</td>
<td>Il'ntskii &amp; Rozhnova 103</td>
</tr>
<tr>
<td>Reservoirs (England)</td>
<td>0.7-3.8, 9.1-43.2</td>
<td>Lewis 99</td>
</tr>
<tr>
<td>Rain water (Koblenz, GFR)</td>
<td>4-80, 200-4000</td>
<td>Hellmann 96</td>
</tr>
<tr>
<td>Lake Constance (GFR)</td>
<td>0.2-11.5, 25-234</td>
<td>Borneff &amp; Kunte 95</td>
</tr>
<tr>
<td>Lake Erie at Buffalo, New York, USA</td>
<td>0.3, 4.7</td>
<td>Basu &amp; Saxena 101</td>
</tr>
</tbody>
</table>

Compiled from Neff 9
hydrophobic nature of the compounds resulting in partitioning from the aqueous phase onto suspended solids. Published literature on adsorption is summarised in Chapter III. Environmental factors likely to be of primary importance in controlling these processes are suspended particulate concentrations, composition and structures; salinity; pH and temperature. Estuaries typically exhibit gradients for all of these variables thus identifying them potentially as areas of exceptional importance in discerning the environmental behaviour and fate of PAH. There is no information presently available on the environmental chemistry of PAH in estuaries.

In coastal and marine environments, where sediment deposition is biologically controlled, Prahl and Carpenter have suggested the importance of zooplankton in transport of PAH via consumption of PAH contaminated materials from atmospheric or riverborne origins and subsequent deposition to the bed in faecal pellets.

1.3.3. PAH in surface sediments

PAH concentrations in sediments are orders of magnitude higher than those of the overlying water. Concentrations vary widely (Table 1.5.). In remote pristine areas, typical concentrations recorded for benzo(a)pyrene (Table 1.5.) are below 10 ng. (g dry sediment)^{-1} \textsuperscript{109-112}. In contrast, sediments in close proximity to urban and industrial conurbations contain substantially higher concentrations, with recorded benzo(a)pyrene levels up to 17000 ng. (g dry sediment)^{-1} (Table 1.5.). Urban rivers would appear especially susceptible to chronic PAH contamination. Factors demonstrated of importance in controlling sedimentary anthropogenic
<table>
<thead>
<tr>
<th>Location</th>
<th>B(a)P (ng(g dry sediment)^-1)</th>
<th>Reference</th>
</tr>
</thead>
<tbody>
<tr>
<td><strong>Fresh water</strong></td>
<td></td>
<td></td>
</tr>
<tr>
<td>River Seine mud</td>
<td>15000</td>
<td>Dauvis 113</td>
</tr>
<tr>
<td>River sediment below a coke byproduct plant, USSR</td>
<td>8200-17000</td>
<td>Fedorenko 114</td>
</tr>
<tr>
<td>River Sunzha, USSR 3-4 km below oil-refinery discharge</td>
<td>9.2-19.0</td>
<td>Szaszlovich &amp; Red'kin 96</td>
</tr>
<tr>
<td>25 km below discharge</td>
<td>trace-3.1</td>
<td></td>
</tr>
<tr>
<td>Askov region, USSR remote from exogenous sources</td>
<td>1-2</td>
<td>Bukhova 111</td>
</tr>
<tr>
<td>Grosser Zveneer See, GFR 100 m from north shore</td>
<td>1610</td>
<td>Grimmer &amp; Bohnke 115</td>
</tr>
<tr>
<td>100 m from south shore</td>
<td>280</td>
<td>MLLer et al. 116</td>
</tr>
<tr>
<td>Lake Constance, GFR</td>
<td>443</td>
<td>Eglinton et al. 117</td>
</tr>
<tr>
<td>River Usk, UK</td>
<td>11000</td>
<td>Heit et al. 118</td>
</tr>
<tr>
<td>Sagamore Lake, USA</td>
<td>128</td>
<td></td>
</tr>
<tr>
<td>Woods Lake, USA</td>
<td>690</td>
<td></td>
</tr>
<tr>
<td><strong>Marine</strong></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Polynesian atolls</td>
<td>0.3-6.5</td>
<td>Miascoat et al. 109</td>
</tr>
<tr>
<td>Godthaab, Greenland</td>
<td>5</td>
<td>Mallet et al. 110</td>
</tr>
<tr>
<td>Port of Dunkerque</td>
<td>400</td>
<td></td>
</tr>
<tr>
<td>Canal d’lIle-jeanty</td>
<td>1750</td>
<td></td>
</tr>
<tr>
<td>Southern regions of the North Sea</td>
<td>traces-122.5</td>
<td></td>
</tr>
<tr>
<td>Rhone River delta</td>
<td>traces-1500</td>
<td>Lolou 119</td>
</tr>
<tr>
<td>Villefranche Bay, France</td>
<td>0-96</td>
<td></td>
</tr>
<tr>
<td>Mouth of the Var River</td>
<td>0-34</td>
<td></td>
</tr>
<tr>
<td>Port-Vendres</td>
<td>23</td>
<td></td>
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<tr>
<td>Banyuls</td>
<td>150</td>
<td></td>
</tr>
<tr>
<td>Cerbere</td>
<td>200</td>
<td></td>
</tr>
<tr>
<td>Bay of St. Malo, France</td>
<td>160</td>
<td>Mallet et al. 120</td>
</tr>
<tr>
<td>Nicean</td>
<td>1320</td>
<td></td>
</tr>
<tr>
<td>Bassin</td>
<td>170</td>
<td></td>
</tr>
<tr>
<td>Bay of Naples, Italy</td>
<td>1.4-3000</td>
<td>Boucart &amp; Mallet 121</td>
</tr>
<tr>
<td>English Channel coast, France</td>
<td>15000</td>
<td>Mallet et al. 110</td>
</tr>
<tr>
<td>Severn estuary at Aust, UK</td>
<td>470</td>
<td>Thompson &amp; Eglinton</td>
</tr>
<tr>
<td>Severn estuary at Foweyhead</td>
<td>300</td>
<td>John et al. 123</td>
</tr>
<tr>
<td>Severn beach</td>
<td>700</td>
<td></td>
</tr>
<tr>
<td>Aust</td>
<td>2700</td>
<td></td>
</tr>
<tr>
<td>Saudafjord, Norway increasing distance from a ferro alloy smelter</td>
<td>7700-26</td>
<td>Bjornseth 124</td>
</tr>
<tr>
<td>Boston Harbour, Mass. Bay, USA</td>
<td>300</td>
<td>Cohnswini &amp; Hites 125</td>
</tr>
<tr>
<td>Sones Sound, Maine, USA</td>
<td>40</td>
<td></td>
</tr>
<tr>
<td>Near oil-refinery tanker jetty, USA</td>
<td>80</td>
<td>Gump et al. 126</td>
</tr>
</tbody>
</table>

26
PAH concentrations, are the pollutant flux, sedimentation rate, and particle size. Sediments have a substantial integrating effect on temporal patterns of PAH, although seasonal variations have been reported. The mechanisms responsible for these variations, whether changes in pollutant flux or "in situ" microbial degradation and biosynthesis, were not resolved in these studies.

Concentrations of PAH decrease approximately logarithmically with distance from point source emissions. This is demonstrated by the results of Dunn and Stich (Fig. 1.5).

![Figure 1.5. Concentrations of benzo(a)pyrene in sediments in the vicinity of the Iona Island sewage treatment plant, Vancouver, Canada. Values are ng. (g dry sediment)^{-1}. From: Dunn and Stich (reference 88).](image)

Benzo(a)pyrene distribution in surface sediments originating from a sewage treatment plant varied from 121 ng. (g dry sediment)^{-1} near the outfall to approximately 1 ng. (g dry sediment)^{-1} at a distance...
of 4 - 5 km downstream. Björseth also reports rapid sedimentation of PAH from a ferro-alloy smelter with a reduction of 99% in sedimentary PAH at a distance of 8.5 km from the source. This author also notes a more rapid sedimentation of benzo(a)pyrene relative to that of phenanthrene and attributes this to the higher solubility of the latter. These findings endorse the importance of particulate association in the transport of PAH (as discussed in Section 1.3.2 (a)) and demonstrate that good geographical resolution of point source inputs can be obtained by sediment analysis.

The composition of individual PAH in the sedimentary assemblage is highly dependent on the origins of the pollutants. The composition in the sediment may exhibit differences from that of the PAH source owing to differential partitioning of PAH between the sediment and aqueous phases and degradation. Once deposited in sediments, PAH are less subject to photo-chemical or biological oxidation, especially if the sediment is anoxic, resulting in persistence of the compounds. Sedimentary assemblages are complex, often containing thousands of different homologues, the majority of which are alkylated derivatives. Techniques utilised to compare environmental and source assemblages include subjective comparison of chromatograms; assay of the relative content of PAH parent (ie. non-alkylated) molecules; alkylation patterns of these homologues; and ratios between selected PAH. Even though locally significant sources and combinations of origins have been identified, there appears to be a nearly ubiquitous assemblage that dominates many of the environmental sediments analysed. An example of this assemblage (obtained by Giger and Schaffner) is shown in Fig. 1.6. Parent hydrocarbons dominate the alkylated homologues,
Figure 1.6.
Gas chromatogram of a fraction isolated from a river sediment (Monchalttorfer Aa, Switzerland). Numbers refer to:
1. acenaphthene; 2. fluorene; 3. phenanthrene; 4. anthracene;
5. methylphenanthrenes; 6. 4,5-methylenephenanthrene;
7. fluoranthene; 8. pyrene; 9. benzo(a)fluorene;
10. benzo(b)fluorene; 11. benzo(a)anthracene; 12. chrysene/
triphenylene; 13. benzofluoranthenes; 14. benzo(e)pyrene;
15. benzo(a)pyrene; 16. perylene; 17. dibenzanthracenes;
18. indeno(1,2,3-cd)pyrene; 19. benzo(ghi)perylene;
20. coronene; S. internal standard (1-chlorotetradecane).
G.C. conditions: 20 m x 0.3 mm i.d. glass capillary column with
barium carbonate interlayer and coated with SE-52, 0.8 atm.
hydrogen as carrier gas, flame ionization detector, column at
ambient temperature during injection and elution of the solvent
then programmed from 60°C to 250°C at a rate of 2.5°C.min⁻¹.
From Giger and Schaffner (reference 133).
indicative of a combustion origin (Section I.2.2.a). Opinion in
the literature regards anthropogenic combustion of fossil fuels as
being primarily responsible for the ubiquity of this type of
assemblage (see also Chapters IV and V).

I.3.4. PAH distribution in sediment cores

Much work has been done on the distribution and composition of
PAH in dated sediment cores in order to evaluate sources and
diagenesis of the compounds. Aspects of the results obtained have been previously discussed
(Sections I.2.1., I.2.2.). Findings indicate that two groups of PAH
exist in the sediments:

1. Compounds of biogenic origin eg. perylene and retene, of which,
significant concentrations are recorded throughout cores (Section
I.2.1.).

2. Anthropogenic PAH usually attributed to fossil fuel combustion.
The distribution of these homologues show negligible concentrations
up to the late 1800s when levels increase substantially towards the
present. In many of the cores analysed a maximum is reached in the
1950/60s with a slight decrease occurring towards the surface (this
has been attributed to a reduction in coal combustion in favour of
oil and gas burning which emit less PAH on combustion). In all but
the most remote pristine environments, anthropogenic homologues
contribute the vast majority of the PAH to modern sediments.

Individual core data are reviewed in context with results
obtained from a sediment core sampled from the Tamar Estuary
(Chapter IV).
I.3.5. **PAH in aquatic organisms**

Concentrations of PAH in aquatic organisms are generally intermediate between those in the sediment and levels encountered in the water column. Studies of PAH in aquatic biota have been reviewed \(^6,7,9\). Subsequent to the most recent review by Neff (1979) \(^9\) further papers have been published \(^136-141\). Most literature has concentrated on benzo(a)pyrene as a representative PAH, and in particular report levels in commercially important bivalve molluscs in coastal marine environments. Neff \(^9\) has summarised and tabulated published benzo(a)pyrene concentrations in aquatic organisms which range from 0.1 to 5000 \(\mu\)g \((\text{kg dry organism})^{-1}\). There are few trends exhibited in the data. Samples collected in close proximity to urban and industrial regions generally contain higher concentrations than those in remote areas. Few apparent correlations exist between benzo(a)pyrene concentrations and the phylitic position, trophic level or habitat preference of the respective organisms.

Many interacting factors potentially affect PAH in organisms. Proximity to discharges, and hence the environmental PAH levels in immediate contact with the biota, is of paramount importance. Concentrations in selected species have been shown to decrease with increasing distance from point source emissions \(^124,83\). The form of the pollutant PAH, whether in solution, emulsified or strongly particulate bound, will influence uptake. Dunn and Young \(^142\) have suggested that particle-bound benzo(a)pyrene may not be biologically available to filter-feeding bivalves. The habit of the organisms in question, whether sedentary, free living or migratory, will affect
exposure. Tissue accumulation of PAH is balanced by the relative rates of uptake and depuration. Knutzen and Sortland have recently demonstrated that different species sampled from the same locality exhibited different PAH profiles. Routes of uptake, whether via the water or diet, are likely to affect PAH concentrations and assemblages. Uptake from water might result in enrichment of the more soluble lower molecular weight homologues. Differences also occur in the ability of organisms to metabolise PAH. For example, mussels lack, or have a poorly developed aryl hydrocarbon hydroxylase enzyme system required for PAH metabolism. Stresses, such as salinity gradients encountered by estuarine species, will also affect metabolic activity, as would temperature and seasonality. Age structures and relative degrees of development of the individual organisms sampled will add to intra-species variability. When assessing PAH concentrations in organisms, influences owing to the above factors should be considered.

I.4. ENVIRONMENTAL FATES OF PAH

Howard et al. have defined environmental fate as encompassing any transport or degradation processes which describes the behaviour of a chemical in the environment. Several review articles discuss fates of PAH.

The particulate affinity of PAH is of importance in their environmental chemistry, effectively controlling the availability of the compounds for other physical, chemical and biological processes.
Owing to the fact that most natural degradation processes require oxygen (Sections I.4.1., I.4.2.), sedimentation and subsequent burial of particulate associated PAH into anoxic environments may result in preservation of the compounds.

The role of volatilisation in removing PAH from aquatic environments has been assessed most recently by Southworth. Losses from aqueous systems by this route are almost restricted to soluble forms of the compounds and are controlled by the surface area relative to water volume and, current and wind velocities. The relatively high solubilities and vapour pressures of the lower molecular weight PAH (e.g., naphthalene and anthracene) render these most susceptible to volatilisation, and Southworth predicts that in riverine situations their half lives would be less than 100 hours. For the larger molecular weight carcinogenic homologues it was calculated that volatilisation is of minor importance and does not significantly reduce aquatic concentrations. Additional factors not assessed by Southworth but likely to affect volatilisation are the presence of surface films, waves and aerosol formations.

PAH present in the aquatic environment are subject to degradation. The most important natural processes are photo-oxidation and biological transformation by aquatic bacteria, fungi and animals. Chemical oxidation of PAH has also been described in the literature, mainly in context with water purification using chlorine and ozone. PAH can also react with additional organic and inorganic oxidants including various electrophiles, peroxides, nitrogen oxides and sulphur oxides. These reactions are likely to be of significance in
atmospheric oxidation of PAH but are generally unimportant in aqueous environments.

1.4.1. Photo-oxidation

Photo-oxidation is potentially important in the breakdown of PAH in the atmosphere and aquatic environments. Photo-induced oxidation reactions of PAH in the aqueous phase by singlet oxygen, ozone and HO radicals have been reviewed. Most commonly endoperoxides are formed (Fig. 1.7.(a)). Photolysis of these peroxides through ring cleavage and dealkylation, produces a variety of products (Fig. 1.7.(a)). This process proceeds by a free radical mechanism (cleavage of the O-O bond) and initiates auto-oxidation. When, for steric reasons, no endoperoxide can be formed (e.g. in the case of benzo(a)pyrene), photo-oxidation yields a variety of diones (Fig. 1.7.(b)). Photo-oxidation involves an energy transfer from the triplet state of PAH yielding singlet oxygen $^{1}O_{2}$ which reacts with excited PAH to yield the peroxide or dione. Singlet oxygen from other sources can also react with PAH. Photo-reactivity of sorbed PAH has been suggested to be greater than that of PAH in solution and does not appear to involve an endoperoxide intermediate.

Anthracene adsorbed onto silica gel or alumina is photo-oxidised to anthraquinone and by subsequent oxidation to 1,4 dihydroxy-9,10-anthraquinone (Fig. 1.7.(c)). The presence of suspended particulates, however, are generally thought to decrease rates of photo-oxidation by increasing light attenuation. Early work predicting the influence of photo-oxidation in aqueous media often involved the experimental presence of traces of known photo-
Figure 1.7.
Types of reactions proposed for the photo-oxidation of polycyclic aromatic hydrocarbons.
(a) photo-oxidation of 9,10-dimethylanthracene through a 9,10-endoperoxide intermediate to yield several oxygenated products.
(b) photo-oxidation of benzo(a)pyrene to the 6,12-, 1,6-, and 3,6-diones.
(c) photo-oxidation of anthracene adsorbed to silica gel or alumina to 1,4-dihydroxy-9,10-anthraquinone.
From 'Particulate Polycyclic Organic Matter' (reference 81).
sensitizers (for example acetone) and have subsequently been criticized \(^{152}\). Most recent calculations of the effects of photo-oxidation on aquatic PAH concentrations result in the theoretical half-lives of selected PAH (in an inland water body (5 m depth) integrated over a full summer day (latitude 40° N)) \(^{152}\) listed below:

<table>
<thead>
<tr>
<th>PAH</th>
<th>No partitioning</th>
<th>With partitioning (20 mg dm(^{-3}))</th>
</tr>
</thead>
<tbody>
<tr>
<td>Naphthalene</td>
<td>550</td>
<td>550</td>
</tr>
<tr>
<td>Phenanthrene</td>
<td>59</td>
<td>69</td>
</tr>
<tr>
<td>Anthracene</td>
<td>4.5</td>
<td>5.2</td>
</tr>
<tr>
<td>Fluoranthene</td>
<td>160</td>
<td>200</td>
</tr>
<tr>
<td>Pyrene</td>
<td>4.2</td>
<td>5.9</td>
</tr>
<tr>
<td>Benz(a)anthracene</td>
<td>3.7</td>
<td>9.2</td>
</tr>
<tr>
<td>Benzo(a)pyrene</td>
<td>3.2</td>
<td>13</td>
</tr>
<tr>
<td>Naphthacene</td>
<td>0.2</td>
<td>0.95</td>
</tr>
</tbody>
</table>

These results show the greater sensitivity of linear homologues to photo-oxidation compared to their angular isomers, as discussed in Section I.1.3. It is stressed that these are calculated values and their accuracy remains unproven although relative photo-reactivity is likely to be precise.

I.4.2. Biological Degradation of PAH

Some microbes have been shown capable of complete oxidation of simple aromatic hydrocarbons to carbon dioxide and water \(^{153}\). Others 'co-oxidise' aromatics in the presence of alternative substrates into various oxygenated metabolites \(^{154}\). The mineralisation of naphthalene \(^{153}\) by \textit{Pseudomonas putida} is shown in Fig. 1.8 (a).

This involves oxidation to \textit{cis}-1,2-dihydroxy-1,2-dihydronaphthalene. The dihydrodiol is next oxidised to 1,2-dihydroxymethyl naphthalene and
Metabolism of polycyclic aromatic hydrocarbons by bacteria.

(a) metabolism of naphthalene by *Pseudomonas putida* to carbon dioxide and water (from Gibson, reference 153).

(b) initial reactions in the metabolism of benzo(a)pyrene by *Beijerinckia* sp. (from Gibson, reference 154).
Following ortho-cleavage of the dihydroxylated benzene ring, yields (via intermediates) salicylic acid, which is subsequently degraded through catechol to carbon dioxide and water. Larger molecular weight homologues, for example benzo(a)anthracene and benzo(a)pyrene, are inadequate sole carbon sources (apparently because of the inability of microbes to oxidise them completely to carbon dioxide and water). Some species of bacteria oxidise these PAH to various phenolic and acidic metabolites. Cis-hydroxylation is also the principal mechanism for the degradation of these larger molecular weight compounds, as demonstrated by Gibson (Fig. 1.8. (b)) using a mutant strain of a cultured *Beijerinckia* sp. initially isolated from a polluted river. The dihydrodiols formed from benzo(a)pyrene were shown to be reduced non-enzymatically to 7- and 9-hydroxy benzo(a)pyrene (Fig. 1.8. (b)). Wild type *Beijerinckia* sp. are capable of further degradation of the dihydrodiols by ring cleavage.

Strains of bacteria isolated from an oil polluted estuary were shown by Dean-Raymond and Bartha to degrade fluorene, phenanthrene and anthracene more readily than larger molecular weight PAH. Some of the oxygenated products produced were identified.

Degradation studies of PAH spiked into environmental waters have been published. Lee and Takahashi assessed degradation rates in a controlled marine ecosystem enclosure before and after the addition of No 2 fuel oil to the system. They demonstrated that the degradation potential increased after spiking with the oil. Lee estimated degradation of PAH by natural populations of microbes in marine and estuarine waters. His results
showed that PAH were degraded most rapidly in estuarine waters (turnover times for naphthalene and benzo(a)pyrene were approximately 40 and 3500 days respectively) and attributed this finding to the high standing stock of bacteria owing to high levels of nutrients in estuaries. Estuarine environments exposed to oil pollution exhibited the highest degradation rates, possibly owing to increased numbers of hydrocarbon degrading microbes in the natural populations. Even in these situations degradation was almost restricted to lower molecular weight PAH. Microbial populations in coastal waters were less efficient at degrading PAH than those in estuarine waters. Marine water samples from the Gulf Stream contained very little microbial degradative activity. Degradation rates were higher during summer than winter. Further filtration experiments suggested that naphthalene and methyl-naphthalenes were degraded by free-living microbes.

Sedimentary degradation of spiked PAH has also been demonstrated. Degradation rates decrease with increasing depth, the oxidation-reduction potential being of primary importance, mineralisation decreasing dramatically with increasing sediment anaerobiosis. pH controls activity to a lesser extent. The physical structure of the sediment particles is another factor for consideration. Gardner et al. demonstrated higher degradation rates in sands compared to marsh 'mud'. The presence of polychaete worms also increase mineralisation rates. Surface sediments previously subjected to oil contamination exhibit a significantly elevated capacity for degradation of PAH. Thompson and Eglinton have shown that sediment particulates incorporated into surface diatom slimes contain significantly less PAH than those in
the underlying sediments. The authors suggest that components of the slimes may actively biodegrade the PAH.

Higher organisms (invertebrates and vertebrates) have been shown to concentrate and degrade PAH \(^9\) and references therein. An important feature of the bacterial degradation pathway is that the cis-dihydrodiol is produced through a dioxetane intermediate. In higher animals a trans-dihydrodiol is produced through an arene oxide intermediate via a mixed function oxygenase (MFO) enzyme system \(^9\). It is these arene oxides or their immediate oxidation products that appear to be the active carcinogenic and mutagenic agents \(^{168,169}\).

I.4.3. The relative contributions of individual processes to the environmental fate of PAH

It should be appreciated that the dynamic nature of aquatic systems (and particularly estuaries) render judgement of individual processes environmentally unrealistic. Selected recent papers have attempted to place in context the relative contributions of individual components contributing to the environmental fate of PAH. Herbes et al. \(^{161}\) have assessed fates of selected homologues in fresh water environments. Examples of their results are reproduced in Figs. 1.9.(a), and (b). Fig. 1.9.(a), demonstrates the importance of volatilisation of naphthalene and photolysis of the larger molecular weight compounds in a shallow rapidly flowing stream depleted in suspended particulates. Fig. 1.9.(b), shows how the relative importance of the parameters assessed change in other fresh water systems using benz(a)anthracene as an example. For this high molecular weight PAH, sediment sorption and photolysis appear dominant.
Figure 1.9.
(a) Predicted contributions of major transport processes to the removal of PAH from water of a shallow, rapidly flowing stream. N, A, BA, BP indicate naphthalene, anthracene, benzo(a)anthracene and benzo(a)pyrene respectively.
(b) Predicted transport behaviour of benzo(a)anthracene in several representative aquatic systems.
From Herbes et al., (reference 161).
in controlling the fate.

Results published from experiments by Lee et al.\textsuperscript{159} on the fate of selected PAH in a controlled ecosystem enclosure, indicate that aromatic hydrocarbons in shallow marine waters may have residence times in the order of a few days. For lower molecular weight aromatics such as benzenes, naphthalenes and phenanthrenes, their results suggest that microbial degradation and volatilisation are the primary removal processes. In contrast, the concentrations of higher molecular weight aromatics such as chrysenes, benz-anthracenes and benz-pyrenes were shown to be primarily affected by sedimentation and photo-chemical oxidation (in agreement with Herbes et al.\textsuperscript{161}). Furthermore, Lee et al.\textsuperscript{159} suggest that in open oceans which are characterised by low concentrations of suspended particulates and clear waters, rates of sedimentation would be low and photo-oxidation rates should be high. It follows that under turbid conditions, such as occur in tidal estuaries, movement of suspended solids and sedimentation would be expected to be of primary importance in the transport of these larger molecular weight PAH.

\section*{I.5. THE ENVIRONMENTAL CYCLE OF PAH}

Suess\textsuperscript{72} has summarised the environmental cycle of PAH (Fig. 1.10.). The relative contributions indicated, however, are not necessarily applicable to localised situations. Sources identified include primarily combustion processes, biosynthesis (a topic of some controversy (Section I.2.1.(a))) and direct spillage of PAH rich
Figure I.10. The Environmental Cycle of PAH. From Suess (reference 72).
materials eg. oils. Geological production of fossil fuels and natural leaching of these products are not shown. Routes of entry into the aquatic environment include atmospheric fallout, land runoff and waste effluent emissions. The majority of PAH entering the aquatic environment remain close to sites of deposition, so that lakes, rivers, estuaries and coastal marine environments near centres of human population are primary repositories of aquatic PAH. High particulate association of the compounds leads to deposition, concentration and sediment burial of PAH. Degradation mechanisms reduce concentrations of PAH in aerobic environments, but once buried in anoxic sediments the compounds are persistent. Further transport only occurs via disturbance and relocation of sediments.

I.6. THE TAMAR ESTUARY

A map of the River Tamar catchment and estuary is shown in Plate I. The River Tamar drains relatively pristine moorlands and its associated biota include salmon and trout. Sewage and runoff from villages and small towns (for example Launceston and Gunnislake) represent the major point source pollutant discharges, although these are supplemented by individual domestic effluents. During the 19th century there was extensive mining activity in the catchment and at the head of the estuary. This was associated with the Dartmoor granite metamorphic aureole which is rich in copper, lead, arsenic, tin, manganese and zinc minerals. No significant mining activity has, however, occurred since the turn of the century. Present day activity in the Tamar Valley is mainly confined to
Plate I
Reduced copy of part of the Plymouth and Launceston Ordnance Survey 1:50000 Map (Sheet 201). The grid represents 1 m squares. Reproduced with the permission of the Controller of Her Majesty's Stationery Office, Crown Copyright reserved.
agriculture. Although the Lynher and Tavy tributaries enter the estuary, the Tamar represents the major fresh-water source.

In contrast, urban Plymouth is situated adjacent to the lower estuary (Plate II). Industry associated with the city is dominated by H.M. Dockyards at Devonport. Other light industries and a small oil terminal at Wilcove are the only other potentially important sources of industrial pollution into the Tamar. Shipping associated with the dockyards and oil jetty, and recreational boating represent further pollutant sources. Oil contamination in the lower estuary would appear inevitable. Domestic and industrial waste water from treatment works and by direct emission, together with urban runoff (particularly storm water overflows that often bypass sewage works) will further pollute this region of the estuary. The Tamar road bridge is a major route into Cornwall and carries an exceptionally high volume of traffic. This is potentially an important source of motor vehicle associated emissions such as oil, PAH and lead.

The close proximity of the Institute for Marine Environmental Research and the Marine Biological Association to the Tamar Estuary has resulted in a substantial amount of research being directed towards the chemistry, hydrodynamics and biology of the Tamar. Relevant data is discussed in context with the results presented in this thesis.
Plate II

Aerial photograph of the lower urban region of the estuary taken from Grid Reference SX 463527, viewing in a north-westerly direction.
There is little data in the literature concerning PAH in estuaries and none discussing PAH distribution in context with classical estuarine chemistry. The objective of this research was to elucidate sources, distribution, transport mechanisms and fates of PAH in the Tamar Estuary.

To perform this task it is necessary to evaluate many interacting factors, for example, to assess the environmental contributions of PAH from unresolved sources, both spatial and temporal variability of the PAH flux must be assessed and fate mechanisms that act to differentially change the individual compounds once they are released into the environment must be accounted for. Most frequently the substantial integrating capacity of sediments for these hydrophobic compounds has been exploited in determining the aquatic distribution and sources of these pollutants.

In this thesis, the approach chosen to discuss the estuarine chemistry of PAH begins by characterisation and quantification of the association of PAH with suspended particulates in the water column. Factors potentially important in controlling sorption throughout the estuary are experimentally investigated and are discussed in context with analyses of the aqueous and particulate phases of fractionated environmental samples.

As a preliminary investigation of sources, the historical record of inputs of PAH are evaluated. Selected homologues were quantified throughout a $^{210}$Pb dated sediment core removed from a 'stable' region of the lower estuary in order to determine compositional differences with depth and hence assess temporal
changes in inputs and diagenesis.

In order to further assess sources and investigate transport mechanisms and fates throughout the entire estuarine system, the approach was directed towards three areas of research. To gain an understanding of the behaviour of PAH in the water column, individual homologues (spanning a range of molecular weights) were analysed in water samples removed during an axial traverse of the estuary. Concentrations encountered were then compared with changes identified in environmental variables including salinity, suspended particulate load (total, mineral and organic constituents), pH, dissolved oxygen, temperature and ammonia. Secondly, surface sediments throughout the Tamar catchment and estuary were characterised and analysed for PAH. Finally, selected experiments were designed to further assess potentially important processes identified by the previous surveys.

The environmental and experimental data obtained are summarised and correlated in Chapter VI of this thesis.
REFERENCES


35. W.E. May. In 'Petroleum in the Marine Environment'.


82. M.R. Guerin. In Reference 12, 1, p.3.


CHAPTER II

EXPERIMENTAL
II.1. ENVIRONMENTAL ANALYSES

II.1.1. Water Chemistry

(a) Salinity, Temperature, pH, Turbidity, Dissolved Oxygen, Ammonia.

These variables were monitored aboard a Rotork Seatruck sampling vessel. Water was pumped from a depth of 0.5 m using a submersible (Flygt B 2040) centrifugal pump. Salinity, temperature, pH, turbidity and dissolved oxygen were measured as described by Morris et al. The pumped water was passed through a constant-levelled reservoir (flushing time 20 secs.) containing the detecting sensors. Salinity and temperature were recorded discretely using an Electronic Instruments Ltd., Salinity Temperature Bridge Type MC 5. The instrument salinity response was calibrated routinely in the field by comparison with working standard sea waters (previously checked against I.A.P.S.O. Standard Sea Water using an Autolab inductively-coupled salinometer). At very low salt concentrations, chloride ion activity was measured using a Philips IS 550 - Cl− chloride selective electrode in conjunction with a Philips pH 44/2 - SD/1 double junction reference electrode. The output was recorded via a Corning - Elmet Model 113 pH/mV meter. This system was calibrated directly with dilutions of Standard Sea Water. pH was determined continuously with a Rye combination glass electrode (401E7) in conjunction with a compensating thermistor (625) activating a Rye Model 292 Mk2 pH/mV meter. Calibration was achieved with N.B.S. buffers, disodium hydrogen phosphate (pH 6.87 at 25°C) and borax (pH 9.18 at 25°C). A Partech Electronics Ltd. Suspended Solids Monitor, Model LP - 740 - 3R, supplied a direct
recording of turbidity. Dissolved oxygen concentrations were obtained with a Yellow Springs Instrument Co. Inc. Model 57 Oxygen Meter. Calibration was checked with a range of saline and fresh waters which had been equilibrated with the atmosphere. Percentage saturation values were calculated with the aid of tables prepared by U.N.E.S.C.O. Ammonia was measured by an automated version of the indophenol blue method employing a modified buffered chlorine-donor system.

(b) Particulate characterisation

Estimates of suspended particulates via turbidity as described in Section II.1.1.(a), were inadequate for some aspects of this research. Gravimetric determinations were therefore obtained. Total suspended solids were determined by filtering known volumes of estuarine water through ashed (450°C, 12 h) preweighed Whatman GF/C filters (47 mm), washed with distilled water to remove residual salts, dried (110°C, 12 h) and reweighed. Mineral suspended solids were approximated by heating the filters (450°C, 12 h). Organic suspended solids were then calculated by difference. The inadequacies of this latter technique are discussed in Section II.1.2.(a). Particulate carbon and nitrogen contents were determined as described for sediments (Section II.1.2.(a)).

Suspended particulate size distributions were obtained directly from well mixed estuarine water sub-samples using a Malvern Instruments Ltd. 2200/3300 computerised Laser Particle Sizer V13 according to the Methods Manual.
II.1.2. Elemental Analyses

(a) Carbon and Nitrogen

Freeze dried sediment samples were ground (5 min) in an agate ball mill (Glen Creston Ltd.). Aliquots (approximately 6 mg) were weighed into precleaned (soaked in chloroform (1 h), rinsed in acetone, then distilled water and dried) aluminium foil 'boats'. Weighings were recorded to ± 1 µg, (Cahn electro-balance). Acetanilide standards (approximately 1 mg) of known carbon and nitrogen content were also prepared together with foil container blanks. Samples were analysed using a Carlo Erba Elemental Analyser (Model 1106). Peak areas were recorded by integration and corrected for blanks. Percentages of carbon and nitrogen were then calculated relative to the instrument response for analyses of the standard acetanilide.

'Organic' carbon determinations are subjective and reflect the ashing or digestion process used prior to elemental analysis 6. Low temperature ashing (International Plasma Corporation Low Temperature Asher - Model 1101) was selected 6 to remove organic constituents from sediments. Organic carbon was then calculated by difference from the carbon content of the ashed residue, and carbon in an untreated sediment sub-sample.

(b) X-ray Fluorescence

Silicon, aluminium, zinc and copper were determined in sediments using a Philips 1220 X-ray fluorescence spectrometer. Dried, ground sediment samples were pressed into tablets (2 cm in diameter). Silicon and aluminium were analysed using a Cr target
X-ray tube and a LiF analysing crystal. A LiF (200) analysing crystal with a gold target X-ray tube were used for zinc and a tungsten target X-ray tube for copper.

(c) **Atomic Absorption Spectroscopy**

Lead was analysed by flame atomic absorption spectroscopy following a digestion and leaching procedure of Van Loon et al. 7. Dried, ground sediment (2 g) was heated (1 h) with concentrated hydrochloric (9 cm$^3$) and nitric (3 cm$^3$) acids. After allowing to cool, distilled water (3 cm$^3$) was added and the samples gravity filtered (Whatman No. 42) with acid washings into volumetric flasks (25 cm$^3$). Volumes were made up with dilute hydrochloric acid. Quantification was obtained using an Instrumentation Laboratory Incorporated Atomic Absorption Spectrophotometer in accordance with the directions in the company's Methods Manual.

II.1.3. **Analyses of Polycyclic Aromatic Hydrocarbons**

The proven carcinogenic properties of some PAH render analyses involving standards and extracts HAZARDOUS and every precaution must be implemented to ensure that contact, either direct or indirect (eg. via the atmosphere), is avoided.

(a) **A Review of Current Methods**

Analyses of PAH are widely documented. A recent review article "Modern Analytical Methods for Environmental Polycyclic Aromatic Compounds" 8 cites over 400 references including 'Polycyclic Aromatic Hydrocarbons: Occurrence and Analysis - A Partial
Bibliography which itself includes a bibliography of more than 1000 references. Other texts further emphasise the interest in analysis of this class of compounds.

Environmental PAH are present as complex mixtures including numerous isomeric compounds with multi-substituted alkylated homologues. Relative concentrations of individual components vary greatly, primarily as a function of source. Analytical methods must ideally be selective, sensitive and overcome effects of the sample matrix. In the analysis of environmental PAH it is necessary to investigate and optimise the extraction, clean-up, chromatographic and quantification stages.

**Extraction**

**WATER:** The analysis of PAH in water has been recently reviewed. PAH may be extracted from water by liquid - liquid partition using a suitable solvent, chosen to combine high solubility for PAH, low miscibility with water and high volatility. Examples of solvents used include dichloromethane, chloroform, benzene and cyclohexane. Extraction can be facilitated by use of a separating funnel, vigorous stirring as with a mixer - homogeniser or by counter - current extraction. Acheson et al. investigated factors affecting the efficiency of solvent extraction from environmental water samples. Reductions in extraction efficiencies of spiked PAH were observed for experimental waters dosed with Fuller's earth. An alternative to solvent extraction is pre-concentration from water onto activated carbon, Amberlite XAD macroreticular cross-linked polystyrene resins, Tenax-G.C., ion exchange resins, polyurethane foam and
HMC precolumns. Turbid samples also present problems with these pre-concentration techniques; filtration being a pre-requisite. Filtration gives rise to the potential for contamination and PAH losses by volatilization and photo-degradation. Adsorption of PAH onto the filter and trapped particulates and desorption of the more soluble homologues during filtration of sample water might also perturb the environmental partition equilibrium of PAH. Other concentration techniques available including head-space analysis, freeze drying, steam distillation and reverse osmosis have been discussed and evaluated by Futoma et al. 12.

SEDIMENT: Sonication and ball-milling has been applied to sediment extraction. Thompson and Eglinton have used ultra-sonication of wet sediment in a dichloromethane-methanol azeotrope. Aizenshtat applied a method using homogenization of wet sample with 0.1 M HCl in benzene : methanol (70 : 30). Soxhlet extraction is most widely used. Wet sediments can be efficiently extracted with benzene-methanol, or with primarily methanol alone and then with the addition of benzene, or alternatively using methanol : dichloromethane. Soxhlet extraction of dried samples (freeze or air dried and dried with excess anhydrous sodium sulphate) with dichloromethane is also efficient. Digestion of wet sediments by saponification with ethanolic KOH has been favoured by those scientists working additionally on lipid-rich biological material (saponification of which is necessary for efficient extraction). A study by Wong and Williams compared selected extraction methods for hydrocarbons in sediments and indicated that analytical precision is enhanced by extraction of dried sediments although drying may result in losses of the more volatile components.
Clean-up

Extracts of environmental samples invariably contain substantial amounts of other non-PAH materials which can interfere with subsequent analyses. The quantities and nature of contaminants are dependent on the material extracted and the selectivity of the chosen solvent. Clean-up procedures must therefore be tailored to suit the particular extract in addition to the subsequent chromatographic procedure and selectivity/sensitivity of the detection system. For example, thin-layer chromatographic separations of water extracts with fluorescence detection as applied by Borneff and Kunte require little or no clean-up. Extracts analysed by high-performance liquid chromatography with relatively specific UV or fluorescence detection require less clean-up than if they were to be analysed by capillary gas chromatography (G.C.) with flame-ionization detection (FID), because of the limited G.C. column capacity and its susceptibility to contamination together with the non-specificity of the FID detection system.

Initial clean-up frequently involves selective partitioning of PAH between solvents. A study of the extraction characteristics of PAH based on the variation of partition coefficients for a variety of solvents has recently been published. Additional literature on this topic has been reviewed.

Co-extracted elemental sulphur can be removed by percolation of the extract through precipitated copper.

PAH extracts generally contain traces of aliphatic compounds and considerable amounts of polar aromatics which are conveniently removed by column chromatography on silica gel, or alumina. Combinations of these have been employed. Other
adsorbents such as Florisil \(^{42,50}\) and cellulose acetate \(^{51}\) have been recommended. Thin-layer chromatographic clean-ups have also been employed \(^{35}\).

More recently lipophilic gels have been used in preparatory stages. With benzene as eluent, Bio-Beads SX-12 (porous styrene divinylbenzene co-polymer) yield fractions containing compounds of increasing ring number \(^{52}\) whilst also separating multi-alkylated PAH from parent plus mono-alkylated PAH \(^{53}\). PAH are also eluted in sequence of increasing ring number from Sephadex LH-20 using isopropanol \(^{54,55}\). Lee et al. \(^{47,56}\) have reviewed the use of these gels.

**Quantification**

Methods range from simple spectrophotometric estimation of PAH \(^{14,57}\) to computerised high resolution capillary gas chromatography/mass spectrometry (GC/MS). A wide range of intermediary techniques have been published in the literature.

Column chromatography, as described in the previous section, has been used in conjunction with selective UV and fluorescence for the analysis of eluted fractions. TLC \(^{20}\) and reverse phase high-performance TLC \(^{58}\) with fluorescence detection also enable isolation and quantification of selected PAH.

High-performance liquid chromatography (HPLC) has become popular in recent years and offers distinct advantages in the analysis of PAH, including unique separations of isomers and alkylated homologues and highly sensitive and selective on-line UV and fluorescence detection. Initial HPLC in the early 1970's utilised adaptations of simple column chromatography based on
adsorbents such as silica and alumina. The introduction of bonded phases has revolutionised HPLC as a reproducible and high resolution technique. Polar, chemically bonded stationary phases used in the normal phase, achieve PAH separations similar to those of silica and alumina whilst eliminating the problem of deactivation by traces of water. Amino-bonded phases result in alkylated homologues coeluting with the parent compounds in order of increasing aromaticity. This factor linked with a volatile non-polar eluent renders this technique suitable for pre-fractionation. Chmielowiec and George have recently evaluated polar bonded phases (amine, nitrile, diol, ether, diamine and quaternary ammonium) for normal phase separations of PAH.

Octadecylsilane (C18) (ODS) used with gradient elution affords selective separation of parent molecule isomers and alkyl-substituted species. Recent studies have described differences in the efficiency, selectivity and retention characteristics on C18 columns supplied by various manufacturers and also between production batches from the same company. Fundamental chromatographic theory of separation of PAH on C18 columns has been investigated including the selection of mobile phase composition and temperature effects. Systems affording the highest resolution to date are the Perkin Elmer ODS (PAH specific) and the Vydac 201 TP (now marketed by Perkin Elmer in the U.K.) columns with acetonitrile gradient elution.

To increase resolution an extension into capillary columns has been advocated. Separations of PAH with packed and wall coated capillary HPLC columns have appeared in the literature, although, to date, suffer from excessively long retention times.
Technological developments involving the re-designing of current HPLC instrumentation are required to facilitate microbore work.

HPLC has been employed for the determination of PAH in water, sediments, marine biota, air particulates, automobile exhaust, and petroleum and related fuels.

The selectivity and sensitivity of UV and fluorescence spectroscopic detectors in HPLC have been a major advantage of the technique. Variable wavelength UV has been used to achieve sensitivity and selectivity. Ratios of absorbance at selected wavelengths have been applied to aid identification of eluting peaks and Readman et al. have discussed the attributes of stop-flow UV scanning for identification of PAH in HPLC separations. Fluorescence detection has proven both sensitive and selective in detection of the compounds. The performance of filter and monochromator instruments have been compared, together with UV detectors. Multi-channel rapid scanning spectrometers, eliminating the stopping of flow for peak identification, have most recently been applied. LC-MS techniques and current applications have been reviewed by McFadden.

Other spectroscopic techniques employed in PAH analysis include phosphorimetry, low-temperature luminescence using Shpol'skii spectra, laser excitation luminescence, synchronous excitation and emission luminescence and infra-red analysis. These are discussed and reviewed by Lee et al.

Gas chromatography (GC) has been used extensively for the separation of PAH. Racked-column GC, although initially employed, has been replaced by analysis using glass and fused silica capillary columns which afford far superior resolution.
A variety of stationary phases have been used with SE52 and SE54 gaining perhaps most wide acceptance. For most PAH analytical work, capillary columns of 10-25 meters length and 0.2-0.3 mm diameter with film thicknesses of 0.3 μm have been suggested as most suitable. The development of 'on-column' injection has essentially eliminated discrimination in GC analysis caused by differences in volatility and concentration of the individual components injected. With this mode of sample introduction, however, clean-up procedures must provide samples relatively free of non-volatile material which would otherwise reduce column life. Flame-ionization detection (FID) is most frequently used for quantification of PAH. The advantages of FID include its linear response, sensitivity and reliability, although its lack of selectivity does present problems in peak identification. Bjørseth and Eklund have used electron capture (EC)/FID response ratios to successfully differentiate between some isomers. The photo-ionization detector (PID) can also be used selectively for PAH. A powerful approach for identification of PAH constituents separated by capillary GC is computerised capillary GC/mass spectrometry (GC/MS). Numerous applications of this can be found in the literature.

(b) Techniques applied and developed for the analysis of PAH in estuarine samples

(i) Extraction and clean-up

All solvents were of Fisons HPLC grade. Extractions were performed under subdued light to minimise possible photo-oxidation. All glassware was acid-cleaned and pre-rinsed with solvent prior to use.
Estuarine Water: Aliquots (2.00 dm³) at the time of sampling were transferred to pre-cleaned Winchester bottles containing cyclohexane (30 cm³). The sample bottles were then sealed with solvent rinsed aluminium foil or teflon lined caps and thoroughly shaken. On return to the laboratory each sample in turn was shaken and transferred to a separating funnel. Vigorous agitation (5 min) was resumed to enhance extraction. After phase separation (10 min) the aqueous layer was transferred back to the sampling bottle. Isopropanol (2 cm³) was added to the funnel to reduce emulsification at the interface and the cyclohexane collected. This procedure was repeated twice, adding further aliquots of cyclohexane (15 cm³) to the extraction vessel. The combined extract was dried by passage through a column of ashed (450°C, 6 h) AnalaR anhydrous sodium sulphate (10 g). The extract, including column washings (10 cm³ cyclohexane) was then reduced in volume by rotary evaporation (ambient temperature) to approximately 1 cm³ and quantitatively transferred with hexane washings to a vial (2 cm³). The volume was further reduced to 0.5 cm³ by gently passing a stream of nitrogen over the surface. To assess efficiencies, selected water samples were re-extracted for analysis and then known quantities of PAH standards added. Following thorough shaking and a period of equilibration (3 h) the samples were re-extracted to estimate recoveries of compounds. Overall reproducibility was assessed from analysis of triplicate water samples.

Estuarine Sediments: Sediment samples (of previously determined water content) were thoroughly homogenised and extracted by a method similar to that of Giger and Schaffner. Accurately weighed portions (ca. 7 g wet weight) were ground with ashed AnalaR
anhydrous sodium sulphate (7 g) to form a dry powder. The resulting mixtures were loaded into pre-extracted (8 h, dichloromethane) cellulose soxhlet thimbles (Whatman 28 mm id./80 mm). Soxhlet extraction was then performed into dichloromethane (100 cm³) for 8 hours in darkness. A weighed portion of the resulting extract was removed and reduced in volume by either rotary evaporation or using a gentle stream of nitrogen (dependent on the volume selected for analysis). Following quantitative transfer with washings to a vial (2 cm³) the volume was reduced further to 0.5 cm³. The dichloromethane was then displaced with hexane and the volume reduced to 0.5 cm³. Recoveries of spikes and re-extracts were also performed.

Clean-up: A micro-column alumina clean-up procedure was developed to produce an extract compatible with final HPLC analysis whilst minimising sample treatment and hence potential contamination and losses. Aluminium (BDH, neutral, Brockman grade 1) columns were prepared using Pasteur pipettes plugged with solvent rinsed glass wool. The pipettes were loaded immediately prior to use with a column (5 mm i.d. x 50 mm) of 7% deactivated (optimised for the technique) ashed (450°C, 6 h) alumina. Prior to sample treatment, columns were washed with hexane (2 cm³). The volume of hexane required to completely recover all the PAH from the alumina was determined before each group of analyses by monitoring the elution of indeno(1,2,3-cd)pyrene from representative columns. The procedure selected to monitor the elution of this standard was by collection of the eluent on TLC plates followed by visualisation of the dried plates under UV light. Samples (both water and sediment extracts) were loaded onto the alumina columns and eluted into vials.
with hexane (3.5 cm$^3$ approx.). Volumes were then reduced to 0.5 cm$^3$ using a stream of nitrogen, the hexane displaced with acetonitrile (0.2 cm$^3$) and the volume further reduced to 0.1 cm$^3$. Prior to analysis, the samples were stored in darkness at 0°C.

(ii) High-performance Liquid Chromatography (HPLC)

It is only recently that significant advances in the analysis of PAH by HPLC have been published. In the initial period of this research (1978/9) comparatively few papers were available concerning high resolution HPLC separations of environmental PAH extracts. It was necessary to develop analytical techniques capable of resolving, identifying and quantifying selected individual PAH utilising the equipment available. The approach adopted was initially directed towards evaluation of suitable phases.

A silica (Hypersil 5-7 μm) HPLC column (100 mm x 5 mm i.d.) with hexane elution was found to suffer from low retention capacity for PAH (even after substantial conditioning) and poor resolution. In order to reduce the influence of deactivation, polar bonded phases were investigated. Picric acid polymer was bonded onto micro-particulate silica (Hypersil 5-7 μm) and then slurry packed into an HPLC column (250 mm x 5 mm i.d.). With hexane elution only slight improvement in separation was obtained, although reproducibility was enhanced. Ethylamidepropyltrichlorosilane (EPS) bonded onto micro-particulate silica was demonstrated by Hunt et al. to separate the 6 representative PAH recommended for analysis by the World Health Organisation (W.H.O.) (fluoranthene, benzo(b)fluoranthene, benzo(k)fluoranthene, benzo(a)pyrene, benzo(ghi)perylene and indeno(1,2,3-cd)pyrene). This phase was
prepared as directed by Hunt et al. and packed into an HPLC column (250 mm x 5 mm i.d.). Elution with 10% toluene in hexane provided separation of the six W.H.O. PAH, although overall resolution was unsatisfactory since other PAH coeluted.

Octadeylsilane (ODS) columns used in the reverse phase produced superior separations of standards when compared to the above mentioned phases. Columns initially available were pre-packed Spherisorb ODS (10 μm) and Whatman ODS 2 phases. The latter with propan-2-ol: distilled water step gradient elution yielded most promising results, although elution times required to maintain resolution were extensive (approximately 2 h). Resolution was improved and analysis time was reduced by using a Hypersil ODS (5-7 μm) phase which was slurry packed into columns using a Shandon Southern HPLC column packer according to directions in the Methods Manual. Various eluents were tested including methanol: distilled water, propan-2-ol: distilled water and acetonitrile: distilled water, each in conjunction with isocratic, step and linear gradient programmes. The system favoured, and that applied to the analyses of environmental samples, utilised a Hypersil 5-7 μm ODS column (250 mm x 5 mm i.d.) with acetonitrile: distilled water gradient elution at a flow rate of 3 cm³.min⁻¹ (delivered by Perkin Elmer Series 2 high-performance liquid chromatograph with modified check-valves). The solvent programme consisted of an isocratic stage (50% acetonitrile : 50% distilled water) for 15 minutes prior
to injection and 9 minutes after injection, followed by a gradient of 1.5% min\(^{-1}\) increase in acetonitrile up to 99%. Samples (20-30 mm\(^2\)) were introduced onto the column using a Rheodyne 7125 injector. Examples of separations are shown in Fig. 2.1. Column parameters as defined by Knox\(^{99}\) were routinely assessed to ensure maintenance of column performance. Although separations of environmental extracts exhibited peaks with corresponding retention times to those of the standards (confirmed by co-injections), as with all chromatographic systems a technique capable of identifying and assessing peak purity was required. The HEC detector available at the time (a Perkin Elmer LC 55 variable wavelength UV spectrophotometer) was adapted, using a suitable motor and gearing, to perform stop-flow UV scanning. This system was successfully used to identify some PAH in environmental samples. Following the introduction of the Perkin Elmer LC 75 scanning UV spectrophotometer (for HPLC), data obtained resulted in the publication of 'Use of Stop-flow Ultraviolet Scanning and Variable-wavelength Detection for Enhanced Peak Identification and Sensitivity in High-performance Liquid Chromatography', \textit{Analyst}, \textbf{106}, p.122 (1981)\(^{83}\). Using this information a variable wavelength programme for detection of compounds at pre-selected wavelengths was designed for use with the Hypersil ODS separations (Fig. 2.1.) in order to maximise sensitivity and/or selectivity.

Superior resolution of PAH was subsequently obtained using a Perkin Elmer HG-ODS (PAH specific) column which was donated by Dr. K. Ogan of the Perkin Elmer Corporation (Norwalk, U.S.A.) prior to its commercial release. Elution conditions selected were similar to those suggested by Ogan et al.\(^{75}\) with an eluent flow
Figure 2.1.

Typical HILIC chromatograms showing standard and environmental PAH separations using a Hypersil 5-7 µm ODS column (250 mm x 5 mm i.d.) with acetonitrile : distilled water gradient elution at a flow rate of 3 cm³.min⁻¹. Elution and detection conditions are indicated at the bottom of the figure. Peak numbers correspond to 1. naphthalene; 2. phenanthrene; 3. anthracene; 4. fluoranthene; 5. pyrene; 6. chrysene; 7. benzo[a]anthracene; 8. benzo(b)fluoranthene; 9. benzo(k)fluoranthene; 10. benzo(a)pyrene. 'T' is an unresolved mixture (discussed in Section II.1.3.(b).(ii)). Unidentified peaks in the standard separation correspond to co-eluting compounds. Additional peaks in the extract separations include unidentified parent PAH and alkylated homologues.
rate of 0.5 cm$^3$.min$^{-1}$ and a solvent programme consisting of isocratic conditioning (40% acetonitrile:60% distilled water) for 15 minutes, prior to injection, and on injection a gradient increase of 3% acetonitrile.min$^{-1}$ was introduced. Once the solvent composition of 99% acetonitrile:1% distilled water was reached, this concentration was maintained until completion of the chromatogram. Examples of separations are shown in Fig. 2.2. With increased resolution obtained using the Perkin Elmer column, the stop-flow UV scanning technique was further refined and improved (Fig. 2.3.). The separation was, however, incompatible with detection using wavelength programming (as previously described for the Hypersil ODS column) owing to increased resolution of isomers reducing the spaces between PAH groupings with differing numbers of conjugated benzene rings (Fig. 2.2.). Additional detectors (Pye LC UV variable wavelength detectors and a Kratos Fluoromat Fluorometer FS 950) were employed in series with the LC 75 UV detector to provide constant information on peak purity via ratios of absorbance at different wavelengths (254 and 280 nm) and fluorescence (Table 2.1.). Coincident perylene (Fig. 2.2. Peak 9) was selectively quantified at 408 nm.

The large peak associated with the separations of environmental extracts (retention times 57 minutes on the Hypersil ODS column (Fig. 2.1.) and 25 minutes on the Perkin Elmer ODS column (Fig. 2.2.) exhibited a UV spectrum atypical of PAH (Fig. 2.4.). GC/MS analysis (courtesy of Professor G. Eglinton and Mrs. A. Gower, Bristol University) identified the peak as a mixture of phthalates and phenyl esters/ethers (Figs. 2.5. & 2.6.).

Calibration of the HPLC system was performed using high purity
Figure 2.2.

Typical HPLC chromatograms showing PAH separations of standards and a sediment extract using a Perkin Elmer 10 μm HC ODS column (250 mm x 2.6 mm i.d.)/acetonitrile:distilled water gradient elution at a flow rate of 0.5 cm³.min⁻¹. Elution conditions are shown at the bottom of the figure. Detection was by UV absorbance at 254 nm. Peak numbers correspond to 1. naphthalene; 2. phenanthrene; 3. anthracene; 4. fluoranthene; 5. pyrene; 6. benz(a)anthracene; 7. chrysene; 8. benzo(e)pyrene; 9. coincident perylene and benzo(b)fluoranthene; 10. benzo(k)fluoranthene; 11. benzo(a)pyrene; 12. dibenz(ah)anthracene; 13. benzo(ghi)perylene; 14. indeno(1,2,3-cd)-pyrene. 'X' is an unresolved mixture (discussed in Section II.1.3.- (b).)

The peaks numbered in the sediment extract were identified by their respective retention times, co-injections with authentic standard PAH, stop-flow UV scanning (to assess peak purity by comparison with UV spectra of authentic standards (Fig. 2.3.; Section II.1.3.(b). (ii.)) and routinely by UV and fluorescence response ratios (Table 2.1.). Additional peaks in the sediment extract separation include unidentified parent PAH and alkylated homologues. Although the parent compounds are shown in the analysis to dominate the chromatogram, the unresolved small peaks and area under the chromatogram undoubtedly contain a complex mixture including alkylated PAH often resolved by capillary gas-chromatography (Section I.3.3., Fig. 1.6.). Alkylated homologues are, however, selectively separated from their parent hydrocarbons on ODS phases 6.
Figure 2.3.

Analyses of a PAH standard mixture and a sediment extract utilising stop-flow UV scanning between 200 and 400 nm. Separations were obtained using a Perkin Elmer 10 µm HC ODS column (250 mm x 2.6 mm i.d.) with acetonitrile : distilled water gradient elution at a flow rate of 0.5 cm³.min⁻¹ (as detailed in Section II.1.3.(b). (ii)). The chromatograms shown centrally represent UV absorbance at 254 nm. UV scans were performed by initially calibrating the electronic memory of the Perkin Elmer LC 75 detector (+ autocontrol) at the base of each peak in turn, resuming flow and stopping on the peak in order to perform the scan. Absorbance ranges were selected to obtain on-scale deflection. Standard PAH scans are identified by figures and correspond to, 1. phenanthrene; 2. anthracene; 3. fluoranthene; 4. pyrene; 5. benzo(a)anthracene; 6. benzo(e)pyrene; 7. benzo(k)fluoranthene; 8. benzo(a)pyrene; 9. dibenz(ah)anthracene; 10. benzo(ghi)-perylene; 11. indeno(1,2,3-cd)pyrene. Peaks with retention times corresponding to the above standards are identified consecutively by letters and their respective scans are arranged opposite the corresponding standard spectra. Comparison affords a high degree of identification and, in the case of dibenz(ah)anthracene (peak 9), demonstrates a co-eluting interference in the equivalent peak (i) of the sediment extract.
Table 2.1.
UV absorbance and fluorescence ratios used routinely for verification of peak purity in HILC separations of environmental extracts. The responses recorded were obtained from individual detectors arranged in series. The table is divided into two sections. The upper portion reports peak heights (for UV absorbance at 254 and 280 nm, and fluorescence (Section II.1.3b(ii)) and selected ratios, for three individual HILC separations of PAH standards. The average values and standard deviations of the ratios calculated from these injections are shown in the boxed section. For comparison, the corresponding results from the analyses of three independent estuarine sediment extracts are shown in the lower section. 'n' indicates that the component was not quantified.
<table>
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<th></th>
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<th>Pyrene</th>
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<td>1</td>
<td>9</td>
<td>6</td>
<td>n</td>
<td>1</td>
<td>19</td>
<td>2</td>
<td>6</td>
<td>4</td>
<td></td>
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<tr>
<td>Peak height (Fluor.)</td>
<td>22</td>
<td>43</td>
<td>23</td>
<td>6</td>
<td>n</td>
<td>9</td>
<td>n</td>
<td>38</td>
<td>11</td>
<td></td>
<td></td>
</tr>
<tr>
<td>F : 280</td>
<td>4</td>
<td>6</td>
<td>1.3</td>
<td>2.0</td>
<td>-</td>
<td>12</td>
<td>0.3</td>
<td>1.5</td>
<td>2</td>
<td>1.5</td>
<td></td>
</tr>
<tr>
<td>254 : 280</td>
<td>5.5</td>
<td>43</td>
<td>2.5</td>
<td>1</td>
<td>9</td>
<td>-</td>
<td>-</td>
<td>6</td>
<td>2.7</td>
<td></td>
<td></td>
</tr>
<tr>
<td>280 S.D.</td>
<td>3.0</td>
<td>1.3</td>
<td>0.2</td>
<td>0.2</td>
<td>-</td>
<td>3.0</td>
<td>0.5</td>
<td>0.1</td>
<td>0.1</td>
<td>0.2</td>
<td></td>
</tr>
<tr>
<td>280</td>
<td>5.6</td>
<td>44.3</td>
<td>1.9</td>
<td>1.1</td>
<td>-</td>
<td>7.2</td>
<td>0.4</td>
<td>-</td>
<td>6.0</td>
<td>2.4</td>
<td></td>
</tr>
<tr>
<td>254 : 280</td>
<td>5.4</td>
<td>5.1</td>
<td>0.5</td>
<td>0.2</td>
<td>-</td>
<td>1.6</td>
<td>-</td>
<td>-</td>
<td>0.7</td>
<td>0.3</td>
<td></td>
</tr>
<tr>
<td>280 S.D.</td>
<td>4.4</td>
<td>2.1</td>
<td>0.1</td>
<td>0.1</td>
<td>-</td>
<td>1.6</td>
<td>-</td>
<td>-</td>
<td>0.7</td>
<td>0.3</td>
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<table>
<thead>
<tr>
<th>SEDIMENT EXTRACTS</th>
<th>254 %</th>
<th>280 %</th>
<th>F %</th>
<th>280 S.D.</th>
</tr>
</thead>
<tbody>
<tr>
<td>Peak height @ 254 nm</td>
<td>18</td>
<td>5</td>
<td>8</td>
<td>5</td>
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<tr>
<td>Peak height @ 280 nm</td>
<td>4</td>
<td>1</td>
<td>8</td>
<td>8</td>
</tr>
<tr>
<td>Peak height (Fluor.)</td>
<td>26</td>
<td>40</td>
<td>12</td>
<td>8</td>
</tr>
<tr>
<td>254 : 280</td>
<td>6</td>
<td>40</td>
<td>1.5</td>
<td>1</td>
</tr>
<tr>
<td>280 %</td>
<td>4.2</td>
<td>6.2</td>
<td>1.1</td>
<td>2.0</td>
</tr>
<tr>
<td>280 %</td>
<td>0.3</td>
<td>1.3</td>
<td>0.2</td>
<td>0.1</td>
</tr>
<tr>
<td>F %</td>
<td>5.6</td>
<td>44.3</td>
<td>1.9</td>
<td>1.1</td>
</tr>
<tr>
<td>280 S.D.</td>
<td>0.4</td>
<td>5.1</td>
<td>0.5</td>
<td>0.2</td>
</tr>
</tbody>
</table>
Figure 2.4.
(a) Stop-flow UV spectrum of the large peak denoted by the letter 'X' (retention time 57 minutes) in the sediment extract chromatogram shown in Fig. 2.1. Similar spectra were obtained for the peak with a retention time of 25 minutes in Perkin Elmer ODS HPLC separations of environmental extracts (denoted by 'X' in Fig. 2.2.).
(b) Stop-flow UV scans of peak 'X' in some environmental extract separations obtained using the Perkin Elmer column, showed a chrysene spectrum superimposed onto that typical of peak 'X' (as shown in part (a) of this figure).
Figure 2.5.

GC-MS analysis of peak 'X' - trapped from the HPLC separation of an estuarine sediment sample (Fig. 2.1.) and extracted into dichloromethane.

GC-MS analysis was performed by Mrs. A.P. Gowar, Bristol University.

System: Finnigan 4000 capillary GC-MS; INGOS data system.

Column: Flexsil Hewlett Packard 25 m methyl silicone fluid 0.2 mm i.d.

Temperature Programme: 50 - 260°C @ 5°C.min⁻¹, isothermal at 260°C.

Injector temperature 280°C; Interface temperature 280°C.

M.S. Mode: Centroid positive ion, Scans 1 to 265.

Electron multiplier 1980V; Emission current 350 µA.

Electron voltage 40 eV.

Reconstituted ion traces (RIC) are shown for the majority of the GC separation (scans 300-2300) and for an expanded portion (scans 1200-2300). Base peak m/e 149 is highly significant for phthalates and in the top diagram selection for m/e 149 isolates three peaks. Most peaks eluting after 20 minutes (lower diagram) give base peak m/e 49 (suggested by the INGOS library to be indicative of phenyl esters). The poorly resolved peak (retention time approximately 17 minutes) is identified by its mass spectra to be sulphur (Fig. 2.6.). Letters (a), (b) and (c) refer to scans 1152, 1785 and 1052 respectively, the mass spectra of which are shown in Fig. 2.6.
Phthalates

Sulphur

Phenyl Esters / Ethers
Selected mass spectra from the GC-MS analysis (Fig. 2.5.) of peak 'X' (Fig. 2.1.). The mass spectra (a), (b) and (c) correspond to scans 1152, 1785 and 1052 respectively of the GC-MS analysis. The peak m/e 149 in (a) is highly indicative of a phthalate. Mass spectrum (b), with base peak m/e 49 was identified by the INCOS library to be a phenyl ester although could possibly have resulted from a phenyl ether. (c) is representative of the poorly resolved peak in Fig. 2.5, and identifies the peak as sulphur.
(a) Phthalate

(b) Phenyl Ester / Ether

(c) Sulphur
standards (donated by various institutes) prepared at known concentrations in acetonitrile. The use of peak area integration was attempted but instability of the base lines, particularly with wavelength programmes (Fig. 2.1.), presented difficulties. Linear calibration graphs were, however, obtained using peak height measurements. Calibrations were performed routinely at the start of each analytical session and dispersed between analyses of environmental samples. The concentrations of PAH in the environmental extracts (calculated from the chromatograms) were corrected for blanks and extraction efficiencies (Section II.1.3.(b),(iii).) prior to final calculation of concentrations of PAH in the environmental samples.

(iii) Work-up efficiencies

To estimate the analytical efficiencies for the procedures summarised above, reproducibilities, re-extraction blanks and recoveries of spiked PAH were determined using water and sediment samples from the Tamar Estuary. Water samples were not filtered in these experiments for the reasons discussed in Section II.1.3.(a). Efficiencies of the combined operations of extraction, clean-up and HPLC analysis (using a Hypersil ODS column - Section II.1.3.(b),(ii)). for analyses of water and sediment samples are summarised in Tables 2.2. and 2.3., respectively.

Recoveries of triplicate spikes of the individual compounds (excluding naphthalene) from pre-extracted estuarine waters varied between 78 to 110% with average standard deviations of ± 8% (Table 2.2.). Comparable extraction efficiencies were obtained by Sorrell and Reding using a similar technique. Re-extracts were
Table 2.2.

Analytical efficiencies for the extraction and analysis of PAH in waters of the Tamar Estuary

<table>
<thead>
<tr>
<th>Compound</th>
<th>Reproducibility (1) (ng.dm(^{-3}))</th>
<th>Re-extraction blank (2) (% of initial extract)</th>
<th>Recovery of spikes (3) (% ± s.d.)</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>mean ± s.d.</td>
<td></td>
<td></td>
</tr>
<tr>
<td>Naphthalene</td>
<td>15.3 ± 20.0</td>
<td>b</td>
<td>40 ± 20</td>
</tr>
<tr>
<td>Phenanthrene</td>
<td>5.1 ± 0.5</td>
<td>b</td>
<td>105 ± 15</td>
</tr>
<tr>
<td>Anthracene</td>
<td>4.5 ± 0.4</td>
<td>10</td>
<td>110 ± 15</td>
</tr>
<tr>
<td>Fluoranthene</td>
<td>9.6 ± 0.5</td>
<td>b</td>
<td>106 ± 10</td>
</tr>
<tr>
<td>Pyrene</td>
<td>15.0 ± 2.2</td>
<td>7</td>
<td>84 ± 8</td>
</tr>
<tr>
<td>Chrysene</td>
<td>2.7 ± 0.1</td>
<td>b</td>
<td>95 ± 7</td>
</tr>
<tr>
<td>Benzo(a)anthracene</td>
<td>5.5 ± 0.1</td>
<td>b</td>
<td>96 ± 8</td>
</tr>
<tr>
<td>Benzo(b)fluoranthene</td>
<td>6.4 ± 1.1</td>
<td>b</td>
<td>100 ± 10</td>
</tr>
<tr>
<td>Benzo(k)fluoranthene</td>
<td>3.1 ± 0.4</td>
<td>b</td>
<td>97 ± 10</td>
</tr>
<tr>
<td>Benzo(a)pyrene</td>
<td>7.5 ± 1.4</td>
<td>b</td>
<td>78 ± 4</td>
</tr>
</tbody>
</table>

1. Overall reproducibility of triplicate samples including the contributions from sampling, extraction, clean-up and HPLC analysis.
2. Re-extraction of an estuarine water sample providing an assessment of the initial extraction efficiency and includes contributions from contamination via the analytical procedures.
3. Indicates that any compound present was below the limits of detection.

Table 2.3.

Analytical efficiencies for the extraction and analysis of PAH in sediments of the Tamar Estuary

<table>
<thead>
<tr>
<th>Compound</th>
<th>Reproducibility (1) (ng.g(^{-1}) dry sed.)</th>
<th>Re-extraction blank (2) (% of initial extract)</th>
<th>Recovery of spikes (3) (% ± s.d.)</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>mean ± s.d.</td>
<td></td>
<td></td>
</tr>
<tr>
<td>Naphthalene</td>
<td>290 ± 185</td>
<td>5</td>
<td>72 ± 29</td>
</tr>
<tr>
<td>Phenanthrene</td>
<td>580 ± 35</td>
<td>2</td>
<td>100 ± 5</td>
</tr>
<tr>
<td>Anthracene</td>
<td>190 ± 7</td>
<td>b</td>
<td>103 ± 3</td>
</tr>
<tr>
<td>Fluoranthene</td>
<td>1820 ± 83</td>
<td>2</td>
<td>98 ± 5</td>
</tr>
<tr>
<td>Pyrene</td>
<td>1810 ± 93</td>
<td>2.5</td>
<td>94 ± 4</td>
</tr>
<tr>
<td>Chrysene</td>
<td>1040 ± 106</td>
<td>1</td>
<td>74 ± 5</td>
</tr>
<tr>
<td>Benzo(a)anthracene</td>
<td>640 ± 18</td>
<td>1</td>
<td>85 ± 6</td>
</tr>
<tr>
<td>Benzo(b)fluoranthene</td>
<td>820 ± 99</td>
<td>b</td>
<td>82 ± 4</td>
</tr>
<tr>
<td>Benzo(k)fluoranthene</td>
<td>570 ± 9</td>
<td>b</td>
<td>86 ± 8</td>
</tr>
<tr>
<td>Benzo(a)pyrene</td>
<td>1210 ± 30</td>
<td>b</td>
<td>86 ± 10</td>
</tr>
</tbody>
</table>

1. Overall reproducibility of triplicate sub-samples of a homogenised sediment including contributions from the analytical techniques.
2. Re-extraction of a sediment sample, providing an assessment of the initial extraction efficiency and includes contributions from contamination via the analytical procedures.
3. Indicates that any compound present was below the limits of detection.

35
low (Table 2.2.) and for most compounds were below the detection limits for the technique, which were typically 0.1-0.6 ng dm⁻³ for individual compounds. Owing to the high volatility of naphthalene, extraction efficiencies and reproducibilities for this compound were low (Table 2.2.) but were considered adequate to obtain an indication of the behaviour of the compound in the estuary.

Recoveries of triplicate spikes of PAH from pre-extracted sediment varied between 72 to 103% with average standard deviations of ± 3% (± 2% for naphthalene). Attempts to improve the precision of the naphthalene analysis by adopting the analytical method of Gschwend and Hites were inconclusive. The generally improved analytical precision compared to water sample analyses, results from the higher concentrations of PAH extracted from sediments. Re-extracts of sediments amounted to less than 1% of PAH in the original extract. Detection limits (taken as 2 x baseline noise) for the HPLC system, with detection using a variable wavelength UV programme (Section II.1.3.(b).(ii).), were below 1 ng injected for all compounds analysed. Recoveries from sediments spiked with compounds not separated by the Hypersil ODS column (and hence not shown in Table 2.3.) but determined using the Perkin Elmer ODS column (Section II.1.3.(b).(ii).) (including benzo(a)pyrene, perylene, dibenz[ah]anthracene, benzo(ghi)perylene and indeno(1,2,3-cd)pyrene) were typically in excess of 90%.

II.1.4. $^{210}$Po activity

$^{210}$Po geochronology was used in the dating of a sediment core sampled from the Tamar Estuary (Chapter IV). $^{210}$Po
activity was measured by counting the $\alpha$-activity of its daughter product $^{210}\text{Po}$. The method used was essentially that of Clifton and Hamilton. Freeze-dried, lightly ground samples (2 g) were spiked with $^{208}\text{Po}$ as an internal standard. The sediments were then dried, treated with 5 cm$^3$ concentrated $\text{HNO}_3$, warmed, concentrated $\text{HCl}$ added to expel the $\text{HNO}_3$ and then leached with 6 M-$\text{HCl}$ (1 h, 90°C). The volume was then adjusted to 20 cm$^3$ and the extract centrifuged. The separated $^{210}\text{Po}$ was plated onto silver planchets according to the procedures of Flynn. The $^{210}\text{Po}$ activity was determined by alpha spectrometry using an Ortec $^R$ 200 mm$^2$ silicon surface barrier detector coupled to a Canberra (Series 80) Multi-Channel Analyser. Samples were counted for 10$^3$ minutes and results corrected for background activity. $^{210}\text{Po}$ activity was then calculated based on its counts relative to the recovery of $^{208}\text{Po}$ internal standard counts.

II.1.5. Statistical treatment of results

The large number of possible paired correlations between individual PAH and associated environmental variables (Chapters IV and V) require non-parametric procedures for their statistical appraisal. Initially, Pearson product-moment correlation coefficients ($r$) were calculated for all pairs of variables to assess the covariability of individual constituents. The resultant correlation matrix can be further analysed by correlation profile analysis (C.P.A.) clustering and multi-dimensional scaling (M.D.S.) C.P.A. allows a simple graphical comparison of the correlation matrix derived from the data set with matrices of
identical size but containing randomly generated numbers. In effect, this technique is a global test of whether there are any significant correlations in the data and, if so, how many (Section V.3.1.(a).). Cluster analysis graphically summarises the correlations between individual variables in similarity dendrograms (Section V.3.1.(a).). M.D.S. summarises the correlations in the data by representing the variables (PAH and environmental data) as points in the two dimensional M.D.S. space; the closer the points, the stronger the correlation between those variables (Sections IV.4.6. and V.3.1.(e).). The origin, orientation and the scale of the M.D.S. plot are arbitrary. Mantoura et al. have critically evaluated the use and merits of M.D.S. in environmental organic chemistry.

Dr. K.R. Clarke (I.M.E.R.) performed the complex computing essential in the application of the techniques.

II.2. EXPERIMENTAL ANALYSES

II.2.1. Radiochemical Experiments

(a) Microbial Heterotrophic Degradation Studies

Microbial degradation of soluble organic compounds and hydrocarbons in natural waters has been assessed by incubating the radiolabelled material in environmental samples. Heterotrophic degradation of $^{14}$C-labelled naphthalene and benzo(a)pyrene was determined in selected water samples from the Tamar Estuary using a radiochemical technique similar to that of Lee.
Compounds used were $1(4,5,8)^{14}$C naphthalene (5 mCi/m mole, Amersham) and 3,4-benz, 7,10-$^{14}$C pyrene (18.6 mCi/m mole, Amersham). Dissolution of the crystalline naphthalene supplied was performed by shaking the ampoule (delabelled and pre-cleaned with acetone and suitably scored) in a robust, sealed, pre-cleaned glass vessel containing distilled water (30 cm$^3$). The crystalline benzo(a)pyrene supplied was prepared by dissolution in acetone through the broken neck of the ampoule and transferred with washings into a secure vial. The purities of radiochemical stocks were assessed by thin-layer chromatography (on silica gel-G plates developed in hexane) with subsequent analysis using a Berthold Automatic Scanning 2π-détector for TDC. β-activities and hence concentrations of aliquots of stock solution were assessed by liquid scintillation counting (Packard Model 3255) using a suitable scintillant and appropriate quench correction. Dilutions were performed to produce suitably concentrated stock solutions allowing minimal spike volumes (50 mm$^3$, naphthalene; 10 mm$^3$, benzo(a)pyrene).

Naphthalene and benzo(a)pyrene were spiked at 3 μg.dm$^{-3}$ and 20 μg.dm$^{-3}$ respectively into triplicate environmental water samples (100 cm$^3$) in sealed glass flasks (100 cm$^3$) and incubated at 10°C in darkness for 20 hours (naphthalene) and 165 hours (benzo(a)pyrene). Duplicate controls, consisting of sub-samples poisoned with mercuric chloride (1 mg.dm$^{-3}$), were treated in the same way as experimental samples.

Following the incubation period, samples were poisoned with mercuric chloride (100 mm$^3$ of 1 g.dm$^{-3}$ HgCl$_2$). An aliquot of the sample water (20.0 cm$^3$) was transferred into a separating funnel (50 cm$^3$) and extracted (2 min) with cyclohexane (10 cm$^3$). After
phase separation (10 min) the hexane layer was transferred into a scintillation vial in order to assess the undegraded PAH remaining in solution. The $^{14}$CO$_2$ in the remaining sample was then collected following acidification (1 cm$^3$ M HCl) using the apparatus shown in Fig. 2.7. Experiments with Na$_2^{14}$CO$_3$ solutions demonstrated that >95% $^{14}$CO$_2$ activity (liberated by acidification) was trapped following the passage of nitrogen (flow rate 400 cm$^3$.min$^{-1}$) for 7 minutes. $^{14}$CO$_2$ was trapped in $^{14}$C-absorber P scintillation cocktail. The trapping apparatus was rinsed thoroughly (three successive washings with methanol) between samples. An aliquot (5.0 cm$^3$) of the remaining acidified solution was then transferred to a scintillation vial to determine the amount of hydrocarbon metabolites by difference. Scintillants were added to the cyclohexane (Fisons Fisofluor 3, 10 cm$^3$) and aqueous (Triton Cocktail N, 15 cm$^3$) samples, and following thorough mixing, activity in all vials was measured using a liquid scintillation counter (Packard Model 3255). Corrections for quenching by method of external standards were applied. Prior to interpretation of the data, activities were balanced to account for relative volumes of sample assayed.

(b) Adsorption of PAH onto estuarine particulates

Radiolabelled naphthalene and benzo(a)pyrene were also used in experiments to assess adsorption of spiked PAH onto suspended particulates in estuarine water samples. The standard $^{14}$C-labelled compounds (of proven high purity, as prepared in Section II.2.1.(a).) were spiked in minimal volumes into triplicate aliquots of estuarine water (100 cm$^3$), to produce concentrations of 7 and 0.8 µg.dm$^{-3}$ for
Figure 2.7.
Apparatus used to trap the $^{14}$C-CO$_2$ produced by microbial heterotrophic degradation of 'spiked' $^{14}$C-labelled PAH in estuarine water samples. The CO$_2$ is liberated by acidification of the sample and is then carried into the spiral CO$_2$ trap (containing Absorber P scintillation cocktail) by flushing with nitrogen (Section II.2.1.(a)).
naphthalene and benzo(a)pyrene respectively. Duplicate control experiments were conducted using filtered sub-samples. Following constant agitation (Gallenkamp orbital shaker) (20°C in darkness) for pre-determined equilibration times (Section III.3.), the PAH remaining in solution was assayed: Aliquots (2 cm³) of the incubated water samples were removed and centrifuged (2000 rpm., 5 min) in pre-cleaned, sealed centrifuge tubes. Measured volumes of the supernatant were then transferred into scintillation vials, scintillant (10 cm³, Fisofluor 2) added, and the contents mixed thoroughly. Following β-activity counting (Packard Model 3255 Scintillation Counter) results were corrected for quenching (Section II.2.1.(a)).

II.2.2. Direct analysis of PAH in experimental waters by High-Performance Liquid Chromatography (HPLC).

In order to monitor the fate of PAH in model aqueous systems, a rapid HPLC technique was developed, capable of separating and quantifying mixtures of dissolved compounds. PAH were selected to span a range of molecular weights and hence physical and chemical properties. Those chosen were, naphthalene, anthracene, fluoranthene, benz(a)anthracene and benzo(a)pyrene.

(a) Analytical Design

With the exception of naphthalene, fluorescence detection provides the most sensitive and selective form of quantification. Elution conditions were designed to minimise analysis time whilst retaining baseline resolution of the individual components.
Acetonitrile : distilled water elution was chosen (Section II.1.3. (b).(ii).). Isocratic elution was selected because, with the fluorescence detector adjusted for maximum sensitivity, step and linear gradients were found to produce unacceptable baseline shifts. A suitable separation was obtained in 8 minutes using a Hypersil 5-7 μm ODS column (100 mm x 5 mm i.d.) with 70% acetonitrile : 30% distilled water elution at a flow rate of 2 cm³.min⁻¹ (Fig. 2.8.(a)). A Kratos FS950 Fluoromat Fluorometer was found to provide higher sensitivity than either the Perkin Elmer M4F 3 spectrofluorometer fitted with an HHC micro-flow cell or the Dupont 836 UV/fluorescence HPLC detector. Excitation at 254 nm with an interference filter, and emission detection >320 nm proved most suitable. Excitation at 360 nm with detection at >418 nm resulted in slightly increased sensitivity for the larger molecular weight PAH, but considerably reduced sensitivity for the lower molecular weight homologues. Standard solutions were prepared by dissolving known amounts of individual high purity PAH in ethanol. A standard mixture (balanced for the detection system) for spiking into the environmental waters was prepared (Table 2.4.). For calibration purposes an aliquot (50 mm³) of this mixture was spiked into 50% acetonitrile : 50% distilled water (100 cm³) (100% acetonitrile injections were found to deleteriously modify the chromatography). A range of injection volumes was introduced into the chromatographic system (Fig. 2.8.(b)) using a Waters U6K injector. Syringe washings (50 mm³, 50% acetonitrile : 50% distilled water) were co-chromatographed with sample injections.
Figure 2.8.

(a) HPLC chromatogram of the PAH selected for experimental study. The chromatographic system employed a Hypersil 5-7 μm ODS column (100 mm x 5 mm i.d.) with elution by 70% acetonitrile : 30% distilled water at a flow rate of 2 cm².min⁻¹. Detection was by a Kratos Fluoromat fluorometer with excitation at 254 nm, emission > 320 nm and adjusted for maximum sensitivity. The peaks correspond to 2000, 3, 600, 210 and 130 pg injected respectively for naphthalene, anthracene, fluoranthene, benzo(a)anthracene and benzo(a)pyrene.

(b) Calibration graphs for the individual PAH using the chromatographic system described above.
(b) Experimental Design

Although potentially useful for a number of experimental applications the 'direct injection' system described above was primarily used to assess adsorption of PAH onto estuarine particulates suspended in the water column. Gas-tight borosilicate glass incubation vessels (100 cm$^3$) were shown to adsorb less PAH (particularly the higher molecular weight homologues) than other glassware tested and PTFE-coated centrifuge tubes. Bottles were acid washed and rinsed with sample water immediately prior to incubation. Triplicate sub-samples (100 cm$^3$) of selected estuarine waters were spiked with a standard mixture of PAH (50 mm$^3$) (Table 2.4). Duplicate controls of filtered (Whatman GF/C) sub-samples were treated in the same way as the experimental samples. Poisoning of waters to prevent microbial degradation was not performed owing to the potential for the poisoning agent to interfere with the natural sorption chemistry. The vessels were agitated (Gallenkamp orbital shaker) (in darkness at 20°C) for a predetermined period of time (selection of the incubation period is discussed in Section III.3.). Each sample was then vigorously shaken and an aliquot (5.00 cm$^3$) transferred into a precleaned centrifuge tube which was immediately sealed with an aluminium foil lined bung to prevent volatilization of naphthalene. Particulates were separated by centrifugation (2000 rpm., 5 min) and a sub-sample (500 mm$^3$) of the aqueous phase transferred into a vial (2 cm$^3$) containing acetonitrile (500 mm$^3$), added to prevent degradation and sorption of PAH onto the vessel walls during subsequent storage.

For selected samples, PAH adsorbed onto the centrifuged particulates were analysed by decanting the remaining aqueous phase
in the centrifuge tube and extracting the particulates with acetonitrile (1.00 cm$^3$). Extraction was facilitated by thorough vortex mixing (2 min). The tube walls were then rinsed with distilled water (1.00 cm$^3$). Particulates were centrifuged down and an aliquot (1 cm$^3$) of the supernatant transferred into a vial (2 cm$^3$) ready for HPLC. All vials awaiting analysis were sealed with aluminium foil lined caps and retained in darkness. Aliquots (200 mm$^3$ of the aqueous sample, 40 mm$^3$ of the sediment extract) were analysed by HPLC according to Section II.2.2.(a).

Table 2.4.

<table>
<thead>
<tr>
<th>Compound</th>
<th>PAH concentrations in standard stock mixture (ng.cm$^{-3}$)</th>
<th>PAH concentrations in experimental waters (μg.dm$^{-3}$)</th>
<th>Solubility (μg.dm$^{-3}$)</th>
<th>Detection limit (μg injected)</th>
</tr>
</thead>
<tbody>
<tr>
<td>Naphthalene</td>
<td>40000</td>
<td>20</td>
<td>31300(a)</td>
<td>400</td>
</tr>
<tr>
<td>Anthracene</td>
<td>67</td>
<td>0.03</td>
<td>44.6(b)</td>
<td>0.3</td>
</tr>
<tr>
<td>Fluoranthene</td>
<td>12000</td>
<td>6</td>
<td>206(c)</td>
<td>75</td>
</tr>
<tr>
<td>Benz(a)anthracene</td>
<td>4270</td>
<td>2.1</td>
<td>9.8(c)</td>
<td>35</td>
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<tr>
<td>Benzo(a)pyrene</td>
<td>2670</td>
<td>1.3</td>
<td>0.172(d)</td>
<td>25</td>
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</table>

1. Standard mixture prepared in ethanol (balanced for detection).
2. Concentrations in estuarine waters and calibration standard after dosing of 50 mm$^3$ of stock PAH mixture.
3. Literature values for solubility in distilled water (25-30°C).
   (a) Eganhouse and Calder (reference 115), (b) May et al. (reference 116), (c) May et al. (reference 117),
   (d) Lu et al. (reference 118).
4. Detection limit = 2 x baseline noise.
REFERENCES


47. Chapter 5 in Reference 9.
56. Chapter 4 in Reference 9.


90. Chapter 7 in Reference 9.


CHAPTER III

THE ASSOCIATION BETWEEN PAH AND ESTUARINE PARTICULATES
The transport and fate of hydrophobic pollutants in natural water systems are highly dependent on the sorptive behaviour of the compounds onto particulate material. Börnfeld \(^1\) demonstrated that suspended solids in natural waters accumulated PAH to concentrations several thousand-fold greater than those observed in the water. Adsorption of PAH has been shown to occur in the presence of substrates such as activated carbon \(^2\), minerals \(^2\), clays \(^3,4\), humic acids \(^5\) and microbes \(^5\). Herbes \(^6\) has demonstrated the importance of suspended organic material in sorption of PAH.

The chemical variability and complexity of natural sediments, together with the potential for a large number of sorptive interactions, foiled attempts to produce predictive adsorption models until comparatively recently. Initial work on systemization and estimation of sorption in natural systems was performed by soil chemists working on pesticides. Lambert \textit{et al.} \(^7,8\) demonstrated that sorption of hydrophobic pesticides could be correlated to organic carbon content of soils. They postulated that hydrophobic sorption may be analogous with partitioning of organic compounds between aqueous and organic solvent phases. Briggs \(^9\) developed a regression equation relating the sorption of herbicides on soils to their octanol-water partition coefficients. Marickhoff \textit{et al.} \(^10\) applied these findings to PAH and sediments, and demonstrated a high correlation between organic carbon content of the sediments and PAH sorption. Means \textit{et al.} \(^11\) confirmed this relationship and also suggested that adsorption of PAH on sediments and soils is only marginally affected by the pH of solution, cation exchange capacity,
texture (particle size distribution) and clay mineralogy. A recent paper by Karickhoff showed that partition coefficients of hydrophobic pollutants, normalised to their organic carbon content, were highly invariant over a wide range of sediments and soils. Equations for estimating adsorption of PAH from data on water solubility and octanol-water partition coefficients were developed and successfully tested against sorption data in the literature. Schwarzenbach and Westall and Chiu et al. have derived similar equations and discuss the use of models in estimating sorption of hydrophobic compounds. Southworth and Herbes et al. have investigated sorption of PAH in context with other environmental fates (Section 1.4.3.).

Most of the above studies investigated adsorption of PAH in relation to particulate chemistry and were performed in distilled or fresh water systems rather than saline waters. Karickhoff et al. suggested an approximately linear increase between the adsorption of pyrene and aqueous salt (NaCl) concentration. In studies on the environmental fate of selected PAH in estuarine waters, Lee and co-workers identified the importance of adsorption in the turbid conditions often associated with estuaries.

There have been no systematic investigations discussing particulate sorption of PAH with respect to the physico-chemical and hydrodynamic gradients encountered in estuaries. The multiplicity of particulate sources and characteristics represents another important estuarine feature for consideration in assessing sorption. This chapter discusses the aforementioned factors in relation to the particulate sorption of PAH in the Tamar Estuary. Results from sorption experiments, which predict the equilibrium
partition of PAH, are then discussed in context with results obtained from analyses of in situ environmental PAH associated with the aqueous and particulate phases of fractionated estuarine water samples.

III.2. SAMPLE COLLECTION

The positions of the sample sites are shown in Fig. 3.1. Sampling was performed on the ebb tide on the 14th January 1982. High tide on this day was 9.01 am. Sampling commenced at 10.30 am. Surface water samples were collected during an axial traverse of the estuary (fresh water to marine) aboard a Rotork Seatruck. Samples were taken using a stainless steel bucket pre-rinsed with sample water. Aliquots (2 dm$^3$) for adsorption studies and particulate characterisation were transferred to Winchester bottles (pre-rinsed with concentrated hydrochloric acid and then distilled water and, immediately prior to sampling, with sample water). Sub-samples (2 x 2.5 dm$^3$) of water from stations 3 and 7 were also removed into pre-cleaned Winchester bottles for PAH analysis of the dissolved and particulate phases. Possible contamination from the vessel's stern engines was avoided by sampling at the extreme bow of the Seatruck. Sample flasks were sealed with solvent rinsed aluminium foil lined caps and retained in darkness throughout transport back to the laboratory. During the transect of the estuary, salinity, turbidity and temperature were continuously recorded.
Figure 3.1.
Description of the Tamar Estuary and sampling stations. Water sample numbers (1-9) are identified by the large figures with their respective locations indicated by crosses. Distances upstream from the narrows (point O) are shown in 5 km intervals. Plymouth is indicated by the hatched area with the dockyards located along the estuarine foreshore demarked by stippled.
Salinity, temperature and turbidity were monitored according to Section II.1.1.(a). Total suspended particulates were determined gravimetrically (Section II.1.1.(b).) and particulate organic carbon and nitrogen (POC; PON) were measured on sub-samples of the isolated particulates using a Carlo Erba Elemental Analyser (Section II.1.2.(a).). Particle size analyses were performed using a Malvern Laser Particle Sizer (Section II.1.1.(b).).

The analytical and experimental designs for assessment of PAH-partitioning between the aqueous and particulate phases of estuarine water samples are described in Sections II.2.2.(a), and II.2.2.(b). Karickhoff et al. have demonstrated that, providing the adsorption capacity is not exceeded, PAH in a mixture sorb independently of one another. This enables the adsorption characteristics of several PAH to be determined simultaneously.

In order to take full advantage of the speed of direct injection analyses, the adsorption of PAH onto particulates was assessed by monitoring the losses of PAH from solution. The validity of this approach was confirmed in selected samples after recovering PAH from suspended particulates (Section II.2.2.(b).) and constructing a mass balance. Calculation of recoveries by both methods agreed within 10% ± 6%.

Prior to the environmental survey it was necessary to (i) determine the times required to achieve adsorption equilibrium, & (ii) to demonstrate linearity of the sorption isotherms.
(i) Determination of equilibration time:

Times required to achieve adsorption equilibrium were evaluated by monitoring the removal rates of spiked PAH from the aqueous phase of an estuarine water sample relative to a filtered control. It can be shown that the fraction adsorbed \( F \) is equal to:

\[
F = \frac{M_W^C - M_W}{M_W^C} = \left( 1 - \frac{M_W}{M_W^C} \right)
\]

where \( M_W \) and \( M_W^C \) are the masses of dissolved PAH in the experimental and control incubations, respectively.

Figure 3.2.

Graph showing the approach to partition equilibrium for benzo(a)pyrene, fluoranthene and naphthalene. The fraction of spiked PAH associated with the particulate phase of an estuarine water sample is plotted against the incubation time from spiking of the sample (taken as \( t = 0 \)). The vertical bars indicate precision of the analytical technique. The results of triplicate radio-chemical adsorption experiments (for \(^{14}C\) naphthalene and benzo(a)pyrene, incubated individually) performed on sub-samples of the same estuarine water are shown by the dashed lines.
Fig. 3.2. shows that adsorption was rapid in the initial minutes of incubation and then decreased to approach equilibrium within 6 hours. Extending the incubation periods to 10 hours resulted in marginal differences in sorbed PAH, which were within the experimental precision. An equilibration period of 7 hours was adopted since extended periods may lead to microbial degradation of PAH (Section V.3.5.). Fig. 3.2. also shows results from radiochemical experiments performed on sub-samples of the same estuarine water according to the method described in Section II.2.1.(b). Adsorption results from the two techniques compared favourably and further confirmed the validity of the HPLC technique. Precision of the HPLC method was notably reduced for benzo(a)pyrene determinations owing to the measurement of small peaks associated with reduced aqueous concentrations when adsorption was high. Precision of the naphthalene radiochemical assay was lower than that attained by the HPLC technique.

(ii) Adsorption Isotherms:

It is necessary to confirm that the adsorption experiments are conducted within the linear region of the adsorption isotherm. Fig. 3.3. shows that over the concentration range of PAH used in the experiments, the isotherms are linear and agree with results reported by Karickhoff et al. 10. This implies that classical non-linear adsorption isotherms (eg. Langmuir and Freundlich) 19 reduce to the form:

\[ C_s = K_p C_w \]

where \( C_s \) and \( C_w \) are the equilibrium concentrations of PAH sorbed onto particulates (ng.g\(^{-1}\)) and in solution (ng.cm\(^{-3}\)) respectively, and \( K_p \) is the linear adsorption (partition) coefficient.
Figure 3.3.
Equilibrium adsorption isotherms of PAH in a Tamar Estuary water sample (suspended particulate load 160 mg.dm$^{-3}$). Points are plotted as circles to approximate analytical precision. Resultant $K_p$ values derived from the graphs are 1740, 3000, 8330 and 28750 respectively for anthracene, fluoranthene, benz(a)anthracene and benzo(a)pyrene. Naphthalene demonstrated negligible particulate association and hence the relationship is not shown.
III.4. RESULTS AND DISCUSSION

III.4.1. Environmental Variables

The distribution of environmental variables and the physical and chemical properties of the suspended particulates are shown in Fig. 3.4. (and are listed in the Appendix - Table I). In agreement with previous studies of the Tamar Estuary, there is a tidally generated turbidity maximum \( \text{20} \). Compared to other stations, suspended solids in this region contain a higher proportion of large particulates (50-200 \( \mu \text{m} \)). Organic carbon composition decreases from the fresh water to marine stations. The particulates originating from the marine environment appear enriched in nitrogen relative to carbon as indicated by the decrease in C : N ratio. Factors controlling the physical and chemical characteristics of suspended particulates throughout the Tamar, have been discussed by Bale et al. \( \text{21} \) and Morris et al. \( \text{22} \).

III.4.2. Sorption of spiked PAH onto estuarine particulates

Results from the sorption experiments are listed in Table 3.1. The fraction of spiked PAH adsorbed onto particulates was calculated as previously described in Section III.3.

(a) The relationship between adsorption of spiked PAH and the suspended particulate loading

Plots of percentage adsorption against suspended particulate loadings are shown in Fig. 3.5.(a). The relatively high solubility
Figure 3.4.
Axial profiles of environmental variables and $K_p$ values for individual PAH. Sample stations are indicated at the top and bottom of the figure and represent the transition from fresh-water to the marine environment proceeding from left to right across the figure. Locations of the sample sites are shown in Fig. 3.1.). Ranges of the experimentally derived $K_p$ values (from triplicate incubations) are shown as vertical bars which are joined by a continuous line through the mean values. Owing to the low levels of suspended particulates in water sample 1, selected parameters could not be evaluated.
| Sample Site | Phthalates | n adsorbed | mean | range | Antipyrine | n adsorbed | mean | range | Fluoresphene | n adsorbed | mean | range | Bensal- | n adsorbed | mean | range | Summary of results from the adsorption experiments. The partition coefficients \( K_p \), \( K_{oc} \) and \( K_{oc} \) (sal.) and their respective derivations are discussed in the text (Section III.4.2.). | 113 |
of naphthalene (Table 3.3.) results in the vast majority of the compound remaining in solution. Higher percentages of PAH are adsorbed with increasing molecular weight of the compounds.

Figure 3.5.(a.) shows that the percentage of PAH adsorbed is highly dependent on the suspended particulate loading of the water sample. This variable is likely to exert the principal control on sorption of the individual PAH throughout the estuary. Since there is a wide range of suspended solid concentrations in the estuarine samples, it is useful to derive an expression relating the adsorption of PAH to suspended particulates. This can be derived from the linear adsorption isotherm 6 (Section III.3.):

\[ C_s = K_p C_w \] .............. 1

where \( C_s \) and \( C_w \) are the equilibrium concentrations of the compound in the particulate and aqueous phases respectively and \( K_p \) is the linear partition coefficient.

If \( M_s \) and \( M_w \) are the masses of the compound adsorbed and in solution respectively, \( C \) is the concentration of particulates and \( V \) the volume of solution then:

\[ C_s = \frac{M_s}{C.V} \quad \text{and} \quad C_w = \frac{M_w}{V} \] .............. 2

Substituting into equation 1 results in:

\[ \frac{M_s}{C} = K_p M_w \] .............. 3

The fraction of an individual PAH adsorbed (\( F \)) can be expressed as:

\[ F = \frac{M_s}{M_s + M_w} \] .............. 4
Combining equations 3 and 4:

\[ F = \frac{K_p C}{K_p C + 1} \]  

A linear expression is obtained by inversion of equation 5:

\[ \frac{1}{F} = \frac{1}{K_p} \left( \frac{1}{C} \right) + 1 \]  

If the adsorptive properties for any one PAH were identical for all the particulate materials encountered throughout the estuary, then a plot of \( \frac{1}{F} \) against \( \frac{1}{C} \) should be linear with a slope of \( \frac{1}{K_p} \), and an intercept of 1 on the \( \frac{1}{F} \) axis. Plots of \( \frac{1}{F} \) against \( \frac{1}{C} \) for the experimental results are shown in Fig. 3.5. A degree of linearity is demonstrated. Some of the deviation arises from samples containing low concentrations of suspended particulates, which results in reduced experimental precision. Linear regressions of the mean values are shown as dashed lines in Fig. 3.5. Overall \( K_p \) values calculated from the gradients of the regression lines are: anthracene, 5100; fluoranthene, 7900; benz(a)anthracene, 22200; benzo(a)pyrene, 39000. Individual \( K_p \) values (Table 3.1.) are shown in Fig. 3.4. together with environmental data associated with the individual sample stations. General trends indicated, are a reduction in particulate affinity for PAH from the riverine to mid-estuarine samples, with increasing \( K_p \) values in more saline and marine samples. Organic carbon content of sediments has been demonstrated to be of particular significance in PAH sorption. Organic carbon values decrease from the riverine input towards the sea, and could potentially be responsible for the reduction in \( K_p \) values towards the mid-estuarine region. Increase in salt concentration lowers the solubility of PAH, and would therefore
Figure 3.5.

(a) Graphs showing the percentage of spiked PAH associated with the particulate phase of the estuarine water samples, plotted against their respective suspended particulate concentrations. Station numbers associated with the individual samples are identified at the top of the diagram. Vertical bars indicate the range of adsorption recorded in triplicate samples, with the mean values identified by 'o'.

(b) Graphs showing plots of the reciprocal of the fraction of spiked PAH associated with the particulate phase of the estuarine water samples, plotted against the reciprocal of their respective suspended particulate concentrations. Individual sampling locations are indicated by the numbers inset on the individual PAH graphs. The vertical bars plotted indicate ranges of 1/fraction adsorbed for triplicate samples, with the mean values identified by 'o'. Linear regressions of the mean values are shown as broken lines. Kp values calculated from the gradients of the regressed lines are anthracene, 5100; fluoranthene, 7900; benz(a)anthracene, 22200; and benzo(a)pyrene, 39000.
increase partitioning onto particulates and may be responsible for the increase in $K_p$ towards the marine end. These two properties throughout the estuary will tend to counteract each other. Particle size differences could also be of significance.

(b) The influence of organic carbon on adsorption

To assess the effect of organic carbon on the adsorption of PAH, and its contribution to explaining the deviations shown in Figs. 3.4. and 3.5. (b), $K_p$ values were plotted against their respective fraction organic carbon ($f_{oc}$) values (Fig. 3.6.). These graphs do not show any consistent relationship with $f_{oc}$, suggesting that organic matter cannot, on its own, account for all differences in $K_p$. However, with the exception of benzo(a)pyrene, the PAH in samples 2-6 show the expected increase in adsorption with increasing $f_{oc}$ $^{10,11,12}$. The high salinity samples (7,8,9), although demonstrating high $K_p$ variability, show significant increases in particulate affinity for PAH.

The contribution of organic carbon in controlling adsorption of PAH onto estuarine particulates was removed by converting the $K_p$ data (Table 3.1.) into $K_{oc}$ values $^{10,11,12}$ using the expression:

$$K_{oc} = \frac{K_p}{f_{oc}}$$

The results are listed in Table 3.1. Fig. 3.8. shows variations in $K_{oc}$ throughout the estuary. Compared to the uncorrected $K_p$ data (Table 3.1., Fig. 3.4.), variability in the low salinity samples (1-6) is reduced. Anomalies with Samples 7,8 and 9 are increased by the organic carbon correction, owing to their low organic carbon content. The fact that these three samples (7-9) are saline ($>20\%$),
Figure 3.6.
Plots of Kp values against fraction organic carbon in particulates of the corresponding estuarine water samples. Respective sample stations are identified at the top of the diagram. Ranges of Kp values (derived from triplicate incubations) are shown as vertical bars, with each bar joined to adjacent plots through the mean value.
identifies salinity as a potentially important variable in controlling sorption.

(c) The effect of salinity on adsorption

The fundamental relationship describing the solubility \((S)\) of a non-polar compound in an electrolyte medium is described by the Setschenow equation

\[
\log \left( \frac{S_o}{S_s} \right) = K_s C_s
\]

where \(S_o\) and \(S_s\) are the solubilities of the solute in distilled and saline waters respectively, \(K_s\) is the Setschenow constant, and \(C_s\) is the molar salt concentration. This equation predicts that the greatest reduction in the solubility (and hence most salting out) would occur at low salinities. Earickhoff et al. \(^{10}\) suggested an approximately linear increase in \(K_p\) for adsorption of pyrene onto suspended sediments with the addition of salt (NaCl) to the aqueous phase (0-20 mg.cm\(^{-3}\) in 2 mg.cm\(^{-3}\) increments). A 15% increase in \(K_p\) was observed for pyrene from fresh to 20 mg.cm\(^{-3}\) saline water.

The results obtained from the Tamar are not compatible with the above. Most significant increases in \(K_{oo}\) are shown to occur in the high salinity samples (Fig. 3.7.).

To quantify the effect of salinity on particulate sorption of PAH, a model for predicting changes in \(K_{oo}\) with salinity was produced. Initially, theoretical PAH solubilities at the salinities of the sample waters were calculated using the Setschenow equation (reported earlier in this Section). (The published values for Setschenow constants and distilled water solubilities are given in Table 3.3.). Results are listed in Table 3.3. Regression equations relating
Figure 3.7.
Graphs showing Koc partition coefficients (Kp values normalised to organic carbon content) plotted against salinity. Respective sample stations are identified at the top of the diagram. Ranges of Koc values (calculated from the results of triplicate incubations) are shown as vertical bars, with each bar joined to adjacent plots through the mean value.
solubility with $K_{oc}$ have been derived from measured $K_{oc}$ values and solubilities of a range of hydrophobic compounds (including PAH):

From Karickhoff et al. $^{10}$ $\log K_{oc} = -0.54 \log S + 0.44$

(where S is the water solubility expressed as a mole fraction)

From Means et al. $^{11}$ $\log K_{oc} = -0.82 \log S + 4.070$

(where S is the water solubility in $\mu$g.cm$^{-3}$)

Additionally Means et al. $^{11}$ cite: $\log K_{oc} = -0.686 \log S + 4.273$

(where S is the water solubility in $\mu$g.cm$^{-3}$)

By substituting the previously calculated solubility data into these equations, $K_{oc}$ values of the individual PAH were estimated for each sampling station (Table 3.2.). Increases in $K_{oc}$ owing to salinity were then calculated by subtracting the fresh water $K_{oc}$ values (samples 1 and 2) from those calculated for the saline samples. These differences, averaged from the three equations, are listed in Table 3.2. Although this application of the regression equations is novel, the values predicted by the model approximate results obtained by sorption experiments $^{10}$. The experimentally derived $K_{oc}$ values for the Tamar samples were compensated for salinity by subtracting the estimated differences in $K_{oc}$ (reported in Table 3.2.) from the observed $K_{oc}$ figures (Table 3.1.). The resultant values ($K_{oc(sal.)}$) are listed in Table 3.1. and are plotted against their respective positions of sampling in Fig. 3.8. Fig. 3.8. shows that compensation for salinity only marginally linearises the anomaly observed for samples 7, 8 and 9, and therefore, is not the major factor responsible for the increased partition of PAH onto particulates in these samples.
Table 3.3.
CALCULATED changes in Koc owing to increases in salinity incurred during the axial traverse from fresh-water to the marine environment. Equations used in the calculation of Koc values are:

\[ \text{log Koc} = -0.54 \log S + 0.44 \] (from Karickhoff et al. reference 10).

\[ \text{log Koc} = -0.82 \log S + 4.07 \] (from Means et al. reference 11).

\[ \text{log Koc} = -0.686 \log S + 4.273 \] (cited by Means et al. reference 11).

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<th>3</th>
<th>4</th>
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**Anthracene**

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<td>4.76</td>
<td>4.76</td>
<td>4.77</td>
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<td>log Koc ((-0.82 \log S + 4.070))</td>
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<td>5.17</td>
<td>5.18</td>
<td>5.19</td>
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**Benz(a)anthracene**

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<td>Mean difference in log Koc from fresh water value</td>
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**Benz(a)pyrene**

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<th>0.172</th>
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<td>6.31</td>
<td>6.34</td>
<td>6.36</td>
<td>6.37</td>
<td>6.39</td>
<td>6.41</td>
</tr>
<tr>
<td>log Koc ((-0.82 \log S + 4.070))</td>
<td>7.16</td>
<td>7.16</td>
<td>7.16</td>
<td>7.19</td>
<td>7.23</td>
<td>7.26</td>
<td>7.28</td>
<td>7.31</td>
<td>7.33</td>
</tr>
<tr>
<td>log Koc ((-0.686 \log S + 4.273))</td>
<td>6.85</td>
<td>6.85</td>
<td>6.86</td>
<td>6.88</td>
<td>6.92</td>
<td>6.94</td>
<td>6.96</td>
<td>6.98</td>
<td>7.00</td>
</tr>
<tr>
<td>Mean difference in log Koc from fresh water value</td>
<td>0</td>
<td>0</td>
<td>0.01</td>
<td>0.03</td>
<td>0.07</td>
<td>0.09</td>
<td>0.11</td>
<td>0.13</td>
<td>0.15</td>
</tr>
</tbody>
</table>

a. S is solubility expressed as a mole fraction.
b. S is solubility expressed as pg.cm\(^{-3}\).
Figure 3.8.
Axial profiles of Koc and Koc (sal.) values for individual PAH. Sample stations are indicated at the top and bottom of the figure and represent the transition from fresh-water to the marine environment proceeding from left to right across the figure. (Locations of the samples sites are shown in Fig. 3.1.). Ranges of the partition coefficients calculated from triplicate incubation experiments are shown as vertical bars that are joined to adjacent plots through the mean value.
(d) **Other factors potentially important in controlling sorption**

Compensation for the effects of organic carbon and salinity on adsorption of PAH onto estuarine particulates has been shown to enhance unity of sorption throughout the Tamar. Discrepancies remain, most notably for the marine influenced samples (7,8,9) (Fig. 3.8.). Although experimental precision for these samples is low, the differences are nevertheless significant. Marine particulates demonstrate higher affinity for PAH than do the riverine or estuarine particulates. This could be explained by a difference in the character of the organic carbon (as suggested by the decrease in $C/N$ ratio, Fig. 3.4.) or perhaps the organic carbon in low salinity samples is less readily available for sorption owing to coatings with iron hydroxides. Sample 7 demonstrates elevated particulate affinity especially for higher molecular weight homologues. This region is susceptible to urban discharges, although sewage contamination is unlikely to be responsible owing to the fact that no significant changes in carbon content were identified. The increase in $K_{OC}$ in the marine samples is also consistent with an observed increased proportion of small (<50 μm) particulates, thereby increasing the surface area for sorptive interactions (Fig. 3.4.). Differences in combinations of organic and mineral components of sorbing mixtures have been shown to result in non-linear changes in partition. In estuarine environments, where there is a multiplicity of sources of particulates, this is of likely significance.
Comparison of the adsorption experiment results with data reported in the literature

The only comparable published \( K_{oc} \) value has been reported for anthracene \(^{10}\) (\( \log K_{oc} = 4.4 \)). This is within the range of values obtained for Tamar Estuary particulates (4.3 to 5.4). To enable a more complete comparison with the literature it is necessary to use published regression equations correlating \( K_{oc} \) with solubility and octanol-water partitioning. \( K_{oc} \) values calculated from solubility regressions (Section III.4.2.(e.)), and octanol-water partition coefficient (Table 3.3.) regressions (using M.rickhoff's equations \(^{12}\):

\[
\log K_{oc} = 0.989 \log K_{ow} - 0.346 \quad ; \quad K_{oc} = 0.411 K_{ow}
\]

are plotted with the comparable Tamar Estuary \( K_{oc} \) values (compensated for salinity, i.e. \( K_{oc} \) (sal.)) in Fig. 3.9. The experimentally derived \( K_{oc} \) values for the estuarine particulates are generally within the ranges of theoretically calculated \( K_{oc} \) values. The span of values predicted by theory arise from the imprecision of the regressions, which are derived from many hydrophobic compounds. Solubility regressed \( K_{oc} \) values, (which are generally higher than those calculated from the more precise \(^{12}\) octanol-water data, Fig. 3.9,) might be reduced for fluoranthene owing to the relatively high solubility of the compound with respect to its molecular weight (Table 3.3.).

Table 3.3. Selected physical properties of the PAH

<table>
<thead>
<tr>
<th>Compound</th>
<th>Molecular Weight</th>
<th>Aqueous Solubility ( \times 10^{-3} )</th>
<th>Setschenow Constants</th>
<th>( K_{oc} ) Log</th>
<th>( H_{oc} ) Log</th>
</tr>
</thead>
<tbody>
<tr>
<td>Naphthalene</td>
<td>138.2</td>
<td>3169 ( \pm ) 290</td>
<td>0.213 ( \pm ) 0.001</td>
<td>3.36 ( \pm ) 0.02</td>
<td>0.001 ( \pm ) 0.001</td>
</tr>
<tr>
<td>Anthracene</td>
<td>178.2</td>
<td>44.6 ( \pm ) 0.01</td>
<td>0.238 ( \pm ) 0.002</td>
<td>4.36 ( \pm ) 0.04</td>
<td>0.002 ( \pm ) 0.002</td>
</tr>
<tr>
<td>Fluoranthene</td>
<td>202.3</td>
<td>206 ( \pm ) 0.1</td>
<td>0.339 ( \pm ) 0.01</td>
<td>5.61 ( \pm ) 0.01</td>
<td>0.002 ( \pm ) 0.002</td>
</tr>
<tr>
<td>Benz(a)-anthracene</td>
<td>228.3</td>
<td>9.4 ( \pm ) 0.1</td>
<td>0.35 ( \pm ) 0.01</td>
<td>5.61 ( \pm ) 0.01</td>
<td>0.002 ( \pm ) 0.002</td>
</tr>
<tr>
<td>Benz(a) pyrene</td>
<td>252</td>
<td>0.172 ( \pm ) 0.004</td>
<td>0.39 ( \pm ) 0.01</td>
<td>6.04 ( \pm ) 0.01</td>
<td>0.002 ( \pm ) 0.002</td>
</tr>
</tbody>
</table>

(a) May (1978) \(^{26}\), (b) Lu et al. (1977) \(^{27}\), (c) Estimated by extrapolation
(d) M.rickhoff et al. (1979) \(^{10}\), (e) E.P.A. (1979) \(^{28}\), (f) Ruddle et al. (1976) \(^{29}\).
Figure 3.9.
Comparison of Koc values determined for the Tamar Estuary particulates and those predicted from literature. 'An', 'Fl', 'B(a)a' and 'B(a)p' refer to anthracene, fluoranthene, benz(a)anthracene and benzo(a)pyrene respectively. '2-9' indicate the salinity compensated Koc values (Koc (sal.)), experimentally determined for particulates in Tamar Estuary water samples of the corresponding numbers. 'x', 'y' and 'z' are comparable, theoretically derived, Koc values calculated for the individual PAH from the solubility regression equations (Section III.4.2.(c).):

\[
(x) \; \log Koc = -0.54 \log S + 0.444 \quad \text{(reference 10)}
\]

\[
(y) \; \log Koc = -0.32 \log S + 4.070 \quad \text{(reference 11)}
\]

\[
(z) \; \log Koc = -0.686 \log S + 4.273 \quad \text{(reference 11)}
\]

'\*' and 'o' are the theoretically derived Koc values calculated for the individual PAH from the octanol-water partition coefficient regressions (reference 12):

\[
(\ast) \; \log Koc = 0.989 \log Kow - 0.346; \quad (o) \; Koc = 0.411 \text{Kow}
\]

126
III.4.3. Fractionation of Environmental Samples

The equilibrium adsorption experiments (Table 3.1.) show that a significant and measurable proportion of PAH should be found in solution. The extent to which this occurs with in situ PAH encountered in the estuary, was tested by the determination of PAH in the particulate and dissolved phases of two samples of estuarine water removed during the survey.

Sub-samples of water from Stations 3 and 7 (Fig. 3.1.) were filtered (G/F) under gentle pressure. The filtrate was extracted according to Section II.1.3.(b). (i). and the particulates soxhlet extracted (Section II.1.3.(b). (i).). Following alumina clean-up (Section II.1.3.(b). (i).) analysis of PAH was by HPLC (Section II.1.3. (b). (ii).). Results are shown in Table 3.4.

<table>
<thead>
<tr>
<th>Sample 3</th>
<th>Aqueous</th>
<th>Particulate</th>
<th>( K_p )</th>
<th>Sorption</th>
<th>Aqueous</th>
<th>Particulate</th>
<th>( K_p )</th>
<th>Sorption</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>PAH (ng.cm(^{-3}))</td>
<td>PAH (ng.g(^{-1}))</td>
<td>( C_p )</td>
<td>( C_s )</td>
<td>PAH (ng.cm(^{-3}))</td>
<td>PAH (ng.g(^{-1}))</td>
<td>( C_p )</td>
<td>( C_s )</td>
</tr>
<tr>
<td>Naphthalene</td>
<td>b</td>
<td>b</td>
<td>-</td>
<td>-</td>
<td>.0045</td>
<td>b</td>
<td>-</td>
<td>-</td>
</tr>
<tr>
<td>Phenanthrene</td>
<td>.0049</td>
<td>690</td>
<td>108900</td>
<td>-</td>
<td>.0025</td>
<td>238</td>
<td>91500</td>
<td>-</td>
</tr>
<tr>
<td>Anthracene</td>
<td>.0005</td>
<td>184</td>
<td>389000</td>
<td>4770</td>
<td>.0005</td>
<td>32.7</td>
<td>63000</td>
<td>1380</td>
</tr>
<tr>
<td>Fluoranthene</td>
<td>.0023</td>
<td>776</td>
<td>337400</td>
<td>4890</td>
<td>.0014</td>
<td>292</td>
<td>208000</td>
<td>5680</td>
</tr>
<tr>
<td>Pyrene</td>
<td>.0035</td>
<td>1052</td>
<td>300500</td>
<td>-</td>
<td>.0030</td>
<td>616</td>
<td>205000</td>
<td>-</td>
</tr>
<tr>
<td>Benzo(a)anthracene</td>
<td>b</td>
<td>112</td>
<td>-</td>
<td>20280</td>
<td>.0011</td>
<td>179</td>
<td>162000</td>
<td>22200</td>
</tr>
<tr>
<td>Chrysene</td>
<td>.0006</td>
<td>165</td>
<td>273000</td>
<td>-</td>
<td>.0001</td>
<td>104</td>
<td>109000</td>
<td>-</td>
</tr>
<tr>
<td>Benzo(e)pyrene</td>
<td>b</td>
<td>1209</td>
<td>-</td>
<td>-</td>
<td>b</td>
<td>268</td>
<td>-</td>
<td>-</td>
</tr>
<tr>
<td>Benzo(k)fluoranthene</td>
<td>b</td>
<td>99</td>
<td>-</td>
<td>-</td>
<td>.0003</td>
<td>86</td>
<td>286700</td>
<td>-</td>
</tr>
<tr>
<td>Benzo(a)pyrene</td>
<td>.0002</td>
<td>381</td>
<td>1503000</td>
<td>49300</td>
<td>b</td>
<td>265</td>
<td>-</td>
<td>-</td>
</tr>
<tr>
<td>Benzo(ghi)perylene</td>
<td>b</td>
<td>829</td>
<td>-</td>
<td>-</td>
<td>b</td>
<td>670</td>
<td>-</td>
<td>-</td>
</tr>
</tbody>
</table>

Table 3.4.
Comparison between \( K_p \) values derived from fractionation and subsequent analyses of environmental PAH in aqueous and particulate phases, and those derived from PAH-spiked sorption experiments. Both analyses and experiments were performed on sub-samples of the same estuarine waters (Samples 3 and 7, Fig. 3.1.). 'b' indicates that these compounds were below the limits of detection.
\( K_p \) values derived from the fractionation analyses are orders of magnitude higher than those obtained by sorption experiments (Table 3.4.). Even accounting for possible analytical problems associated with filtration (Section II.1.3.(a)) and extended periods of sorption equilibration, as discussed by Karickhoff,\(^{28}\), this result is highly indicative that PAH in the Tamar Estuary water column are not in equilibrium between the aqueous and particulate phases. The difference in \( K_p \) values indicates that PAH in the Tamar are considerably enriched in the particulate fraction, and are in a form that renders them unavailable for dynamic exchange with the water. Such forms might include incorporation of PAH into particles, for example as might be expected with particulates resulting from combustion processes or erosion of asphalt surfaces.

The results from the fractionation experiment are in agreement with \( K_p \) values that can be calculated from some of Lewis'\(^{32}\) data on PAH distribution in various aquatic systems in the U.K.
REFERENCES

15. G.R. Southworth. Aquatic Toxicology. ASTM STP 667.


CHAPTER IV

THE DISTRIBUTION OF PAH IN A DATED SEDIMENT CORE

SAMPLED FROM THE TAMAR ESTUARY
IV.1. INTRODUCTION

The characteristically high particulate association of PAH (discussed in Chapter III) led to extensive research being directed towards sediments (Section I.3.3.). Most recently, dated cores have been analysed to assess distribution and compositional changes of PAH in order to determine sources and the effect of man's activities 1-11. Combustion, particularly of fossil fuels, has been identified as a major contributor to the environmental burden and ubiquity of PAH 2-5,8. Primary sources of more localised significance include combustion specifically of coal 1,6 and road runoff/street dust 9,12. These, and other potential sources including oil spillage, domestic and industrial waste water effluents, 'natural' fires and biosynthesis, have been previously discussed (Section I.2.). Wakeham et al. 10 and Tan and Heit 8 further expand on biogenesis as a source of PAH in the environment.

This study involves a detailed investigation of the distribution of PAH and selected elements, including heavy metals, in a $^{210}$Pb dated sediment core from the Tamar Estuary. The objectives were to elucidate the historic record of PAH inputs to the Tamar Estuary, and the origins, diagenesis and compositional changes of the compounds.

IV.2. SAMPLE COLLECTION

The geographical location of the sample site is shown in Fig. 4.1.. Plate III is an aerial photograph of the locality. Selection of the St. John's Lake mud-flats as the sampling area was supported by
Figure 4.1.
Description of the lower Tamar Estuary and sampling site. Urban regions in the area are indicated by open stipples. Dockyards located along the estuarine foreshore are demarked by hatched lines.
Plate III

Aerial photograph showing the mouth and St. John's Lake region of the estuary, taken at high tide from Grid Reference SK 480540 (Plate I), viewing in a westerly direction. 'X' indicates the sample site.
results from previous studies on sedimentation and core dating. Compared to other regions of the estuary, this area is probably least susceptible to sediment disturbance.

An intertidal core was obtained (October 1980) using a hand-held plastic liner (6.6 cm i.d. x 60 cm), (Decon 90 soaked, rinsed in distilled water). Once inserted, the liner was sealed and the surrounding mud physically removed to allow withdrawal with minimum disturbance. The core (stored vertically) was immediately returned to the laboratory.

IV.3. EXPERIMENTAL

An X-ray morphological study of the core was performed upon return to the laboratory. It was then transferred into a nitrogen glove-box, and the liner cut longitudinally on two opposing sides allowing removal of one half of the liner for visual inspection of the sediment. The column was calibrated for depth, and \( \text{Eh} \) determined throughout (Pt/Ag/AgCl electrode). Samples from the central portions (out of contact with the vessel surfaces) of individual sections were transferred into glass jars (solvent rinsed, with solvent rinsed aluminium foil lined screw-caps). Each sample was then homogenised and sub-samples removed to pre-labelled beakers. The glass jars were then sealed and frozen awaiting PAH analysis (Section II.1.3.(b.).) Weighed portions of the sub-samples were removed for freeze drying and determination of water content. Aliquots of the dried samples were then lightly crushed in a plastic ball mill before X-ray fluorescence (Section II.1.2.(b.), atomic
absorption (Section II.1.2.(c)) and \(^{210}\text{Po}\) analysis (Section II.1.4.). Further dried sub-samples were analysed for carbon and nitrogen content, and additionally, organic carbon and nitrogen lost on low temperature ashing (Section II.1.2.(a)).

IV.4. RESULTS AND DISCUSSION

X-ray analysis of the core (Plate IV) identified sparsely distributed gastropod and bivalve mollusc shells, and concentrated shell debris at a depth of between 51-52 cm. Visual inspection revealed a dark-grey anoxic core with a pale brown surface layer (0-0.5 cm). The shells observed by X-ray were identified as Littorina sp. and shallow burrowing Cardium sp. An apparent change in texture from 45 cm downwards was also noted.

IV.4.1. Variability of the parameters monitored with depth

Figure 4.2. shows the monitored variables plotted against depth. Only minor fluctuations occurred in water content of the sediment. Eh decreased exponentially with depth. The ratio silicon : aluminium was uniform throughout the majority of the core, although below 45 cm it increased, corresponding to the change in texture observed during visual inspection. Decreases in carbon, nitrogen, organic carbon and 'nitrogen lost on low temperature ashing' probably result from diagenic processes. Anomalies to these generalised trends were noted in the surface 5 cm of the core, and in a section approximately 8-15 cm depth. Carbon and nitrogen contents in these regions were
Plate IV
X-ray of the sediment core.
Core X-Ray
Figure 4.2. Distribution of selected parameters with depth.

137
lower than expected and in the surface layers an increase in the ratio silicon : aluminium was observed.

Analysis of selected heavy metals showed them to be considerably enriched in the surface sediments (0-10 cm). In the region of the core above 20 cm, these metals covary. Concentrations encountered were 100-200, 20-90 and 30-70 μg.(g dry sediment)⁻¹ for total zinc, copper and lead respectively. An apparent depletion in the metals was noted at approximately 13 cm depth, corresponding to the anomalous region described above. It is of interest that levels of lead were enriched in the section of core below 30 cm.

Concentrations of total PAH were negligible in the lower core (below 30 cm) and increased exponentially from a depth of 15 cm upwards. Concentrations in the surface sediments (4000 ng.(g dry sediment)⁻¹) are more than two orders of magnitude higher than those recorded below 30 cm depth (20 ng.(g dry sediment)⁻¹). In common with the trace metals, PAH were depleted in the region of the core between 10-15 cm depth. Significant correlations (quantified using 'r' - Section II.1.5.) were identified between PAH and zinc (r = 0.96), copper (r = 0.97), lead (r = 0.92), ²¹⁰Po (r = 0.94) and nitrogen lost on low temperature ashing (L.T.A.) (r = 0.87). Unlike carbon, the chemical composition and geochemical significance of the proportion of nitrogen lost on L.T.A. is uncertain.

The individual values for the monitored variables are listed in Table II of the Appendix.
IV.4.2. Dating of the core

$^{210}\text{Pb} \quad (t_\frac{1}{2} = 22 \text{ yr})$ has been found suitable for dating sediments over periods of approximately the last 100 years $^{13,14,15}$. The conventional assumptions made for the technique employed were $^{13}$:

1. $^{210}\text{Pb}$, $^{210}\text{Bi}$ and $^{210}\text{Po}$ are in secular equilibrium and there is no post-depositional migration of any of these radionuclides.

2. The flux of $^{210}\text{Po}$ to the system, and the sedimentation rate are constant.

3. The $^{210}\text{Pb}$ background is constant throughout the sediment column.

The water content of the sediment (Fig. 4.2.) was shown to remain constant throughout the core, thus compaction was negligible and it was thought unnecessary to incorporate depth corrections $^{13}$ in date determination. Fig. 4.3.(a) validates the above assumptions to a high degree by exhibiting the anticipated exponential decrease in activity. This curve tends to linearity at a value of 0.27 pCi g$^{-1}$ which was taken to represent background activity. A log plot of the unsupported $^{210}\text{Po}$ activity against depth (Fig. 4.3.(b).) shows a near linear relationship confirming exponential decrease in $^{210}\text{Po}$.

Conversion of the $^{210}\text{Po}$ activity into dates is shown in Fig. 4.3.(c). The graphs plotted in Fig. 4.3. identify a disturbance in the 8-16 cm region of the core, which correlates with the PAH and trace metal anomaly discussed previously. It is noted from Fig. 4.2. that the observed perturbation is also linked with a positive increase in Eh and a reduction in total carbon, nitrogen and nitrogen lost on L.T.A. The ratio silicon : aluminium, however, remains unchanged possibly ruling out inclusion of sediment from a different origin. The disturbance could be explained by the incorporation of 'older'
Figure 4.3.
(a) Graph of $^{210}\text{Po}$ activity against depth. On extrapolation the background activity supported by the sediment is taken to be 0.27 pCi/g.
(b) Log unsupported $^{210}\text{Po}$ activity plotted against depth (tends towards linearity demonstrating exponential decrease in $^{210}\text{Po}$ in the core).
(c) Calculated dates plotted against depth indicate a general sedimentation rate of 0.8 cm yr$^{-1}$. (A disturbed portion of the core is identified).

The horizontal bar of each cross indicates the depth interval analysed and the vertical bar approximates counting error (taken as ± $\pm$ counts).
sediment containing characteristically lower levels of $^{210}$Po activity, PAH, heavy metals, carbon, nitrogen and nitrogen lost on L.T.A. Mechanisms by which this could occur include bioturbation by a deep burrowing organism eg. Mya sp., although this is unlikely to result in the extent of disturbance observed. A more feasible explanation would be deposition of older sediments on the surface, for example, during baitworm collection by man, or by lateral 'slumping' or shearing of sediments during storm events. Subsequent physical and biological activity at the interfaces followed by further deposition to the present time could result in the observed distribution.

From Fig. 4.3.(c), the overall sedimentation rate was calculated to be 0.8 cm.yr$^{-1}$. The corresponding flux of $^{210}$Po can be calculated as 0.24 pCi.cm$^{-2}$.yr$^{-1}$ (unsupported surface $^{210}$Po activity = 0.47 pCi.(g dry sediment)$^{-1}$; wet sediment density = 1.502 g.cm$^{-3}$; sediment water content = 58% by weight). This compares with a value of 0.28 pCi.cm$^{-2}$.yr$^{-1}$ previously reported for the Tamar Estuary. It is the sedimentation rate that is most frequently used to date sediment cores. In the core sampled from the Tamar, however, dates were assigned to individual sections (from which sub-samples had been analysed for PAH and metals) according to their $^{210}$Po activity relative to that at the surface. This method was developed to improve accuracy of dating estimates, and in addition, to attempt partial correction for the perturbation. Sections analysed without corresponding assigned $^{210}$Po activities were dated using values obtained by extrapolation of the $^{210}$Po depth profile (Fig. 4.3.(c)).
IV.4.3. **Composition of the PAH assemblage**

HRC chromatograms of PAH extracted from three sections of the core are shown in Fig. 4.4. Unsubstituted PAH are clearly dominant over individual alkyl-substituted homologues that may be present in the extracts. To investigate compositional changes of individual PAH in the suite of compounds monitored, PAH compositions at selected depths are shown in Fig. 4.5. Concentrations are plotted on a log. scale to aid comparison. Post-1940 PAH compositions are similar. Sediments deposited prior to this date, (total PAH concentrations typically $<50 \text{ ng. (g dry sediment)}^{-1}$) show marked differences in composition. To further investigate changes throughout the core, percentage compositions of individual PAH are shown in Table 4.1. No trends in the data are apparent, excepting for the greater compositional variability in the lowest four sections (owing to low PAH concentrations and hence, a reduction in the numbers of quantifiable PAH). The generally uniform composition of PAH identified in most sections, together with the absence of trends of change, might suggest that once PAH are incorporated into the sediment, they are subject to negligible chemical or biological modification. As discussed in Section I.4.2., microbial degradation rates of PAH decrease with increasingly anaerobic conditions, thus, in the anoxic Tamar Core, conditions might favour preservation of the compounds. Hinga et al. 16, however, reported benz(a)anthracene degradation in sediments in a Marine Ecosystems Research Laboratory (MERL) microcosm study. Gschwend and Hites 2 (who observed a similar constancy in PAH composition with depth) attribute this contradiction to the form in which the PAH are introduced.
HPLC chromatograms showing examples of PAH separations of sediment extracts throughout the core. Peak numbers correspond to 1. naphthalene, 2. phenanthrene, 3. anthracene, 4. fluoranthene, 5. pyrene, 6. chrysene, 7. benzo(a)anthracene (coincident), 8. benzo(c)pyrene, 9. coincident benzo(b)fluoranthene and perylene, 10. benzo(k)fluoranthene, 11. benzo(a)pyrene, 12. dibenzo(a,h)anthracene (coincident), 13. benzo(ghi)perylene, 14. indeno(1,2,3-cd)pyrene. 'X' is an unresolved mixture (Section II.1.3.(b).(ii)). Selective quantification of perylene was obtained from absorbance at 405 nm. Chromatographic (Perkin Elmer CDS column) elution and detection conditions are detailed in Section II.1.3.(b).(ii).
Figure 4.5

Compositional variations of PAH in selected sections of the core. For each section 12 lines are shown indicating individual PAH concentrations starting with 1. phenanthrene and proceeding towards the right in sequence, 2. anthracene, 3. fluoranthene, 4. pyrene, 5. benzo(a)anthracene, 6. benzo(e)pyrene, 7. benzo(b)fluoranthene, 8. perylene, 9. benzo(k)fluoranthene, 10. benzo(a)pyrene, 11. benzo(g,h,i)perylenne, 12. indeno(1,2,3-cd)pyrene. To aid comparison a log PAH concentration scale has been selected. Short lines extending beneath the base line indicate that these compounds were not quantified.

Table 4.1

| Depth (cm) | 0-1 | 1-2 | 2-3 | 3-4 | 4-5 | 5-6 | 6-7 | 7-8 | 9-10 | 10-11 | 11-12 | 12-13 | 13-14 | 14-15 | 15-16 | 16-17 | 17-18 | 18-19 | 19-20 | 20-21 |
|-----------|-----|-----|-----|-----|-----|-----|-----|-----|-----|------|------|------|------|------|------|------|------|------|------|
| Phenanthrene | 7.0 | 5.2 | 8.6 | 9.2 | 8.9 | 7.3 | 6.3 | 7.8 | 9.9 | 15.5 | 11.4 | 10.5 | 11.9 | b | b | b | |
| Anthracene | 3.7 | 2.1 | 4.0 | 2.5 | 2.1 | 3.2 | 1.5 | 1.6 | 1.6 | 2.3 | 2.0 | 2.2 | b | b | b | |
| Fluoranthene | 20.3 | 17.4 | 17.2 | 20.7 | 18.4 | 12.8 | 13.6 | 14.1 | 13.8 | 6.1 | 34.2 | 10.5 | 19.5 | b | b | b | |
| Pyrene | 25.3 | 21.0 | 25.3 | 26.3 | 26.0 | 18.7 | 25.7 | 30.8 | 42.3 | 21.6 | 31.2 | 25.1 | 33.7 | 47.8 | 57.5 | |
| Benzo(a)anthracene | 7.6 | 8.4 | 8.0 | 7.2 | 8.0 | 5.9 | 10.4 | 7.1 | 5.5 | 4.9 | 5.7 | 6.3 | 4.9 | a | a | |
| Chrysene | a | a | a | a | a | a | 6.5 | 12.3 | a | b | a | a | 4.9 | b | b | |
| Benzo(e)pyrene | 12.9 | 18.9 | 16.4 | 9.6 | 13.9 | 25.1 | 19.7 | 9.0 | 11.4 | 9.3 | 8.0 | 10.3 | 5.2 | b | b | b | |
| Benzo(b)fluoranthene | a | 2.3 | a | a | 2.8 | a | a | 5.3 | 1.6 | 2.6 | 3.6 | 2.6 | 1.4 | a | a | a | |
| Perylene | a | 1.1 | a | a | 1.6 | a | a | 3.6 | 3.0 | 1.6 | 4.5 | 3.2 | 1.9 | 36.9 | 32.3 | 33.6 | |
| Benzo(k)fluoranthene | 2.2 | 2.5 | 2.1 | 1.2 | 1.2 | 1.2 | 2.2 | 3.2 | 1.2 | 1.5 | 4.6 | 5.2 | 8.3 | 7.3 | b | 4.5 | |
| Benzo(a)pyrene | 11.5 | 12.7 | 11.2 | 7.9 | 9.9 | 8.9 | 10.8 | 9.2 | 6.3 | 6.5 | 6.6 | 7.3 | 16.5 | 9.8 | 12.0 | 4.5 | |
| Benzo(g,h,i)perylenne | 6.0 | 5.2 | 5.1 | 1.3 | 4.3 | 5.5 | 5.5 | a | 1.4 | 6.9 | b | 6.5 | 1.4 | b | 9.8 | 8.0 | b |
| Indeno(1,2,3-cd)pyrene | 2.3 | 1.1 | 1.5 | 0.6 | 1.9 | 2.3 | 2.3 | 9.7 | 0.4 | 0.3 | b | 1.3 | 0.8 | b | b | b | |

(a) Compound not quantified owing to interference from coeluting material.
(b) Concentrations below the detection limits of the analytical scheme.
In the MERL experiment PAH were added in solution with oil, whereas in the environment PAH might be associated with particles, with which microbial interactions may not be possible. This explanation further supports the theory of particle incorporated PAH, proposed in Section III.4.3. to explain the non-equilibrium partition of PAH between suspended particulate and aqueous phases in the Tamar Estuary water column.

The general consistancy in PAH composition also suggests that the compounds originate from a single primary source. The apparently random compositional fluctuations observed in Table 4.1. probably arise from subtle temporal changes in the source and additional secondary source inputs of PAH.

IV.4.4. Distribution of PAH and heavy metals with date

Fig. 4.6. shows the distribution of individual PAH and heavy metals with date of deposition. The correction used to alleviate the effects of the disturbance (Section IV.4.2.) appears to have effectively 'smoothed' the PAH distribution. With the exception of perylene, all the PAH monitored exhibited a similar qualitative pattern of distribution, with low levels prior to 1940. A gradual increase in the PAH follows until 1960 when dramatic increases are observed to present day. Inflexions are noted for some PAH in the mid-1970's. Further dramatic increases in all compounds occur at the top of the core. Using pyrene and benzo(a)pyrene as examples, the surface concentrations (1050 and 450 ng. (g dry sediment)^-1 respectively) are more than two orders of magnitude higher than
Figure 4.6.
Distributions of individual PAH and heavy metals with date of deposition.
concentrations in sediments deposited 40 years ago (22 and 4 ng. (g dry sediment)$^{-1}$ for pyrene and benzo(a)pyrene respectively). The present total PAH flux (including all PAH monitored) to sediments in the sample area is 0.021 g.m$^{-2}$.yr$^{-1}$ which compares with 0.00023 g.m$^{-2}$.yr$^{-1}$ in 1940.

Individual PAH concentrations encountered in the surface sediments (top 5 cm) ranged from 30 - 750 ng. (g dry sediment)$^{-1}$. These levels are comparable with those obtained from, for example, Severn Estuary (U.K.) sediments $^{17,18}$ and Narragansett Bay (U.S.A.) sediments influenced by Providence City runoff $^{19}$ (see also Section I.3.3.). The extent to which urban Plymouh contributes to PAH pollution in the Tamar Estuary is discussed in Chapter V.

Perylene shows a unique sedimentary distribution. Although recent increases similar to those exhibited by the other compounds occur, they are less marked. Significant concentrations (5 - 10 ng. (g dry sediment)$^{-1}$) were also found throughout the core prior to 1940 (Fig. 4.6.).

Although substantial concentrations of the heavy metals were recorded throughout the entire core, there are significant recent (post 1950) increases in the elements which generally reflect the observed pattern of PAH pollution (Fig. 4.6.).

IV.4.5. Sources

(a) PAH composition

The dominance of unsubstituted parent hydrocarbons relative to alkylated homologues (Section IV.4.3.) (Fig. 4.4.) precludes native or undegraded oil as a major contributor of PAH $^{20,21}$ (Section
I.2.1.(b). Parent PAH assemblages similar to those observed have been reported in sediments from the Severn Estuary, U.K. 17, Monchalttorfer Aa and Lake Greifensee, Switzerland 12, and Lakes Lucerne and Zurich, Switzerland and Lake Washington, U.S.A. 9, (Section I.3.3.) (Fig. 1.6.). Wakeham et al. 9 identify this sedimentary composition as ubiquitous, citing literature from the marine environment 4,21-23, fresh-water sediments 1,6,12 and in soils 21,23. Giger and Schaffner 12 and Wakeham et al. 9 attribute 'street dust' as the major source. Giger and Schaffner 12 also suggest a similarity of airborne particulates and Wakeham et al. 9 emphasise the importance of asphalt particles in street runoff and exclude exhaust emissions and tyre wear as major contributors. Other primary sources suggested include combustion of fossil fuels 2-5,8,23, combustion primarily of coal 1,6 and 'natural' fires 21,24. Gschwend and Hites 2 calculated individual PAH ratios to investigate sources. Those applicable to this study are phenanthrene: anthracene, fluoranthene:pyrene, and benzo(e)pyrene:benzo(a)pyrene. Graphs of these relationships are shown in Fig. 4.7. Plots of fluoranthene against pyrene and benzo(e)pyrene against benzo(a)pyrene are approximately linear and therefore indicate a constant composition between the paired PAH in the pollutant flux. This is supportive of a uniform primary source. Regression analyses result in values for fluoranthene:pyrene of 0.7 and benzo(e)pyrene:benzo(a)pyrene of 1.4. The fluoranthene:pyrene ratio (0.7) has been previously recorded for airborne particulates 12,2. The benzo(e)pyrene:benzo(a)pyrene value (1.4) has been obtained from analyses of 'street-dust', 12,2. In contrast to the linear relationships observed for these pairs of PAH, phenanthrene plotted
Cross-correlation between selected PAH throughout the core. Plots of fluoranthene against pyrene and benzo(e)pyrene against benzo(a)pyrene result in approximately linear relationships with calculated regressed slopes (shown as broken lines) of 0.70 and 1.37 respectively. The plot of phenanthrene against anthracene is better suited to a curved regression.
against anthracene (Fig. 4.7.) is better described by a curve. This may arise owing to isomeric discrimination as would be expected in microbial degradation, or alternatively, a change in their relative compositions in the PAH flux to the sediments.

In summary, the composition of PAH in the core is indicative of a primary source related to fossil fuel combustion and urban (road) runoff.

(b) Distribution with date

The distribution of PAH with date of deposition is shown in Fig. 4.6. and has been described in Section IV.4.4. Most significant increases in the pollutant flux have occurred within the last twenty years.

Published data proclaiming fossil fuel combustion as the primary PAH source, generally describe a distribution of negligible concentrations prior to the late 1800's when increases occur to reach a maximum in the region of 1950-1960. After this period concentrations gradually decrease to the present day, an affect attributed to the change from coal as the primary fossil fuel to oil and gas which emit less PAH upon combustion. Heit et al. have also suggested fossil fuel combustion as the major source in 137Cs - dated lake cores after obtaining PAH distributions similar to that described for the Tamar.

Since the 1950-1960 period, when the PAH concentrations rise dramatically, changes affecting the PAH concentrations emitted by local industry and increases in domestic fuel burning (particularly of coal), have not occurred. It is also unlikely that the prevailing
south westerly air stream in the region, which originates from the Atlantic Ocean, would contain sufficiently high concentrations of PAH to produce the flux encountered. The most important contributory factor to PAH emission during this time period is probably the increase in motor vehicles. In 1961 the Tamar Road Bridge was opened. This is the major southern route of vehicles into Cornwall and is used extensively.

Elevated PAH concentrations are noted in the top few cm of the core (Fig. 4.2. and 4.6.). It was initially postulated that degradation mechanisms possibly reduced the concentrations to those associated with adjacent levels. It is, however, noted that heavy metals in the surface layers are also high (Fig. 4.2.) indicating an increased pollutant flux. The PAH composition of this input is the same as that associated with the core. A possible explanation is contamination of the core surface by, for example, a road runoff 'slick', which has become incorporated into the surface layers by seepage during transport. This recent enrichment of PAH and heavy metals coincide with a higher ratio of silicon : aluminium and reduced carbon and nitrogen content (Fig. 4.2.). The significance of, and explanations for this latter observation have not been resolved.

(c) Distribution of combustion particulates

Electron microscopy has been used to distinguish between carbon particulates resulting from combustion of various source materials 25-27. This was applied to selected sediment core samples which were digested to isolate the elemental carbon particles according to Smith et al. 28. This involved treatment in a solution of equal
parts of $H_2O_2$ (30%) and KOH (2M) (3 days), dissolution in hot HCl (6M, 2 h) and digestion in HF (28%, 10 days). The resultant sample was separated by centrifugation, rinsed with dilute HCl and dried (110°C, 6 h). Following adhesion onto a sample block (using double back sellotape $^R$) and coating with gold (11Å), the samples were studied using a Jeol JSM 35C scanning electron microscope.

Griffin and Goldberg $^{25}$ have attempted a morphological categorisation of combustion particulates into 3 groups: (i) porous spheroidal, (ii) elongate prismatic, (iii) irregular fragments. In addition, surface textures are characterised into 3 classes: (a) smooth, homogenous, (b) rough, irregular and pitted, (c) etched and convoluted. Spheroidal particles are commonly produced by combustion of oil and coal. Irregular fragments can result from combustion of oil, coal and wood whereas only the latter two produce elongate prismatic structures. Etched convoluted surface layers are unique to charcoals produced from oil combustion. Smooth, and rough irregularly pitted surfaces are indicative of both coal and wood combustion. Oil derived carbon is thus easily identified by the etched convoluted surface, whereas differentiation between wood and coal combustion products is difficult. In contrast with wood, coal produces spherules, but both result in irregular and elongate prismatic particles. Remnants of cellular or woody structures are indicative of vegetation, brush and wood burning, but are also present in charcoal derived from coal.

Charcoals from oil, coal and wood combustion were identified in all three sections of core analysed. Enumeration of the relative contributions from each source was difficult owing to incomplete mineral digestion (Plates V and VI). Elongate prismatic structures
Plate V

Examples of combustion particulates isolated from the Tamar Estuary sediment core (viewed using a scanning electron microscope).

(a) Porous spheroid with an etched convoluted surface. Highly indicative of an oil combustion origin.

(b) Porous spheroid with a smooth surface resulting from combustion of coal.

(c) Porous irregular fragment with a rough pitted surface probably resulting from coal combustion.

(d) Portion of a porous irregular particle showing remnants of cellular or woody structures, probably resulting from combustion of wood (although coal cannot be ruled out).

(e) Irregular porous fragment with a smooth surface. Associated with coal (or possibly wood) combustion.

(f) Elongate prismatic structure with a smooth surface, likely to have resulted from combustion of either wood or coal.
Combustion Particulates....
Plate VI
Carbon particles isolated from different depths of the sediment core, viewed using scanning electron microscopy. Positions in the core (with date) and associated total PAH concentrations are indicated.
appear to be enriched in the lower samples. No qualitative correlation between combustion particulates and the dramatic increase in PAH concentrations is apparent.

(d) Perylene

The anomalous distribution of perylene relative to other PAH has been described in Section IV.4.4. and is shown in Fig. 4.6. Recent increases in the compound, as with the other PAH, might have resulted from the pollutant flux. The significant concentrations in the lower core support previous work suggesting a biogenic origin of the compound (Section I.2.1.(a)). Wakeham et al. 10 and Tan and Heit 8 discuss the biogenic production of perylene, and suggest potential precursors of the compound (Section I.2.1.(a)).

(e) Heavy Metals

Heavy metals are highly correlated with total PAH (r = 0.92 (lead), 0.97 (copper), 0.96 (zinc)) (Figs. 4.2. and 4.6.). This covariation indicates a similar anthropogenic origin for both classes of pollutants. The Tamar Valley drains areas rich in metals which, until the turn of the century, were extensively mined (Section I.6.). It is unlikely that leaching of metals from the moorland catchment could result in the observed high correlation. 'Acid rain' produced as a result of combustion primarily of coal is unlikely to have increased over recent years (Section IV.4.5.(b).). This effectively precludes mineral derived inputs as the major source of the increases recently (1960-1980) observed. Elevated concentrations of lead in the lower portion of the core (below 30 cm) (Fig. 4.2.) are, however, likely to have been derived from leaching of Dartmoor minerals.
This input could also explain the higher levels of metals associated with the 'older' sediment in the lower region of the disturbance observed in the core (Fig. 4.2.).

To differentiate between natural 'background' levels of trace metals and those derived from anthropogenic sources, Suess has advocated the calculation of trace metal : aluminium ratios. This treatment further endorses the enrichment of lead in the lowest section of core analysed which has a reduced aluminium content (Fig. 4.2.; Appendix Table II). Calculation of the ratio for sections in the remainder of the core, does not have a significant affect owing to the uniformity of aluminium composition (9.34 ± 0.23%). Similar treatments applied to zinc and copper have only marginal effects on the patterns of distribution observed in Fig. 4.2..

Sources of metals associated with motor vehicles have been discussed by Harrison. Zinc is used in both rubber tyres and extensively in motor oils. Copper emissions result from wear of thrust bearings, bushings, bearing metals and particularly brake linings which contain several percent by weight copper. The addition of lead to petrol as an 'anti-knock' agent is well documented. Increases in sediment concentrations of zinc, copper and lead from the 1950's to present day (as a result of the anthropogenic source) are 100, 70 and 50 µg.(g dry sediment)⁻¹ respectively. Lead has been shown by Harrison to constitute the major metallic constituent in street dust, followed by zinc and then copper.

Speciation, transport mechanisms/particle association and mode of emission, however, have the potential to affect distribution in the environment. It is of note that zinc and copper are more closely
associated with the road surface than lead which is primarily emitted into the atmosphere.

IV.4.6. Statistical Results

To resolve cross-correlations and co-variability of the environmental parameters, PAH, and elements, cluster analysis and multi-dimensional scaling (M.D.S.) were performed (Section II.1.5.). The M.D.S. plot obtained is shown in Fig. 4.8(a). A major cluster incorporating variables showing similarities > 90% (identified from the cluster analysis) is circumscribed by an unbroken line. Included into this cluster are the heavy metals (lead, zinc and copper) and all PAH with the exception of chrysene and indeno(1,2,3-cd)pyrene. This confirms the co-variability of metals and PAH (Section IV.4.5.(e)). The removal of indeno(1,2,3-cd)pyrene into the >80% similarity cluster (circumscribed by the broken line) is confirmed by the atypical distribution of the compound with date (Fig. 4.6.). Although chrysene is also isolated from the major cluster, owing to a co-eluting peak, the compound was only quantified in a few sections of core, thus no weighting can be placed on this observation. It is of interest that perylene is incorporated into the >90% similarity cluster, possibly indicating that major contributions of the compound are derived from the same anthropogenic source as the other PAH. There are no significant correlations between the PAH and carbon, silicon or aluminium. Nitrogen, however, is incorporated into the >80% similarity cluster. Fig. 4.2. shows that nitrogen content of the sediment increases significantly in the top 10 cm of the core, corresponding with the increases in PAH.
Figure 4.8.

Multi-dimensional scaling (M.D.S.) analyses of PAH and other variables monitored in the Tamar Estuary sediment core. Clusters with similarities >90%, >80% and >60% are circumscribed by '---', '---' and '---' respectively.

(a) M.D.S. derived from Pearson's product-moment correlation coefficients (Stress factor = 0.1627).

(b) M.D.S. derived from Kendall's rank correlation coefficients (Stress factor = 0.1432).

(See Sections IV.4.6., and II.1.5.).
Diagenesis may have produced this pattern of distribution, although in the cultivated Tamar Valley the use of nitrogen based fertilizers has increased dramatically during the past 30 years (corresponding to the increase in nitrogen), and might have supplemented the nitrogen budget of the estuary.

The statistical analysis described above is influenced by the considerable decrease in most variables with depth. This is reflected by the high levels of similarity identified in the M.D.S. plot (typically > 80%). To remove the weighting produced by this distribution, and hence reveal more subtle co-variations in the data, Kendall's rank correlation co-efficients were calculated to produce the correlation matrix (Section II.1.5.) from which cluster analysis and M.D.S. are derived. The resultant M.D.S. plot is shown in Fig. 4.8.(b). The general distribution of points is in agreement with Fig. 4.8.(a). Perylene and lead are, however, isolated from the major cluster containing all other PAH and heavy metals. The anomalous distribution of perylene (Fig. 4.6.) has been discussed in Section IV.4.4. and was attributed to a biogenic origin of the compound (Section IV.4.5.(d)). Elevated concentrations of lead in the lower portion of the core (Fig. 4.2.) (likely to have been derived from leaching of Dartmoor minerals - Section IV.4.5.(e).) are probably responsible for the isolation of lead in the M.D.S..

It is interesting that in the cluster of PAH, phenanthrene, anthracene, fluoranthene and pyrene are sub-divided from the larger molecular weight homologues, although the statistical significance of this division is uncertain.


CHAPTER V

DISTRIBUTION, TRANSPORT MECHANISMS AND FATES OF PAH IN THE TAMAR
No published data is available concerning individual PAH concentrations in estuarine and coastal waters (Section 1.3.2.). The effects of the physical, chemical and hydrodynamic gradients encountered in estuaries on the distribution and fates of PAH have not been systematically evaluated. Of the limited data that has been published on spatial sedimentary distributions of PAH in estuaries (Section 1.3.3.), none discuss distribution in context with classical estuarine chemistry.

The approach adopted in this research involved analysis of individual homologues in a series of water samples removed during an axial traverse of the estuary (fresh water to marine). Concentrations encountered are compared with changes in environmental variables. To supplement the water data, surface sediments throughout the estuary and catchment were also analysed for PAH in order to provide a temporally integrated assessment of their distribution. The data from these surveys together with selected experiments are used to discuss spatial distributions, sources, transport processes and fates of PAH in the Tamar Estuary.

V.1.1. Study Area and sample collection

A description of the sampling area is presented in Section 1.6. Positions of the estuarine sample sites are illustrated in Fig. 5.1. Morris and co-workers have described the master variables and nutrient chemistry of the Tamar. Since the River Tamar represents the major source of river run-off into the Tamar Estuary, sampling
Figure 5.1.
Description of the Tamar Estuary and sampling stations. Water sample numbers and locations are indicated on the right hand side and degradation sub-samples (D1-D22) and sediment samples (El-E6) on the left hand side. Distances upstream from the narrows (N) at the mouth of the estuary are indicated in 5 km intervals and circled. Plymouth is indicated by the hatched area with the dockyards located along the estuarine foreshore demarked by stipples. The position of Calstock is indicated by 'C' and an oil jetty terminal by 'J'.
was concentrated along the main estuarine axis rather than in the Lynher and Tavy tributaries (Fig. 5.1.). Sampling of estuarine water was performed on the ebb tide on 7th May 1980. High tide at Calstock (Fig. 5.1.) on this day was 11.16 am. Sampling commenced at 12.00 am. Surface water samples were collected during an axial traverse of the estuary (fresh water to marine) aboard a 'Rotork Seatruck'\textsuperscript{R}. Samples were taken using a polypropylene bucket pre-rinsed in the sample water. Possible contamination from the vessel's stern engines was avoided by sampling at the extreme bow of the Seatruck. Aliquots (2 dm\textsuperscript{3}) for PAH analysis were transferred to Winchester bottles containing HILG-grade (Fisons) cyclohexane (30 cm\textsuperscript{3}), which had been cleaned by soaking in Decon 90\textsuperscript{R} for 24 hours and rinsed sequentially with distilled water, chromic acid, distilled water and finally HILG-grade cyclohexane. The sample bottles were then teflon-sealed and thoroughly shaken. During this transect of the estuary, salinity, pH, turbidity, dissolved oxygen, temperature and ammonia were recorded continuously. Sub-samples were also obtained for suspended solids analyses and degradation studies.

Estuarine sediment cores (2.5 cm diameter, 10-15 cm depth) were sampled (Fig. 5.1.) using the Butler Gravity Corer\textsuperscript{5}, retaining the top 5 cm for analysis. Sediment samples were also taken throughout the river catchment (Fig. 5.8.) and were sampled using a glass corer (5 cm diameter) to a maximum depth of 5 cm. Sediment samples were placed in acid cleaned jars sealed with solvent-rinsed aluminium foil lined caps, which were immediately stored over solid carbon dioxide and maintained frozen in the laboratory until analysis.
V.2. EXPERIMENTAL

V.2.1. Water column survey

The methodology for the measurement of environmental variables, salinity, temperature, turbidity, pH, dissolved oxygen and ammonia has been described in Section II.1.1.(a). Characterisation of the suspended particulates was performed according to Section II.1.1.(b). Estuarine waters were analysed for PAH as described in Section II.1.3.(b), using a Hypersil 5-7 μm ODS column (250 mm x 5 mm i.d.) for HILC separations (Fig. 2.1.). Analytical efficiencies for the extraction and analysis are summarised in Table 2.2. Overall reproducibility of the entire procedure was assessed from the triplicate samples 7, 8 and 9 (Fig. 5.2.).

Statistical analysis of the results obtained was performed using correlation profile analysis, clustering, and multi-dimensional scaling (M.D.S.) (Section II.1.5.).

V.2.2. Surface sediment survey

Sub-samples of the thawed homogenised sediments were freeze-dried in order to determine water content. Catchment sediments were then sieved to evaluate particle size distribution, and the estuarine 'muds' were ground in an agate ball mill and analysed for carbon content (Section II.1.2.(a)).

Full details of the PAH extraction and clean-up procedures are described in Section II.1.3.(b).(i). Estuarine sediment extracts were analysed by HILC using a Hypersil 5-7 μm ODS column.
This system was modified for the catchment samples to increase chromatographic resolution by use of the Perkin Elmer 10 µm HC-ODS column (250 mm x 2.6 mm i.d.) (Section II.1.3.(b).(ii), Fig. 2.2.). Analytical efficiencies for the extraction and analyses are summarised in Table 2.3..

Sediments were also analysed for lead as described in Section II.1.2.(c).

V.2.3. PAH associated with particle size fractions

Measured volumes of estuarine water sampled from the turbidity maximum (V.3.1.(a).) were passed through nylon mesh sieves to isolate particles in the following size ranges: >100 µm; 100-53 µm; 53-10 µm and <10 µm. The particulates were collected on ashed (450°C, 6 h) Whatman GF/C filters by back-flushing the sieves with pre-filtered (Whatman GF/C) estuarine water. The resulting samples were stored (-4°C, in darkness) awaiting analysis. The procedure was repeated for quantification and characterisation of the fractionated particulates (Section II.1.1.(b).). Thawed samples were extracted according to Section II.1.3.(b).(i)., with subsequent clean-up and quantification as described for the catchment sediment samples (Section V.2.2.).

V.2.4. Microbial Heterotrophic Degradation

Heterotrophic degradation of ¹⁴C-labelled naphthalene and benzo(a)pyrene was determined on selected sub-samples of water
V.3. RESULTS AND DISCUSSION

V.3.1. Aquatic Distribution

(a) Environmental Variables

The axial distribution patterns (Fig. 5.2.) of pH, dissolved oxygen, ammonia and the location of the turbidity maximum and the fresh water/sea water interface were typical of previous surveys of the Tamar Estuary reported by Morris et al. and that described in Chapter III of this thesis. The suspended particulate load at the turbidity maximum, however, was relatively low (70 mg dm\(^{-3}\)) because sampling in this region was performed just after high water slack, when suspended particulates are normally at a minimum. Causes of small local perturbations in the general trends of the environmental variables, for example, secondary maxima in suspended particulates at sample sites 11 and 15 (Figs. 5.1. and 5.2.) are possibly a consequence of hydrodynamic influences and have been discussed by Morris et al. Ammonia concentrations displayed a typical mid-estuarine maximum (Fig. 5.2.) originating mainly from the microbial activity in the large areas of intertidal mud-flats rather than sewage effluents off Plymouth.

(b) PAH concentrations in the water column

Concentrations of individual PAH (listed in Appendix Table III) generally ranged from less than 1 to greater than 50 ng dm\(^{-3}\).
Figure 5.2.
Axial profiles of individual PAH concentrations and a selection of environmental variables in waters of the Tamar Estuary. A source index of Plymouth specific PAH is indicated at the base of the diagram. Distances from the mouth of the estuary are shown at the bottom of the diagram and the position of Plymouth is indicated. A floating scale is used for the PAH concentrations with origins for individual compounds indicated on both sides of the diagram. Deviations from the mean of triplicate samples 7, 8 and 9 are shown as 95% confidence interval bars on the concentration plots of the individual PAH.
Although PAH concentrations in other estuarine or coastal waters are unknown, levels reported here for the River Tamar are comparable to those for the Ohio River, the River Severn, and selected German rivers. Considerably higher concentrations have, however, been recorded in the River Thames at London and in other rivers associated with large urban or industrialised areas (Section 1.3.2.). The PAH monitored (Fig. 5.2.) may be considered as two groups. Group 1 consists of the low molecular weight homologues (naphthalene, phenanthrene and anthracene) whose concentrations appear to be independent of suspended particulates (correlation coefficient, $r < 0.30$, Table 5.1.). Group 2 PAH (fluoranthene, pyrene, chrysene, benz(a)anthracene, benzo(b)-fluoranthene, benzo(k)fluoranthene, benzo(a)pyrene) have concentrations which exhibit significant correlations with suspended particulates ($r > 0.60$, Table 5.1.). The apparent lack of correlation between benz(a)anthracene and suspended particulates ($r = -0.13$) is caused by the biased weighting of three benz(a)anthracene rich samples (16, 17, 18) near the dockyard area. When these contributions are excluded from the remaining estuarine samples, the anticipated high correlation between benz(a)anthracene and suspended solids ($r = 0.97$) is restored (Table 5.1.). When similar treatments are applied to the other Group 2 PAH, correlation coefficients increase from the range 0.64-0.85 to 0.87-0.97 (Table 5.1.). Corresponding correlations with phenanthrene appear to classify the compound as intermediate between Group 1 and Group 2.
(c) Group 1 PAH (naphthalene, anthracene, phenanthrene)

These compounds appear to exhibit a complex distribution throughout the estuary, possibly reflecting a multiplicity of inputs. This is endorsed by the absence of any correlation with salinity (−0.02 < r < −0.27) which also tends to suggest that salting out processes (Chapter III) of these PAH at low salinities are of secondary importance. In addition, the naphthalene and anthracene regressions against suspended particulates, shown in Fig. 5.3., indicate that there are no significant correlations between Group 1 PAH and suspended solids. These PAH possess physical and chemical properties markedly different from those of the larger molecular weight homologues of Group 2 (Table 5.1.), for example, their solubilities and vapour pressures are higher, whereas their hydrophobicities (as reflected by their octanol-water partition coefficients (K_{OW})) are lower (Table 5.1.).

Sorption of PAH onto estuarine particulates has been investigated in Chapter III of this thesis. Experimentally derived K_p values (Table 3.4.) demonstrated a trend of increasing particulate affinity with increasing molecular weight (as is indicated by log K_{OW} values in Table 5.1.). There is however, no distinct break between sorption properties of anthracene and fluoranthene that could fully account for the marked differences observed in their environmental behaviours. The reduced correlation between suspended particulates and Group 1 PAH compared with that shown for the Group 2 homologues is endorsed by the analyses of the fractionated water sample 7 in Section III.4.3., (Table 3.4.), where calculated K_p values for phenanthrene and anthracene are distinctly lower than those of the larger PAH. This is not, however, clearly defined for anthracene in
Figure 5.3.
Plots of selected PAH concentrations (ng.dm\(^{-3}\)) against total suspended particulate load. X indicates sample sites 16-20 in the Plymouth region of the estuary.

Figure 5.4.
Scatter plots between concentrations (ng.dm\(^{-3}\)) of selected PAH. X indicates sample sites 16-20 in the Plymouth region of the estuary.
Table 5.1
Selected properties of PAH and their correlation with suspended solids.

| Compounds            | Molecular weight | Correlation with Total Suspended Solids | Correlation with Total s.s. (Excluding Plymouth samples) | Solubility (µg dm⁻²) | Vapour pressure (mm Hg) | Log. Kow  
|----------------------|------------------|----------------------------------------|----------------------------------------------------------|----------------------|------------------------|-------------------|
| Naphthalene          | 128              | + 0.07                                  | 0.09                                                     | 31 300 (a)           | 0.33 (a)               | 3.36 (h)  
| Phenanthrene         | 178              | + 0.22                                  | 0.73                                                     | 1070 (a)             | 6.8 x 10⁻¹⁷ (e)        | 4.56 (h)  
| Anthracene           | 178              | - 0.15                                  | 0.27                                                     | 44.6 (b)             | 1.55 x 10⁻¹⁷ (g)       | 5.49 (h)  
| Fluoranthene         | 202              | + 0.90                                  | 0.89                                                     | 206 (c)              | na                     | 4.90 (1)  
| Pyrene               | 202              | + 0.64                                  | 0.87                                                     | 132 (c)              | 6.85 x 10⁻¹⁷ (g)       | 5.17 (h)  
| Chrysene             | 228              | + 0.85                                  | 0.98                                                     | 1.8 (c)              | na                     | 5.61 (1)  
| Benz(a)anthracene    | 228              | - 0.13                                  | 0.97                                                     | 9.4 (c)              | 1.10 x 10⁻¹⁷ (g)       | 5.61 (1)  
| Benzo(b)fluoranthene | 252              | + 0.64                                  | 0.95                                                     | na                   | na                     | 6.64 (1)  
| Benzo(k)fluoranthene | 252              | + 0.71                                  | 0.97                                                     | na                   | 9.59 x 10⁻¹⁷ (g)       | 6.04 (1)  
| Benzo(a)pyrene       | 252              | + 0.78                                  | 0.92                                                     | 0.172 (d)            | 5.99 x 10⁻¹⁹ (g)       | 6.04 (1)  

(a) Eganhouse and Calder (12)  
(b) May et al. (16)  
(c) May et al. (15)  
(d) Lu et al. (16)  
(e) Maekay and Wolkoff (17)  
(f) Jordan (18)  
(g) Pupp (16)  
(h) Mencikoff et al. (20)  
(i) EPA (21)  
(j) Radding et al. (22)  
(k) Data not available

The lack of particulate associations of Group 1 compounds implies that significant proportions of these PAH may be in solution and subject to different fates than the Group 2 homologues.

The competitive partitioning of PAH between air and water, as controlled by their vapour pressures (Table 5.1.) is likely to be of importance in accounting for the differences between Group 1 and 2 PAH. The vapour pressures of Group 1 compounds are at least three orders of magnitude greater than those of Group 2 (Table 5.1.). This is in agreement with laboratory based volatilisation experiments reported by Southworth ²³,²⁴ and Herbes et al. ²⁵ (discussed in Section I.4.3.).

The role of microheterotrophic degradation of Group 1 PAH will be discussed in Section V.3.5. Other processes such as photo-chemical oxidation ²³,²⁶ (Section I.4.1.) were not investigated.
between individual Group 1 PAH were not significant (-0.14 < r < -0.42), and only phenanthrene exhibited a cross-correlation with Group 2 compounds as illustrated in the benz(a)anthracene/phenanthrene plot (r = 0.84) shown in Fig. 5.4.

(d) Group 2 PAH (fluoranthene, pyrene, chrysene, benz(a)anthracene, benzo(b)fluoranthene, benzo(k)fluoranthene, benzo(a)pyrene)

The axial distribution of Group 2 PAH generally correlates with suspended particulate distribution throughout the estuary (Fig. 5.2.) exhibiting highest concentrations at the turbidity maximum. Superimposed on this trend is a secondary PAH concentration maximum located off Plymouth and is likely to be associated with urban discharges (Sections I.2.2. and I.2.3.). This secondary PAH maximum does not appear to be entirely associated with particulates but may be present as transient soluble or colloidal forms. PAH composition in these samples is markedly different to that generally observed throughout the estuary. Some additional peaks in their chromatograms (possibly owing to alkylated homologues) might indicate an 'oil' component in the discharge (Section I.2.1.). Benz(a)anthracene is shown to be particularly enriched. A similar PAH composition with high concentrations of benz(a)anthracene has, however, been recorded in the literature for particulate associated emissions from an internal combustion engine. 27

Correlations with respect to salinity are all negative (-0.68 < r < -0.45) pointing to a non-causal correlation via suspended particulates (salinity/total suspended particulates, r = -0.84). As with Group 1 there is no evidence of salting out of Group 2 compounds. Highly significant correlations exist between the
Group 2 PAH and suspended particulates (typically \( r > 0.70 \)) even though the concentrations in the estuarine water are less than 1% saturated. As previously indicated this relationship is strengthened further by excluding samples associated with the localised Plymouth inputs \(( r > 0.87 \)) (Table 5.1.). Fig. 5.3. shows the almost linear plots of benzo(k)fluoranthene \(( r = 0.97 \)) and benz(a)anthracene \(( r = 0.97 \)) against total suspended particulate load. Plymouth input samples exhibit positive concentration (enrichment) anomalies. Extrapolation of the Group 2 PAH plots against suspended particulates all have negative ordinate intercepts, infering the absence of soluble forms of these PAH in the Tamar Estuary. These plots may also indicate the presence of a proportion of particulates that are apparently depleted of PAH. It is unlikely that these high correlations would result from adsorption of PAH from water (Chapter III) and is highly supportive of the hypothesis that PAH are incorporated into the particulates (Section III.4.3.), rendering a high proportion of the PAH unavailable for dissolution. This is also supported by the fact that the correlation coefficients between Group 2 PAH and organic suspended solids \(( r = 0.53 \)) is lower than that between Group 2 PAH and total particulates \(( r = 0.65 \)), organic carbon having been identified as a significant parameter in consideration of sorption of PAH (Sections III.1. and III.4.2.(b)).

Significant correlations existed between most members of the Group 2 PAH (Fig. 5.4.) for example benzo(a)pyrene and chrysene \(( r = 0.92 \)) indicating general consistency of composition. With others eg. benzo(k)fluoranthene and benz(a)anthracene, bimodal distributions were apparent (Fig. 5.4.), illustrating the enrichment of benz(a)anthracene in the Plymouth inputs (see also Fig. 5.2.).
These compositional differences can be exploited (e.g. through benz(a)anthracene : chrysene ratio) to derive source indices (Fig. 5.2.) capable of resolving the Plymouth specific inputs of PAH from those PAH associated with the estuarine particulates. Without further compositional studies it is not possible to characterise the nature of the inputs of PAH from Plymouth.

Irrespective of location, pyrene correlated best with Total PAH \( (r = 0.91) \) (Fig. 5.4.) indicating that pyrene might be utilised as the most representative constituent of the total PAH in the Tamar Estuary water column.

(e) Statistical Analysis

The co-variability between PAH and environmental variables was evaluated by cluster analysis and multi-dimensional scaling (M.D.S.) (Section II.1.5.) of all 23 estuarine samples. These showed that the 4 Plymouth samples (17-20) significantly masked the correlations which would otherwise be present throughout the rest of the estuary. An example of this has already been discussed in Section V.3.1.(c), (see also Table 5.1.). In order to resolve the cross-correlations in the bulk of the estuary, cluster analysis and M.D.S. were repeated on all but the four Plymouth samples and the results are shown in Figs. 5.6. and 5.7. The selection of statistically meaningful clusters in the similarity dendrogram (Fig. 5.6.) was based on the conservative criterion that correlation coefficients derived from twenty simulations of randomly generated data of identical matrix size to the data set, never exceeded values of \( r = 0.83 \) (Fig. 5.5.). This acceptance criterion is indicated by the dashed line in Fig. 5.6. This clearly identifies a major cluster.
Figure 5.5.
Correlation profile analysis of PAH and environmental variables in the Tamar Estuary (excluding Plymouth samples 17-20). The unbroken lines represent correlation coefficients from twenty simulations of randomly generated data of identical matrix size to the environmental data set. No correlations obtained exceed a value of 0.83. This value was therefore adopted as the acceptance criterion of significant correlation. In contrast the broken line represents the plot achieved from the matrix containing the environmental variables and PAH data, demonstrating the existence of a significant number of non-spurious correlations in the data (approximately 65 correlations with coefficients >0.83).
Figure 5.6.
Cluster analysis dendrogram of PAH and environmental variables in the Tamar Estuary (excluding Plymouth samples, 17-20). The dashed line at 83% similarity (Similarity = 100 x absolute value of r) was adopted as the criterion for selecting significant correlations (see text).

'x' - unresolved mixture (Sections II.1.3.(b),(ii), and V.3.1.(e)).
Figure 5.7.
Multi-dimensional scaling analysis of PAH and environmental variables in the Tamar Estuary (excluding Plymouth samples 17-20). The cluster with similarity > 83% in Figure 5.6. is circumscribed. Stress factor = 0.155.

'x' - unresolved mixture (Sections II.1.3.(b),(ii), and V.3.1.(e)).
consisting of all Group 2 PAH (including total PAH), the phthalates and phenylesters (Section II.1.3.(b).(ii).), turbidity, total and mineral suspended solids. Naphthalene, phenanthrene and anthracene did not display significant correlations with either one another or with Group 2 PAH, confirming their random nature in the estuary. The M.D.S. plot (Fig. 5.7.) confirms the results of the cluster analysis but also shows the extent to which naphthalene, phenanthrene and anthracene are disparate and random with respect to other PAH and environmental variables. Aspects of other less significant correlations shown in Figs. 5.6. and 5.7. have been previously discussed. Thus, statistical analyses of the co-variability of PAH and associated environmental data provides an objective and independent confirmation of conclusions derived from chemical knowledge.

The HPLC peak, identified in Section II.1.3.(b).(ii)., as being comprised of phthalates and phenylesters, was semi-quantified for comparison purposes using a calibration assuming an equivalent molar extinction coefficient to the five ring PAH dibenz(ah)anthracene with which it coelutes (Hypersil ODS column. Section II.1.3.(b).(ii).). The resulting distribution demonstrated a remarkably high correlation with suspended particulates (Figs. 5.6. and 5.7.) suggesting that the compounds were particle associated and co-extracted with the PAH. Analytical blanks and extracts of filtered estuarine water samples were almost devoid of the compounds. It is also of note that the 'contaminant' peak was present in sections analysed throughout the dated sediment core (Chapter IV, Fig. 4.4.). Further research is required to fully evaluate these findings.
V.3.2. Sedimentary PAH in the Tamar Estuary and River Catchment

(a) Concentrations

Concentrations of individual PAH in the Tamar Estuary sediments (0-5 cm) varied typically between 30 to 1500 ng.(g dry sediment)$^{-1}$. Individual values are listed in Appendix Table IV. These values are comparable with those obtained for the Severn Estuary, U.K. 28,29 and for estuarine sediments from Narragansett Bay, U.S.A. 30 Tamar river sediments, however, contained considerably lower concentrations ranging typically from less than 1 to 50 ng.(g dry sediment)$^{-1}$ (Appendix Table IV), levels which are indicative of an unpolluted environment (Section 1.3.3.).

(b) Distribution and Transport Mechanisms

Fig. 5.8. shows the spatial distribution of pyrene and benzo(a)pyrene as representative PAH throughout the sample area. The riverine sediments are relatively depleted of PAH, which is consistent with the generally unpolluted state of the catchment. In addition, any small particulates with their associated PAH (Section III.4.3.) (see also Section V.3.4.) and light organic material, onto which PAH would preferentially adsorb20,31,32 (Chapter III), are likely to be selectively removed by the fast flow of the river. The only exception to this trend of low PAH concentrations in fresh water sediments is sample G2, which is exposed to chronic anthropogenic pollution. The PAH levels at station G4 located 5 km downstream of G2, however, return to the low concentrations typical of the catchment. The influence of the PAH discharged is therefore relatively short lived as regards sedimentary burden.
Figure 5.8.
Description of the Tamar catchment and estuary showing sediment sample site locations (Cl-G9 and El-E8). Pyrene, benzo(a)pyrene and lead concentrations and % particle size < 250 μm/ % carbon content data are shown graphically for each sample. Scales for the graphs are indicated on the left hand side of the diagram. Urban regions in the area are demarked by open stipple. Roads crossing the Tamar are shown and denoted by their numbers. Sewage works of particular note are indicated by 'SW'.

181
In Fig. 5.8, the percentages of particles < 2.50 μm are indicated for the catchment survey sediments (composed of a sand and gravel mixture) but are replaced by carbon analyses for the estuarine mud samples. There is a hundred fold increase in the concentrations of PAH from C8 to C9, with high concentrations maintained throughout the estuary (Figs. 5.8. and 5.9.). This surge in the PAH concentrations occurs just downstream from the weir which corresponds to the tidal limit of the estuary. Samples C9, E1 and E2 contain substantially elevated levels which increase sequentially. Settlement and flocculation processes in this intertidal region depositing PAH rich riverine particulates are the mechanisms most likely to produce the observed distribution. This is consistent with the substantial increase in < 250 μm particles at station C9. Maher et al. report a greater than ten fold increase in concentration of benzo(a)pyrene from fresh water sediments to estuarine sediments in the River Yarra, South East Australia. Further towards the mouth of the estuary at sample stations E3 and E4, decreases in PAH content are observed probably owing to dilution with less polluted marine sediments, together with degradation and physico-chemical processes (see Section V.3.2.(c).). This seaward decrease is also mirrored by an overall trend of decreasing carbon content (Figs. 5.8. and 5.9.).

Associated with the urbanised portion of the estuary are again elevated levels originating from chronic anthropogenic sources. Highest concentrations in this area were found beneath the Tamar Road Bridge (Figs. 5.8. and 5.9.).
Axial profile of concentrations of PAH and percentage carbon content in surface 5 cm of sediment cores in the Tamar Estuary. Distances from the 'narrows' (at the mouth of the estuary) are indicated at the bottom of the diagram. The position of Plymouth is shown. Symbols: o, naphthalene; a, phenanthrene; +, anthracene; v, fluoranthene; v, pyrene; x, chrysene; △, benzo(a)anthracene; ●, benzo(b)fluoranthene; △, benzo(k)fluoranthene; ■, benzo(a)pyrene.
(c) **Compositional Structure**

The dominance of unsubstituted parent hydrocarbons was apparent in all HGC chromatograms (Figs. 2.1. and 2.2.) (Section IV.4.3.). Compositional variations in the suite of compounds quantified are illustrated in Fig. 5.10. These are plotted on a log concentration scale to aid comparison. PAH composition of the estuarine sediments E1-E8 and the two high PAH concentration samples from the catchment (C2 and C9) are similar. Compositionally similar sedimentary PAH assemblages have been reported as ubiquitous (Section I.3.3.) (Section IV.4.5.(a)). Differences are, however, noted with samples E1 to E4 (Figs. 5.8., 5.9., 5.10.). PAH concentrations decrease seaward but the lower molecular weight compounds decrease at a greater rate than the higher molecular weight homologues. Sample E4 is thus enriched with, for example, benzo(a)pyrene or, alternatively, relatively depleted of phenanthrene, anthracene, fluoranthene and pyrene. Owing to the gradation observed, it is likely that an in situ change is responsible rather than changing source inputs. Degradation and/or solubilisation are probably responsible for the preferential reduction in the lower molecular weight PAH. The general compositional structure is restored in the urban portion of the estuary. Catchment samples C1 and C3-C8 exhibit differences from this general trend to varying degrees, C1 appearing particularly anomalous.

(d) **Sources**

For comparison purposes, the compositional PAH assemblages associated with various source emissions reported in the literature are illustrated in Fig. 5.11. Included are: motor vehicle exhaust, airborne particulates, street dust, sewage, domestic and industrial
Figure 5.10.

Compositional variations of PAH in Tamar sediments. For each sample 10 bars are shown indicating individual PAH concentrations starting with 1. phenanthrene at the top and proceeding downwards in sequence 2. anthracene; 3. fluoranthene; 4. pyrene; 5. benzo(a)anthracene; 6. benzo(e)pyrene; 7. benzo(k)fluoranthene; 8. benzo(a)pyrene; 9. benzo(ghi)perylene; 10. indeno(1,2,3-cd)pyrene. To aid comparison between samples a log PAH concentration scale was selected. Short lines extending beyond the base line indicate that these compounds were not quantified.
PAH compositions of selected sources. For comparison purposes Tamar sediment sample C9 is included. For each assemblage 10 bars are shown indicating individual PAH concentrations starting with 1. phenanthrene at the top and proceeding downwards in sequence - 2. anthracene; 3. fluoranthene; 4. pyrene; 5. benzo(a)anthracene; 6. benzo(e)pyrene; 7. benzo(k)fluoranthene; 8. benzo(a)pyrene; 9. benzo(g,h,i)perylene; 10. indeno(1,2,3-cd)pyrene. To aid comparison between samples a log PAH concentration scale was selected. Units of concentration vary and are individually noted. Short lines extending beyond the base line indicate that these compounds were not quantified.

(a) For these samples further qualitative information of compounds not quantified is available from chromatograms published by the authors.

(b) The authors suggest that the sampling procedure is responsible for loss of the lower molecular weight homologues.
waste water and coke oven emissions, and are likely to be representative of the major sources of PAH in the Tamar area. The dominance of unsubstituted parent hydrocarbons in the chromatograms is generally indicative of combustion sources (Section 1.2.2.) since crude oils display more complex assemblages \(^24\) (Section 1.2.1.(b)).

It is worthy of note that the PAH compositional patterns at the riverine (C2) and lower estuarine sampling stations are similar, tending to suggest similarity of the primary source.

As discussed in Sections 1.3.3., and IV.4.5.(a), other researchers, for example, Giger and Schaffner \(^35\) (Figs. 1.6. and 5.11.) and Wakeham et al. \(^36\) have recorded similar PAH assemblages to those observed in the Tamar. These authors attribute 'street dust' as the major source. Giger and Schaffner \(^35\) also suggest a similarity of composition of airborne particulates (Fig. 5.11.) and Wakeham et al. \(^36\) emphasise the importance of asphalt particles in street runoff.

Sample stations C2 and C9 are located in close proximity to major roads and are likely to receive polluted runoff. In addition, sample E5, situated below the Tamar road and rail bridges (Fig. 5.8.) showed the highest PAH concentrations of the lower estuary. The pattern of lead distribution, a possible indicator of road-runoff \(^37\), is shown in Fig. 5.8. Lead plotted against benzo(a)pyrene (Fig. 5.12) shows a near linear relationship \((r = 0.90)\) suggesting that both pollutants might originate from the same source. In combination with results discussed in Section IV.4.5., concerning distribution of PAH in a dated sediment core, road/urban runoff is likely to be a primary source of PAH into the Tamar. The exhaust emission shown in Fig. 5.11. is clearly not entirely responsible for the road runoff composition.

It is probable that a combination of exhaust emissions, asphalt
Figure 5.12.
Graph showing the relationship between lead and benzo(a)pyrene concentrations (µg.(g dry sediment)^{-1}) in surface sediments sampled from the Tamar catchment and estuary. Sediment samples containing significant concentrations of benzo(a)pyrene/lead are individually identified (positions of sampling are shown in Fig. 5.8.). Linear regression of the individual points is shown by the dashed line (correlation coefficient 'r' = 0.90). The intercept on the lead axis approximates the background, mineral derived lead. Regression calculations estimated this value as 17 µg.(g dry sediment)^{-1}.
particles, sump oil and tyre wear are involved.

Other potential sources investigated included sewage. Sample sites G2, G9 and W6 are close to sewage works (Fig. 5.8.) and the overall influence of sewage on the urban portion of the estuary (as indicated by increases in sediment carbon contents - Figs. 5.8. and 5.9.) rendered these emissions of interest. PAH distribution in the lower estuary, however, appears axially more confined than the sewage influenced area (Fig. 5.9.). Analysis of raw sewage from Plymouth (analysed using the technique described for sediments - Section II.1.3.(b).) resulted in a more complex chromatogram enriched with lower molecular weight compounds (Fig. 5.11.) and alkylated homologues. As can be seen from other sewage analyses (Fig. 5.11.), considerable variability of composition can be expected dependent on the degree of domestic and industrial waste and the extent to which street-runoff contributes to storm overflows (Section I.2.3.).

An important proportion of PAH from the sewage works is likely to originate from road runoff during heavy rain.

The PAH assemblage from coke oven emission (Fig. 5.11.) (taken to be generally indicative of coal combustion) resembles, to an extent, the sedimentary composition. Airborne combustion particulates from domestic heating in the area would not result in the observed sediment core distribution of the compounds in the lower estuary (Section IV.4.5.(b).), although might act to increase PAH burden in the catchment. Electron microscopic examination of isolated charcoal particles (according to Section IV.4.5.(c).) from sediments in the upper estuary failed to identify a qualitative correlation with PAH distribution. Point source emissions (for example at G2 and G9) are likely to be of more significance in PAH pollution. It is also
unlikely that the prevailing south westerly air stream in the region, which originates from the Atlantic Ocean, would contain high concentrations of PAH.

The distribution of coal particles at the top of the estuary have qualitatively been linked to the dilution described for PAH. The compositional patterns of PAH derived from coal do not, however, resemble those observed in this study \(^{28,40}\) (Section I.2.1.(b)).

V.3.3. **Comparison of PAH in estuarine surface sediments and suspended particulates in the water column**

In general, suspended particulates in the water column contain levels of the larger molecular weight (Group 2) PAH (expressed as ng.(g dry suspended particulates)\(^{-1}\)) similar to those recorded in the surface sediments (Table 5.2.). In contrast, the lower molecular weight (Group 1) PAH are significantly enriched in the water column relative to the sediments (Table 5.2.) confirming the presence of non-sedimentary sinks (Sections V.3.1.(c) and V.3.5.).

Benz(a)anthracene appears enriched in the water column owing to high concentrations encountered at the Plymouth input.

Percentage compositions of PAH associated with the water column and sediments are shown in Table 5.3. The general uniformity of the sedimentary PAH assemblage (as discussed in Section V.3.2.(c)) is further demonstrated. A similar composition is also recorded for PAH associated with particulates in the water column (as exemplified by the turbidity maximum sample (3) in Table 5.3.). These data suggest that the primary fate of the larger molecular weight (Group 2) homologues is sediment incorporation. It would also appear
Table 5.2.
Average concentrations of PAH and comparative enrichments in water and surface sediments of the Tamar Estuary.

<table>
<thead>
<tr>
<th>Compound</th>
<th>Water concentration (ng dm(^{-3}))</th>
<th>Sediments ng(g dry weight)(^{-1})</th>
<th>Enrichment relative to surface sediments</th>
</tr>
</thead>
<tbody>
<tr>
<td>Naphthalene</td>
<td>13.9</td>
<td>1080 a</td>
<td>245</td>
</tr>
<tr>
<td>Phenanthrene</td>
<td>8.8</td>
<td>1042 a</td>
<td>363</td>
</tr>
<tr>
<td>Anthracene</td>
<td>4.9</td>
<td>497 a</td>
<td>120</td>
</tr>
<tr>
<td>Fluoranthene</td>
<td>10.4</td>
<td>760</td>
<td>802</td>
</tr>
<tr>
<td>Pyrene</td>
<td>18.0</td>
<td>1597</td>
<td>878</td>
</tr>
<tr>
<td>Chrysene</td>
<td>3.5</td>
<td>283</td>
<td>345</td>
</tr>
<tr>
<td>Benz(a)anthracene</td>
<td>15.2</td>
<td>2710</td>
<td>601</td>
</tr>
<tr>
<td>Benzo(b)fluoranthene</td>
<td>9.3</td>
<td>871</td>
<td>475</td>
</tr>
<tr>
<td>Benzo(k)fluoranthene</td>
<td>4.2</td>
<td>362</td>
<td>396</td>
</tr>
<tr>
<td>Benzo(a)pyrene</td>
<td>9.1</td>
<td>741</td>
<td>724</td>
</tr>
</tbody>
</table>

(a) Calculated to illustrate that even if these PAH were particle associated they are significantly enriched in the water column compared with the sediment.

Table 5.3.
PAH compositions of selected water and sediment samples.

<table>
<thead>
<tr>
<th>Sample Code</th>
<th>Turbidity Maximum</th>
<th>Plymouth Input</th>
<th>Fresh Water</th>
<th>Tamar Bridge</th>
<th>Oil Jetty</th>
</tr>
</thead>
<tbody>
<tr>
<td>Naphthalene</td>
<td>1.8</td>
<td>0.2</td>
<td>3.7</td>
<td>4.3</td>
<td>2.9</td>
</tr>
<tr>
<td>Phenanthrene</td>
<td>8.0</td>
<td>11.6</td>
<td>7.0</td>
<td>8.1</td>
<td>10.0</td>
</tr>
<tr>
<td>Anthracene</td>
<td>1.4</td>
<td>1.5</td>
<td>2.3</td>
<td>3.4</td>
<td>3.8</td>
</tr>
<tr>
<td>Fluoranthene</td>
<td>14.6</td>
<td>1.9</td>
<td>17.4</td>
<td>15.6</td>
<td>13.6</td>
</tr>
<tr>
<td>Pyrene</td>
<td>21.7</td>
<td>16.0</td>
<td>17.4</td>
<td>17.7</td>
<td>18.6</td>
</tr>
<tr>
<td>Chrysene</td>
<td>5.5</td>
<td>2.0</td>
<td>7.5</td>
<td>6.8</td>
<td>7.2</td>
</tr>
<tr>
<td>Benz(a)anthracene</td>
<td>9.9</td>
<td>46.2</td>
<td>13.3</td>
<td>13.0</td>
<td>12.5</td>
</tr>
<tr>
<td>Benzo(b)fluoranthene</td>
<td>12.4</td>
<td>9.3</td>
<td>10.7</td>
<td>10.0</td>
<td>8.6</td>
</tr>
<tr>
<td>Benzo(k)fluoranthene</td>
<td>7.0</td>
<td>4.1</td>
<td>6.6</td>
<td>6.6</td>
<td>7.9</td>
</tr>
<tr>
<td>Benzo(a)pyrene</td>
<td>17.7</td>
<td>7.2</td>
<td>14.2</td>
<td>14.5</td>
<td>15.0</td>
</tr>
</tbody>
</table>
that with the exception of the preferential reduction of lower molecular weight PAH discussed in Section V.3.2.(c.), degradation processes which might differentially act on individual homologues in the water column, such as photo-oxidation (Sections I.4.1. and I.4.3.) and heterotrophic degradation (Section V.3.5.) are generally unimportant with regard to particle bound PAH. The sediment uniformity might also suggest compositionally similar inputs (as discussed in Section V.3.2.(a.).) This is not supported by the water column survey which identified a pollutant discharge in the Plymouth region with a different composition (Table 5.3.). The large sedimentary reservoir could act to buffer compositional changes in recent inputs of PAH, although the Plymouth input was shown to be present as transient soluble and colloidal forms and as such may be more susceptible to diffusive transport and degradative processes (Section IV.4.3.).

V.3.4. PAH associated with suspended particulate size fractions

Individual particle size fractions: >100 μm; 53 μm to 100 μm; 10 μm to 53 μm; <10 μm (corresponding approximately to sand, very fine sand, silt and clay respectively according to Wentworth classification) were isolated (Section V.2.3.) from estuarine water sampled from the turbidity maximum of the estuary (Section V.3.1.(a.).) Results are shown in Fig. 5.13(a). All four fractions show similarity of PAH composition (Fig. 5.13(b.).) Concentrations of PAH in particles >100 μm were substantially higher (6 fold) than those in the 53 μm-100 μm fraction and more than an order of magnitude greater than levels associated with particles <53 μm. A proportion
Figure 5.13.
Histograms showing PAH concentrations and compositions in particulate size fractions of suspended solids sampled from the turbidity maximum of the estuary. For each fraction 10 bars are shown indicating individual PAH concentrations starting with
1. phenanthrene on the left and proceeding right in sequence
2. anthracene, 3. fluoranthene, 4. pyrene, 5. benzo(a)anthracene,
6. chrysene, 7. benzo(e)pyrene, 8. perylene, 9. benzo(k)-fluoranthene, 10. benzo(a)pyrene. Short bars extending beyond the base line indicate that these compounds were not quantified.
(a) PAH concentrations in isolated particulates (expressed in µg.(g dry particulates)⁻¹).
(b) PAH concentrations corrected for the relative proportions of the particulate fractions in the estuarine water sampled and expressed as ng.dm⁻³.
of the > 100 μm fraction was identified as low density organic debris. It might be expected that this fraction would contain charcoal particles and charred organic material, the densities of which are characteristically low. A loss in weight of 40% on ignition (450°C, 6 h) confirmed the associated high organic content. Prahl has also reported enrichment of PAH in low density fractions of sediment. In agreement with Brassell and Eglinton the sand fraction contains an order of magnitude higher concentrations of PAH than silt and clay sized fractions. Weight loss on ignition (450°C, 6 h) for the very fine sand was 20% compared to 10% for particles < 53 μm indicating higher organic content of the sand fraction. Further microscopic studies in conjunction with fractionations are, however, required to identify the particles responsible for this PAH distribution. Analyses for asphalt particles (shown to contain a PAH assemblage remarkably similar to that of combustion origin) might prove of particular interest.

The relative importance of the individual fractions in PAH composition of the total suspended particulates sampled are indicated in Fig. 5.13(b). Although highest concentrations were recorded in the >100 μm fraction, within the sample this is the fraction of least importance with regard to the total PAH content.

The PAH content of these fine particulates (which originate from the catchment area and are transported in the fast flow of the river) support the theory that settlement and flocculation processes produce the elevated PAH concentrations in sediments at the head of the estuary (Section V.3.2.(b)).
V.3.5. **Microbial Heterotrophic Degradation**

An indication of the relative microbial degradation rates of spiked PAH may be derived from the radiochemical data (Section II.2.1.(a.)) by calculating the "turn over times" as shown by Lee. The axial distribution of degradation rates of spiked \(^{14}\)C-naphthalene and \(^{14}\)C-benzo(a)pyrene (as monitored by \(^{14}\)C-CO\(_2\) production) in the Tamar Estuary are plotted in Fig. 5.14. In general, degradation rates in the estuary are considerably higher than those in marine waters with rates for naphthalene far exceeding those for benzo(a)pyrene. For example, naphthalene degradation rates off the dockyard (D16) and in Plymouth Sound (D22) were 72% day\(^{-1}\) and 3% day\(^{-1}\) respectively, whereas the corresponding rates for benzo(a)pyrene were only 0.035% day\(^{-1}\) and 0.011% day\(^{-1}\) respectively.

Naphthalene degradation rates were independent of both naphthalene concentrations in the estuary and the suspended particulates (Fig. 5.15). This suggests that free living bacteria are responsible. However, it is not certain whether the high mid-estuary rates arise from higher microbial biomass or from a microbial population rich in hydrocarbon degraders. The corresponding naphthalene turn over times of 1-2 days, calculated for the mid-estuarine region (D10-D16) are comparable to the flushing times of the estuary 3-5 days. Clearly microbial degradation of naphthalene, at least at the 1 \(\mu g.dm^{-3}\) level, is efficient and rapid and may be a principal fate of naphthalene in the Tamar Estuary.

In contrast, the degradation rates of benzo(a)pyrene peak at the turbidity maximum and correlate with suspended particulates (Fig. 5.15.) suggesting that particle associated bacteria are
Figure 5.14. Axial profiles of naphthalene and benzo(a)pyrene concentrations and microbial heterotrophic degradation rates in the Tamar Estuary water column. 95% confidence interval bars are indicated for triplicate concentration samples (7, 8 and 9) and for each triplicate degradation sub-sample. The profile for total suspended solids is also shown.

Figure 5.15. Plots of naphthalene (a), and benzo(a)pyrene (b), microbial heterotrophic degradation rates against total suspended particulates. Individual points are labelled with sub-sample numbers to indicate their respective positions in the estuary (Fig. 5.1.).
responsible. The slow degradation rates of benzo(a)pyrene (turn over times 2000-9000 days) together with its high particle association (Section V.3.2.(d)), (Chapter III) point towards sediments as the final repository of benzo(a)pyrene in the Tamar Estuary, (Chapter IV).

In addition to $^{14}$C-CO$_2$ production, other parameters assessed after the incubation periods included assay of undegraded parent hydrocarbons and estimation of polar metabolites (Section II.2.1.(a)). $^{14}$C-CO$_2$ production yielded the most precise indication of degradation, particularly for benzo(a)pyrene where degradation was minimal and was typically within the analytical precision of the latter two parameters described. For naphthalene, the partitioning of $^{14}$C activity as shown in Fig. 5.16., demonstrates that a significant proportion of the parent hydrocarbon is degraded to polar metabolites which are not accounted for in 'turn-over time' calculations based on $^{14}$C-CO$_2$ production.
Figure 5.16.
Percentage distribution of $^{14}$C activity following incubation (20 h in darkness) of $^{14}$C-labelled naphthalene spiked into estuarine water samples (D1-D6, Fig. 5.1.). Parent hydrocarbon remaining in solution, polar metabolites and $^{14}$C-CO$_2$ activities are represented as dense shading, hatched lines and open stipples respectively. For comparison purposes a control (C) (mercuric chloride poisoned) incubation is also shown.
REFERENCES


High-performance liquid chromatographic (HPLC) techniques capable of resolving the major individual PAH extracted from environmental samples were developed. Identification of eluting peaks was performed utilising a stop-flow UV scanning system (Readman et al., Analyst, 106, p. 122. (1981)). In addition, an HPLC procedure was developed to enable rapid and precise measurement of selected dissolved PAH in experimental waters by direct injection of sub-samples, thus avoiding time-consuming pre-concentration.

Opinion in the literature has attributed the high particulate association of PAH in aquatic environments to the hydrophobic properties of the compounds leading to adsorption. Equilibrium partition of spiked PAH between the aqueous and particulate phases of Tamar Estuary water samples was experimentally quantified and $K_p$ (equilibrium partition coefficient) values calculated. Analyses of the in situ environmental PAH present in the dissolved and particulate phases of sub-samples from the same estuarine waters, resulted in $K_p$ values orders of magnitude higher than those derived from the sorption experiments. This suggests that environmental PAH in the water column are not in equilibrium between the aqueous and particulate phases, and are considerably enriched in the particulates, apparently in a form that renders them unavailable for dynamic exchange with the water. It follows that in the Tamar Estuary a large proportion of the PAH (especially the larger molecular weight carcinogenic homologues) are particle incorporated and may not be readily available for physico-chemical fates and possibly biological cycling.
The vertical distribution of PAH in a \(^{210}\)Pb dated sediment core (sampled from the lower region of the estuary) showed a rapid exponential decrease with depth. Concentrations of individual compounds at the surface were typically two orders of magnitude higher than concentrations in sediments deposited prior to 1940. Most significant increases in the PAH flux were shown to have occurred since 1960. The generally uniform composition of PAH in the anoxic core suggests that a constant primary pollutant source was responsible and that once PAH were incorporated into the sediments, degradation mechanisms were of only minor importance. The PAH composition is indicative of a source related to fossil fuel combustion or urban (road) runoff. No qualitative correlation between combustion particulates and PAH was identified. In the sampling area, the distribution of PAH with date suggests an association with motor vehicles, probably via road runoff. Correlations with elements including heavy metals are discussed. Of the PAH analysed, perylene was the only homologue to exhibit an anomalous distribution with depth, which could be attributed to a biogenic origin of the compound.

A survey of the distribution of PAH throughout the water column of the Tamar Estuary resulted in the first published data available on individual PAH concentrations in estuarine waters (Readman et al., Estuarine Coastal Shelf Sci., 14, p.369, (1982)). Concentrations varied typically between 1 and 50 ng dm\(^{-3}\) for individual compounds. Distribution of the larger molecular weight PAH (MW > 200) was highly correlated (correlation coefficient 'r' > 0.87) with the suspended particulate loading of the water samples, even though concentrations of PAH in the estuary were less than 1% saturated. This relationship is supportive of the theory of non-equilibrium
partition, confirming considerable particulate enrichment. Highest concentrations of these compounds were associated with the turbidity maximum of the estuary.

In contrast, lower MW homologues (MW < 200) demonstrated relatively low correlations with suspended particulates. Naphthalene and anthracene were shown to exhibit particularly anomalous distributions, although phenanthrene demonstrated higher correlations with the larger MW PAH (MW > 200). The low correlation with particulates indicates enrichment of soluble states, which are likely to be available for physico-chemical and biological fates. Microbial heterotrophic degradation of 'spiked' naphthalene in estuarine water samples yielded turn-over times between 1 and 30 days, with maximum degradation associated with the mid-estuarine region. Degradation possibly rates were independent of suspended particulates, suggesting that free-living microbes are responsible. It is not certain whether the high mid-estuarine rates arise from higher microbial biomass or from a microbial population rich in hydrocarbon degraders. Compared with the flushing time of the estuary (3-5 days) microbial degradation is probably important in removal of naphthalene and possibly other low MW homologues. Volatilisation is also proposed as a potentially important process in determining the fate, and hence distribution, of these homologues.

High PAH concentrations were recorded in a series of water samples from the urban portion of the estuary. The PAH composition associated with these samples was different to that identified throughout the remainder of the estuary and was attributed to a pollutant emission, the source of which was not fully characterised. By using selected homologue ratios, an index was derived to isolate samples
A temporally integrated assessment of the spatial distribution of PAH was obtained by surveying PAH in surface sediments throughout the estuary and river catchment (Readman et al., in 'Transfer Processes in Cohesive Sediment Systems'. W.R. Parker and D.J.J. Kinsman (eds.). In press). Concentrations of individual PAH in sediments from the Tamar catchment were typically < 50 ng. (g dry sediment)^{-1} whereas those from the estuary were between 30 to 1500 ng. (g dry sediment)^{-1}. The generally low levels of PAH in the river sediments was attributed to the relatively unpolluted environment and the fast flow of the river removing any PAH rich materials. A point source PAH emission to the river was observed, but its downstream distribution declined over a short distance (< 5 Km). Sedimentation and flocculation processes at the head of the estuary, deposit riverine particulates, increasing the sedimentary PAH concentrations in this region. Levels then decrease towards the mid-estuarine region owing primarily to dilution with less polluted marine sediments. Associated with the urbanised portion of the estuary are elevated levels originating from chronic anthropogenic sources. Highest PAH concentrations in the lower estuary were recorded in sediment sampled from below the Tamar road and rail bridges.

Although substantial variations in PAH concentrations were recorded throughout the Tamar region, PAH composition was generally uniform. Exceptions to the uniform assemblage were most notable in catchment samples containing very low levels of PAH. A trend in change was also observed in sediments at the head of the estuary, where reductions in low MW PAH relative to higher MW homologues were
recorded for sequential samples progressing from the tidal limit towards the mid-estuarine region (Section V.3.2.(c)). This observation might demonstrate that some exchange of low MW PAH from the sediment particulates to the water occurs, which supports results from the water column survey demonstrating enrichment of soluble states of these homologues. The 'typical' PAH composition is restored in the urban region of the estuary.

Suspended particulates in the water column contain similar levels, and compositionally reflect the larger MW PAH (MW > 200) recorded in the surface sediments. This observation endorses the significance of particulate transport (and hence the influence of hydrodynamics) and also suggests that compound-selective degradation processes in the water column (for example photo-oxidation) are of only minor importance, the primary fate of these large MW compounds being sediment incorporation (Chapter IV).

The generally uniform composition of PAH recorded throughout the estuary might also suggest a uniform primary source. As previously discussed for the core, the PAH assemblage is indicative of a combustion origin or road and urban runoff. In the lower estuary evidence supports pollution from road runoff. The proximity of major roads and urban settlements to point source emissions of PAH in the catchment, together with covariation between lead and PAH throughout sediments of the catchment and estuary (correlation coefficient \(r\) between benzo(a)pyrene and lead = 0.90), suggests that road runoff might be a primary source of PAH throughout the entire area. No qualitative correlation between PAH concentrations and combustion particulates was observed in the upper estuary. Particle size
distribution of PAH in suspended particulates sampled from the turbidity maximum of the estuary revealed that highest concentrations were associated with relatively large particulates (<53 μm) (Section 5.3.4.). Further research is required to fully characterise the source, with analyses for asphalt particulates possibly affording the greatest potential for resolving this problem. The approach suggested for this extension would involve high-resolution chromatographic analyses of sediments (and isolated PAH rich particulates) for comparison with equivalent analyses of 'weathered' asphalt particles collected directly from a road-runoff source. Capillary-GC, capable of resolving individual alkylated homologues, would provide the most suitable 'fingerprint' for comparison of the PAH compositions. Analyses and ratioing of metals such as vanadium and nickel (which might be characteristic for asphalts) in sediments could possibly provide additional confirmatory information with regard to pollution from asphalt particulates and road runoff.

Discharge of oil in the Plymouth region (from dockyards and an oil jetty terminal) asserts only a minor influence on sedimentary PAH composition. This might arise from the dominance of the primary source 'buffering' these less significant inputs. Alternatively, oil discharges could be more susceptible than particle associated PAH to diffusive transport and fates such as volatilisation and photo-oxidation.
APPENDIX

Individual values recorded for the variables monitored in estuarine samples

Table I  Data recorded for water samples (and associated particulates) used in sorption experiments (Chapter III).

Table II Sediment Core data (Chapter IV).

Table III Tamar Estuary water column survey (Chapter V).

Table IV Surface sediment survey of the Tamar Estuary and Catchment (Chapter V).
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<th>9</th>
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<td>0.04</td>
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<td>5.71</td>
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<td>18.3</td>
<td>22.3</td>
<td>27.2</td>
<td>31</td>
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<td>Temperature (°C)</td>
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<td>1.98</td>
<td>2.39</td>
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<td>45.6</td>
<td>329.8</td>
<td>282.6</td>
<td>37.2</td>
<td>22.4</td>
<td>18.0</td>
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<td>-</td>
<td>2.7</td>
<td>10.7</td>
<td>5</td>
<td>4.3</td>
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<td>5.2</td>
<td>5.9</td>
</tr>
<tr>
<td>4-50 µm</td>
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<td>89.3</td>
<td>61.3</td>
<td>68.4</td>
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<td>83</td>
<td>85.2</td>
<td>85.5</td>
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<td>50-200 µm (&lt; 200 µm)</td>
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<td>26</td>
<td>0</td>
<td>33.7</td>
<td>27.3</td>
<td>13.2</td>
<td>11.3</td>
<td>9.6</td>
<td>8.6</td>
</tr>
<tr>
<td>FOC (mg.dm^{-3})</td>
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<td>ION (mg.dm^{-3})</td>
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<td>7.89</td>
<td>8.36</td>
<td>9.79</td>
<td>8.47</td>
<td>8.22</td>
<td>7.67</td>
<td>6.78</td>
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</tbody>
</table>

Table I
|     | 1   | 2   | 3   | 4   | 5   | 6   | 7   | 8   | 9   | 10  | 11  | 12  | 13  | 14  | 15  | 16  | 17  | 18  | 19  | 20  | 21  | 22  | 23  | 24  | 25  | 26  |
|-----|-----|-----|-----|-----|-----|-----|-----|-----|-----|-----|-----|-----|-----|-----|-----|-----|-----|-----|-----|-----|-----|-----|-----|-----|-----|-----|-----|
| Depth (cm) | 0 1 | 1 2 | 2 3 | 3 4 | 4 5 | 5 6 | 6 7 | 7 8 | 8 9 | 9 10 | 10 11 | 11 12 | 12 13 | 13 14 | 14 15 | 15 16 | 16 17 | 17 18 | 18 19 | 19 20 | 20 21 | 21 22 | 22 23 | 23 24 | 24 25 | 25 26 |
| Eh | -59.0 | -75.0 | -100.0 | -125.0 | -125.0 | -100.0 | -100.0 | -100.0 | -100.0 | -100.0 | -100.0 | -100.0 | -100.0 | -100.0 | -100.0 | -100.0 | -100.0 | -100.0 | -100.0 | -100.0 | -100.0 | -100.0 | -100.0 | -100.0 | -100.0 | -100.0 |
| Redox | 57.7 | 77.7 | 77.7 | 61.4 | 61.4 | 56.5 | 56.5 | 55.7 | 55.7 | 55.7 | 55.7 | 55.7 | 55.7 | 55.7 | 55.7 | 55.7 | 55.7 | 55.7 | 55.7 | 55.7 | 55.7 | 55.7 | 55.7 | 55.7 | 55.7 |
| Total Carbon (g) | - | 1.09 | - | - | - | 1.00 | - | - | - | - | - | - | - | - | - | - | - | - | - | - | - | - | - | - | - |
| Organic Carbon (g) | - | - | 1.00 | - | - | - | - | - | - | - | - | - | - | - | - | - | - | - | - | - | - | - | - | - | - |
| Total Nitrogen (g) | - | 0.39 | - | - | - | - | - | - | - | - | - | - | - | - | - | - | - | - | - | - | - | - | - | - | - |
| Organic Nitrogen (g) | - | 0.09 | - | - | - | - | - | - | - | - | - | - | - | - | - | - | - | - | - | - | - | - | - | - | - |
| Silicon (g) | 30.9 | 25.3 | 20.9 | 25.7 | 26.1 | 27.3 | 28.3 | 28.5 | 28.8 | 28.8 | 28.8 | 28.8 | 28.8 | 28.8 | 28.8 | 28.8 | 28.8 | 28.8 | 28.8 | 28.8 | 28.8 | 28.8 | 28.8 | 28.8 | 28.8 |
| Copper (µg) | 0.0 | 0.0 | 0.0 | 0.0 | 0.0 | 0.0 | 0.0 | 0.0 | 0.0 | 0.0 | 0.0 | 0.0 | 0.0 | 0.0 | 0.0 | 0.0 | 0.0 | 0.0 | 0.0 | 0.0 | 0.0 | 0.0 | 0.0 | 0.0 | 0.0 | 0.0 |
| Zinc (µg) | 18.5 | 21.5 | 19.5 | 21.5 | 21.5 | 21.5 | 21.5 | 21.5 | 21.5 | 21.5 | 21.5 | 21.5 | 21.5 | 21.5 | 21.5 | 21.5 | 21.5 | 21.5 | 21.5 | 21.5 | 21.5 | 21.5 | 21.5 | 21.5 | 21.5 |
| Lead (µg) | 0.0 | 0.0 | 0.0 | 0.0 | 0.0 | 0.0 | 0.0 | 0.0 | 0.0 | 0.0 | 0.0 | 0.0 | 0.0 | 0.0 | 0.0 | 0.0 | 0.0 | 0.0 | 0.0 | 0.0 | 0.0 | 0.0 | 0.0 | 0.0 | 0.0 | 0.0 |
| Fe 210 Activity (pCi/g) | 0.0 | 0.0 | 0.0 | 0.0 | 0.0 | 0.0 | 0.0 | 0.0 | 0.0 | 0.0 | 0.0 | 0.0 | 0.0 | 0.0 | 0.0 | 0.0 | 0.0 | 0.0 | 0.0 | 0.0 | 0.0 | 0.0 | 0.0 | 0.0 | 0.0 | 0.0 |
| Be 107 Activity (pCi/g) | 0.0 | 0.0 | 0.0 | 0.0 | 0.0 | 0.0 | 0.0 | 0.0 | 0.0 | 0.0 | 0.0 | 0.0 | 0.0 | 0.0 | 0.0 | 0.0 | 0.0 | 0.0 | 0.0 | 0.0 | 0.0 | 0.0 | 0.0 | 0.0 | 0.0 | 0.0 |
| Data | 1900 | 1900 | 1900 | 1900 | 1900 | 1900 | 1900 | 1900 | 1900 | 1900 | 1900 | 1900 | 1900 | 1900 | 1900 | 1900 | 1900 | 1900 | 1900 | 1900 | 1900 | 1900 | 1900 | 1900 | 1900 |
| Nitrates | - | - | - | - | - | - | - | - | - | - | - | - | - | - | - | - | - | - | - | - | - | - | - | - | - |
| Ammonium | - | - | - | - | - | - | - | - | - | - | - | - | - | - | - | - | - | - | - | - | - | - | - | - | - |
| Phosphates | - | - | - | - | - | - | - | - | - | - | - | - | - | - | - | - | - | - | - | - | - | - | - | - | - |
| Pyroh | 0.0 | 0.0 | 0.0 | 0.0 | 0.0 | 0.0 | 0.0 | 0.0 | 0.0 | 0.0 | 0.0 | 0.0 | 0.0 | 0.0 | 0.0 | 0.0 | 0.0 | 0.0 | 0.0 | 0.0 | 0.0 | 0.0 | 0.0 | 0.0 | 0.0 | 0.0 |
| Total B (mg/kg) | 0.0 | 0.0 | 0.0 | 0.0 | 0.0 | 0.0 | 0.0 | 0.0 | 0.0 | 0.0 | 0.0 | 0.0 | 0.0 | 0.0 | 0.0 | 0.0 | 0.0 | 0.0 | 0.0 | 0.0 | 0.0 | 0.0 | 0.0 | 0.0 | 0.0 | 0.0 |
| Table II | | | | | | | | | | | | | | | | | | | | | | | | | | | | | | |
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<tr>
<td>% Carbon</td>
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<td>-</td>
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<tr>
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<td>36</td>
<td>26</td>
<td>26</td>
<td>9</td>
<td>12</td>
<td>16</td>
<td>23</td>
<td>71</td>
<td>213</td>
<td>290</td>
<td>160</td>
<td>163</td>
<td>120</td>
<td>111</td>
<td>185</td>
<td>65</td>
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<tr>
<td>PAH Concentrations (ng (g dry sediment)^{-1})</td>
<td></td>
<td></td>
<td></td>
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</tr>
</tbody>
</table>

| Naphthalene     | -  | -  | -  | -  | -  | -  | -  | -  | -  | -  | -  | -  | -  | -  | -  | -  | -  |
| Phenanthrene    | 5  | 220| 9  | 9  | 31 | 2  | 4  | 3  | 170| 380| 610| 400| 280| 510| 400| 270| 100|
| Anthracene      | 4  | 70 | 3  | 1  | 2  | 1  | 1  | 1  | 70 | 110| 200| 70 | 220| 150| 60 | 70 | 30 |
| Fluoranthene    | 3  | 330| 31 | 11 | 17 | 2  | 5  | 3  | 600| 1300|1520| 700| 390| 1000|550| 590| 270|
| Pyrene          | 4  | 560| 37 | 22 | 14 | 5  | 7  | 8  | 600| 1200|1520| 860| 800| 1100|750| 850| 280|
| Benzo(a)anthracene | 2 | 150| 12 | 10 | 8  | 1  | 5  | 2  | 200| 600| 1160|560| 370| 860| 520| 660| 120|
| Chrysene        | -  | -  | -  | -  | -  | -  | -  | -  | -  | -  | -  | -  | -  | -  | -  | -  | -  |
| Benzo(e)pyrene  | 4  | 240| 14 | 12 | 12 | 2  | 5  | 2  | 460| -  | -  | -  | -  | -  | -  | -  | -  | -  |
| Benzo(k)fluoranthene | - | -  | -  | -  | -  | -  | -  | -  | -  | 570| 930| 440| 720| 660| 350| 600| 120|
| Benzo(k)fluoranthene | 3 | 105| 5  | 3  | 18 | 4  | 4  | 3  | 56 | 390| 530| 330| 230| 480| 320| 70  | 70  |
| Benzo(a)pyrene  | 4  | 210| 14 | 12 | 11 | 2  | 7  | 3  | 450| 690| 1260|720| 520| 920| 610| 660| 150|
| Benzo(ghi)pyrene | 5  | 150| 9  | 8  | 8  | 2  | 5  | 2  | 270| -  | -  | -  | -  | -  | -  | -  | -  | -  |
| Indeno(1,2,3-cd)pyrene | 4 | 60 | 7  | 7  | 7  | 1  | 2  | 1  | 110| -  | -  | -  | -  | -  | -  | -  | -  | -  |

**Table IV**
Aquatic Distribution and Heterotrophic Degradation of Polycyclic Aromatic Hydrocarbons (PAH) in the Tamar Estuary

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Variations in the concentrations and microheterotrophic degradation rates of selected Polycyclic Aromatic Hydrocarbons (PAH) in the water column of the Tamar Estuary were investigated in relation to the major environmental variables. Concentrations of individual PAH varied typically between 1 and 50 ng l⁻¹. Based on their observed environmental behaviour the PAH appeared divisible into two groupings: (i) low molecular weight PAH incorporating naphthalene, phenanthrene and anthracene and (ii) the larger molecular weight homologues (fluoranthene, pyrene, chrysene, benzo(a)anthracene, benzo(b)fluoranthene, benzo(k)fluoranthene, benzo(a)pyrene). Group 1 PAH showed a complex distribution throughout the estuary with no significant correlations with either salinity or suspended particulates. Based on their relatively low particle affinity and high water solubilities and vapour pressures, volatilization is proposed as an important process in determining their fate.

Microheterotrophic turnover times of naphthalene varied between 1 and 30 days, and were independent of suspended solids with maximum degradation rates located in the central and urban regions of the Estuary. When compared with the flushing times for the Tamar (3–5 days), it is probable that heterotrophic activity is important in the removal of naphthalene (and possibly the other Group 1 PAH) from the estuarine environment. In contrast Group 2 PAH concentrations exhibited highly significant correlations with suspended particulates. Highest concentrations occurred at the turbidity maximum, with a secondary concentration maximum localized to the industrialized portion of the estuary and associated with anthropogenic inputs. Laboratory degradation studies of benzo(a)pyrene in water samples taken from the estuary showed turnover times for the compound of between 2000 and 9000 days. Degradation rates correlated positively with suspended solids. The high particulate affinity and microbial refractivity of Group 2 PAH indicate sediment burial as the principal fate of these PAH in the Tamar Estuary. Estuarine sediments contained typically 50–1500 ng g⁻¹ dry weight of individual PAH which were comparable to the levels of
Group 2 PAH associated with the suspended particulates. Highest concentrations occurred at the riverine end of the estuary resulting from unresolved inputs in the catchment. Subsequent dilution by less polluted marine sediments together with slow degradation results in a seaward trend of decreasing concentrations. However, there is a secondary maximum of PAH superimposed on this trend which is associated with urban Plymouth.

Introduction

The carcinogenic properties of some Polycyclic Aromatic Hydrocarbons (PAH) have in recent years led to interest in their distribution and fate in the environment. Many reviews are available on sources, distributions, fates and biological effects of PAH (Andelman & Snodgrass, 1972, Andelman & Suess, 1970, Brown, 1979, Harrison et al., 1975; Jackum & Lake, 1978; Neff, 1979; Radding et al., 1976) In particular, sedimentary PAH have received considerable attention because they may record and integrate the input history of PAH into the aquatic environment (Bier et al., 1978; Hites et al., 1980; John et al., 1979; Lafamme & Hites, 1978; Lake et al., 1979; Platt & Mackie, 1979; Wakeham et al., 1980). Although estuaries are dynamic regions of steep chemical and biological gradients, there have been no systematic field investigations of the effects of environmental variables in estuaries on the aquatic distribution and fate of PAH in estuarine waters. In addition, the proximity of large urban and industrial conurbations render estuaries of particular interest.

Results published from experiments by Lee et al. (1978) on the fate of selected PAH in a controlled ecosystem enclosure indicate that aromatic hydrocarbons in shallow marine waters may have residence times in the order of a few days. For lower molecular weight aromatics such as benzenes, naphthalenes and phenanthrenes, their results suggest that microbial degradation and volatilization (Knapp et al., 1979) are the primary removal processes. In contrast the concentrations of higher molecular weight aromatics such as chrysenes, benzoanthracenes and benz-pyrenes were shown to be affected primarily by sedimentation and photo-chemical oxidation. Furthermore, Lee et al. (1978) suggest that in open oceans which are characterized by low concentrations of suspended particulates and clear waters, rates of sedimentation would be low and photo-oxidation rates should be high. It follows that under turbid conditions, such as occur in tidal estuaries, movement of suspended solids and sedimentation would be expected to be of primary importance in the transport of these larger molecular weight PAH.

This paper investigates the distribution of selected PAH in the water and sediments of the Tamar Estuary and microbial degradation of selected PAH in the water column. These results are compared with variations in salinity, pH, temperature, dissolved oxygen, ammonia and suspended solids.

Study area and sample collection

The geographical location of the sampling area and position of the sample sites are illustrated in Figure 1. Morris et al. (1978, 1981, 1982) have described the master variables and nutrient chemistry of the Tamar. Since the River Tamar represents the major source of river runoff into the Tamar Estuary, sampling was concentrated along the main estuarine axis rather than in the Lynher and Tavy tributaries (Figure 1). Sampling was performed on the ebb tide on 7 May 1980. High tide at Calstock (Figure 1) on this day was 11.16 a.m. Sampling commenced at 12.00 a.m. Surface water samples were collected during an axial traverse of the estuary (freshwater to marine) aboard a 'Rotork Seatruck'. Samples were
Figure 1. Description of the Tamar Estuary and sampling stations. Water sample numbers and locations are indicated on the right-hand side and degradation subsamples (D1–D22) and sediment samples (S1–S8) on the left-hand side. Distances upstream from the narrows (N) at the mouth of the estuary are indicated in 5 km intervals and circled. Plymouth is indicated by the hatched area with the dockyards located along the estuarine foreshore demarked by stipple marks. The position of Calstock is indicated by 'C' and an oil jetty terminal by 'J'.

taken using a polypropylene bucket pre-rinsed in the sample water. Possible contamination from the vessel’s stern engines was avoided by sampling at the extreme bow of the Seatruck. Aliquots (2 l) for PAH analysis were transferred to Winchester, containing HPLC-grade (Fisons) cyclohexane (30 ml), which had been cleaned by soaking in Decon 90 for 24 h and rinsed sequentially with distilled water, chromic acid, distilled water and finally HPLC-grade cyclohexane. The sample bottles were then teflon-sealed and thoroughly shaken. During this transect of the estuary, ammonia, salinity, pH, turbidity, dissolved oxygen and temperature were recorded continuously. Subsamples were also obtained for suspended solids analyses and degradation studies.

Sediment core samples (2.5 cm diameter, 10–15 cm long) were obtained on 10 September 1980 using the gravity Butler Corer (Butler & Tibbits, 1972). The top 5-cm sections of
the cores were sampled and stored in labelled acid-cleaned jars sealed with solvent-rinsed aluminum foil liners. These vessels were immediately stored over solid carbon dioxide, then maintained frozen in the laboratory until extraction.

**Methods**

**Major environmental variables**

The methodology for the continuous measurement of environmental variables, salinity, temperature, turbidity, pH and dissolved oxygen, taken on board the research vessel has been described (Morris et al., 1978, 1982). Ammonia was measured by an automated version of the indophenol blue method (Solorzano, 1969) employing a modified buffered chlorine-donor system (Mantoura & Woodward, in preparation). Total suspended solids were determined by filtering known volumes of estuarine water through ashed (450 °C, 6 h), preweighed Whatman GF/C filters (47 mm), dried (110 °C, 12 h) and reweighed. Mineral suspended solids were approximated by heating the filters (450 °C, 12 h). Organic suspended solids were then calculated by difference. However, subsamples from the thawed sediments were freeze-dried, ground in an agate ball mill and then analysed for carbon content (Carlo Erba Elemental Analyser model 1106).

**PAH analysis**

**Extraction**

All solvents were of Fisons HPLC grade. Extractions were performed under subdued light to minimize possible photo-oxidation. All glassware was acid-cleaned and prerinsed with solvent prior to use.

(1) **Estuarine water.** Water samples (2 l) were extracted three times with cyclohexane (30, 15, 15 ml) shaking vigorously for 5 min to facilitate each extraction. Propan-2-ol (2 ml) was added to reduce emulsification at the interface. The resulting combined extract was dried by passage through a column of ashed (450 °C, 6 h) AnalaR anhydrous sodium sulphate (10 g). This was rinsed with additional cyclohexane (10 ml). The extract was then reduced in volume by rotary evaporation (ambient temperature) to approximately 1 ml and quantitatively transferred with hexane washings to a vial (3 ml). The volume was further reduced to 0.5 ml by blowing a stream of nitrogen over the surface. To assess efficiencies, selected water samples were re-extracted for analysis and then known quantities of PAH standards added, and the samples re-extracted once again to estimate recoveries of extractable compounds. Overall reproducibility of the entire procedure was assessed from the triplicate samples 7, 8 and 9 (Figure 3).

(2) **Estuarine sediment** Sediment samples (of previously determined water content) were thawed, thoroughly homogenized and extracted by a method similar to that of Giger & Schaffner (1978). Accurately weighed portions (ca. 7 g weight) were ground with ashed AnalaR anhydrous sodium sulphate (10 g) to form a dry powder. The resultant mixtures were loaded into pre-extracted (8 h, dichloromethane) cellulose soxhlet thimbles (Whatman 28 mm i.d./80 mm). Soxhlet extraction was then performed into dichloromethane (100 ml) for 8 h in darkness. A weighed portion of the resulting extract was removed (approximately 3 ml) and evaporated, using a gentle stream of nitrogen, to approximately 0.5 ml. The dichloromethane was then displaced with hexane and the volume reduced to 0.5 ml. Recoveries of spikes and re-extracts were also performed.
Clean up

Alumina (BDH, neutral, Brockman grade 1) columns were prepared using Pasteur pipettes plugged with glass wool. The pipettes were loaded with a column (5x50 mm) of 7% deactivated ashed (450 °C, 6 h) alumina. Prior to sample treatment, columns were washed with hexane (2 ml). Samples (both water and sediment extracts) were loaded onto the

![HPLC chromatograms showing PAH separations](image)

Figure 2. Typical HPLC chromatograms showing PAH separations of (a) standards, (b) an estuarine water extract and (c) a sediment extract. Peak numbers correspond to (1) naphthalene, (2) phenanthrene, (3) anthracene, (4) fluoranthene, (5) pyrene, (6) chrysene, (7) benzo(a)anthracene, (8) benzo(b)fluoranthene, (9) benzo(k)fluoranthene, (10) benzo(a)pyrene. x is an unresolved mixture (see text). Unidentified peaks in the standard separation correspond to co-eluting compounds. Additional peaks in the extract separations are suspected to include alkylated homologues. Elution and detection conditions are indicated at the bottom of the figure.
columns and eluted into vials with hexane (3.5 ml). Volumes were reduced to 0.5 ml using a stream of nitrogen, the hexane displaced with acetonitrile (0.2 ml) and the volume further reduced to 0.1 ml. Prior to analysis the samples were stored in darkness at 0 °C.

**Chromatographic analysis**

A Perkin Elmer Series 2 high performance liquid chromatograph (HPLC) with modified check valves, fitted with a Rheodyne 7125 injector, a Perkin Elmer LC 75 variable wavelength detector with auto control and a Perkin Elmer 023 recorder was used. Sample extracts (20–30 μl) were injected onto a Hypersil 5.7 μm octadecylsilane (ODS) column (250 × 5 mm i.d.). An acetonitrile–distilled water gradient elution was chosen for the separations at a flow rate of 3 ml min⁻¹. The solvent programme consisted of an isocratic stage (50% acetonitrile–50% distilled water) for 15 min prior to injection and 9 min after injection, followed by a gradient of 1.5% min⁻¹ increase in acetonitrile up to 99% The variable wavelength u.v. programme used for detection of the compounds at pre-selected wavelengths was chosen to maximize sensitivity and selectivity. Initial identification of peaks was performed by conjection of standards Additional information on peak purity/identity was obtained by comparison of stop-flow u.v. scans of environmental samples with those of authentic standard separations (Readman et al., 1981). Calibration of the system was performed using high purity standards prepared at known concentrations in acetonitrile. Examples of separations are shown in Figure 2. The large peak with a retention time of 57 min in the environmental samples exhibited a u.v. spectrum atypical of PAH. Preliminary analysis by gas chromatography/mass spectrometry (GC/MS) identified the peak as a mixture of phthalates and phenyl esters, which were apparently co-extracted with the PAH.

**Work-up efficiencies**

(1) **Water** Recoveries of triplicate spikes of the individual compounds (excluding naphthalene) from pre-extracted estuarine waters varied between 78 to 110% with average standard deviations of ±8%. Comparable extraction efficiencies were obtained by Sorrell & Redding (1979) using a similar technique. Re-extracts amounted to less than 4% of the original extract Detection limits for the technique were typically 0.1–0.6 ng l⁻¹ for individual compounds. Owing to the high volatility of naphthalene, extraction efficiencies (40±20%) and reproducibilities for this compound were low, but were considered adequate to obtain an indication of the behaviour of the compound in the estuary. Reproducibilities for the PAH are shown in Figure 3, as 95% confidence interval bars.

(2) **Sediment.** Work-up efficiencies for sediment analyses were 72–103%. Re-extracts amounted to less than 1% of the original extract.

**Degradation experiments**

Heterotrophic degradation of ¹⁴C-labelled naphthalene and benzo(a)pyrene was determined on selected sub-samples of water (Figure 1) using a technique similar to that of Lee (1977). Compounds used were 1(4,5,8)⁻¹⁴C-naphthalene (5 mCi mmol⁻¹, Amersham) and 3,4-benz-7,10⁻¹⁴C-pyrene (18.6 mCi mmol⁻¹, Amersham). Naphthalene and benzo(a)pyrene were spiked at 3 μg l⁻¹ and 20 μg l⁻¹ respectively into triplicate water samples (100 ml) in sealed flasks (100 ml) and incubated at 10 °C in darkness for 20 h (naphthalene) and 165 h (benzo(a)pyrene). After incubation the respired ¹⁴CO₂ was liberated by acidification (1 ml M HCl) and collected using a spiral ³⁴CO₂ trap (Barford et al., 1977) containing CO₂ absorber cocktail P Fisons (15 ml) (400 ml min⁻¹ flow of nitrogen for 7 min).
Polycyclic aromatic hydrocarbons in the Tamar Estuary

β activity was measured using a liquid scintillation counter (Packard Model 3255) and corrected for quenching by method of external standards. Duplicate controls, consisting of samples poisoned with mercuric chloride (1 mg l⁻¹) were treated in the same way as experimental samples. Other degradation parameters monitored included the determination

![Diagram](image)

Figure 3. Axial profiles of individual PAH concentrations and a selection of environmental variables in waters of the Tamar Estuary. A source index of Plymouth specific PAH is indicated at the base of the diagram. Distances from the mouth of the estuary are shown at the bottom of the diagram and the position of Plymouth is indicated. A floating scale is used for the PAH concentrations with origins for individual compounds indicated on both sides of the diagram. Deviations from the mean of triplicate samples 7, 8 and 9 are shown as 95% confidence interval bars on the concentration plots of the individual PAH.
of residual hydrocarbon and of polar metabolites. The most reproducible parameter in assessing degradation rates proved to be the production of $^{14}$C-$\text{CO}_2$. Reproducibilities of the triplicate $^{14}$C-naphthalene and benzo(a)pyrene mineralization experiments are shown in Figure 7 as 95% confidence interval bars. $^{14}$C-$\text{CO}_2$ activity of the mercuric chloride poisoned controls averaged 8% (for both naphthalene and benzo(a)pyrene) of the $^{14}$C-$\text{CO}_2$ activity in the environment samples.

**Statistical**

Pearson product–moment correlation coefficients ($r$) for all pairs of variables were calculated and used to construct a correlation matrix. Statistical analysis of these data was then performed using both cluster analysis (Everitt, 1974) and non-metric multidimensional scaling (MDS) (Kruskal & Wish, 1978). MDS is used here to obtain a two-dimensional representation of the covariability of $n$-variables (PAHs and environmental data) in terms of similarity clusters Mantoura et al. (1981) have evaluated critically the use and merits of MDS analysis in environmental organic chemistry.

**Results and discussion**

**Environmental variables**

The axial distribution patterns of pH, dissolved oxygen, ammonia and the location of the turbidity maximum and the freshwater/seawater interface were typical of previous surveys of the Tamar Estuary reported by Morris et al. (1981b). The suspended particulate load at the turbidity maximum, however, was relatively low (70 mg l$^{-1}$) because sampling in this region was performed just after high-water slack, when suspended particulates are normally at a minimum. Causes of small local perturbations in the general trends of the environmental variables, for example, secondary maxima in suspended particulates at sample sites 11 and 15 (Figures 1, 3), are possibly a consequence of hydrodynamic influences and have been discussed by Morris et al. (1982). Ammonia concentrations displayed a typical mid-estuarine maximum (Figure 3) originating mainly from the microbial activity in the large areas of intertidal mud flats rather than sewage effluents off Plymouth (Mantoura & Woodward, in preparation).

**PAH concentrations in the water column**

Concentrations of individual PAH generally ranged from less than 1 to greater than 50 ng l$^{-1}$ (Figure 3). Although PAH concentrations in other estuarine or coastal waters are unknown, levels reported here for the River Tamar are comparable to those for the Ohio River (Basu & Saxena, 1978), the River Severn (Lewis, 1975) and selected German rivers (Borneff & Kunte, 1964, 1965). Higher concentrations have, however, been recorded in the River Thames at London (Acheson et al., 1976) and in other rivers associated with large urban or industrialized areas. The PAH monitored (Figure 3) may be considered as two distinct groups: Group 1 consists of the low molecular weight homologues (naphthalene, phenanthrene and anthracene) whose concentrations appear to be independent of suspended particulates (correlation coefficient, $r<0.30$, Table 1). Group 2 (fluoranthene, pyrene, chrysene, benzo(a)anthracene, benzo(b)fluoranthene, benzo(k)fluoranthene, benzo(a)pyrene) have concentrations which exhibit significant correlations with suspended particulates ($r>0.60$, Table 1). The apparent lack of correlation between benzo(a)anthracene and suspended particulates ($r=-0.13$) is caused by the biased weighting of three benzo(a)anthracene rich samples (16, 17, 18) near the dockyard area. When these contributions are excluded
<table>
<thead>
<tr>
<th>Compounds</th>
<th>Molecular weight</th>
<th>Correlation with total suspended solids</th>
<th>Correlation with total suspended solids (excluding Plymouth samples)</th>
<th>Solubility (μg L⁻¹)</th>
<th>Log $K_{ow}$ octanol/water partition coefficient</th>
<th>Log $K_p$ total suspended solids/water partition coefficient</th>
<th>Vapour pressure (mmHg)</th>
</tr>
</thead>
<tbody>
<tr>
<td>Naphthalene</td>
<td>128</td>
<td>0.07</td>
<td>0.09</td>
<td>31.30</td>
<td>2.36</td>
<td>i.51</td>
<td>0.23</td>
</tr>
<tr>
<td>Phenanthrene</td>
<td>178</td>
<td>0.23</td>
<td>0.73</td>
<td>10.70</td>
<td>4.55</td>
<td>2.37</td>
<td>6.8 × 10⁻¹⁴</td>
</tr>
<tr>
<td>Anthracene</td>
<td>178</td>
<td>-0.15</td>
<td>0.27</td>
<td>4.06</td>
<td>4.54</td>
<td>2.36</td>
<td>1.95 × 10⁻¹⁴</td>
</tr>
<tr>
<td>Fluoranthene</td>
<td>202</td>
<td>0.90</td>
<td>0.89</td>
<td>2.06</td>
<td>4.90</td>
<td>2.62</td>
<td>na</td>
</tr>
<tr>
<td>Pyrene</td>
<td>202</td>
<td>0.64</td>
<td>0.87</td>
<td>1.32</td>
<td>5.19</td>
<td>2.81</td>
<td>6.85 × 10⁻¹⁴</td>
</tr>
<tr>
<td>Chrysene</td>
<td>228</td>
<td>0.85</td>
<td>0.98</td>
<td>1.84</td>
<td>5.61</td>
<td>3.13</td>
<td>na</td>
</tr>
<tr>
<td>Benz(a)anthracene</td>
<td>228</td>
<td>-0.13</td>
<td>0.97</td>
<td>9.4</td>
<td>5.61</td>
<td>3.13</td>
<td>1.10 × 10⁻¹⁴</td>
</tr>
<tr>
<td>Benzo(b)fluoranthene</td>
<td>252</td>
<td>0.64</td>
<td>0.96</td>
<td>na</td>
<td>6.04</td>
<td>3.44</td>
<td>na</td>
</tr>
<tr>
<td>Benzo(k)fluoranthene</td>
<td>252</td>
<td>0.71</td>
<td>0.97</td>
<td>na</td>
<td>6.04</td>
<td>3.44</td>
<td>9.59 × 10⁻¹⁴</td>
</tr>
<tr>
<td>Benzo(a)pyrene</td>
<td>252</td>
<td>0.78</td>
<td>0.92</td>
<td>1.72</td>
<td>6.04</td>
<td>3.44</td>
<td>5.49 × 10⁻¹⁴</td>
</tr>
</tbody>
</table>

References: ¹Schwarzenbach & Westall (1981); ²Eganhouse & Calder (1976); ³May et al. (1978a); ⁴May et al. (1978b); ⁵Lu et al. (1977); ⁶Karickhoff et al. (1979); ⁷Environmental Protection Agency (1979); ⁸Radding et al. (1976); ⁹Mackay & Wolkoff (1973); ¹⁰Jordan (1954); ¹¹Pupp et al. (1974).

na—data not available.
from the remaining estuarine samples the anticipated high correlation between benz(a)-anthracene and suspended solids \( r=0.97 \), is restored. When similar treatments are applied to the other Group 2 PAH correlation coefficients increase from the range 0.64-0.85 to 0.87-0.97 (Table 1). Corresponding correlations with phenanthrene appear to classify the compound as intermediate between Groups 1 and 2.

\[ \text{Figure 4: Plots of selected PAH concentrations (ng l}^{-1}\text{) against total suspended particulate load. } \times \text{ indicates sample sites 16-20 in the Plymouth region of the estuary.} \]

**Group 1 PAH (naphthalene, anthracene, phenanthrene)**

These compounds appear to exhibit a complex distribution throughout the estuary possibly reflecting a multiplicity of inputs. This is endorsed by the absence of any correlation with salinity \(-0.02<r<-0.27\) which also tends to suggest that salting out processes (Eganhouse & Calder, 1976; Gorden & Thorne, 1967) of these PAH at low salinities are of secondary importance. In addition, the naphthalene and anthracene regressions against suspended particulates, shown in Figure 4, indicate that there are no significant correlations between Group 1 PAH and suspended solids, thus precluding adsorption of these compounds as a primary fate. These PAH possess physical and chemical properties different from those of the larger molecular weight homologues of Group 2 (Table 1), for example, their solubilities and vapour pressures are higher, whereas their hydrophobicities, as reflected by their octanol-water partition coefficients, are lower (Table 1). These differences could have profound influences on their environmental behaviour.

Karickhoff et al. (1979) and Schwarzenbach & Westall (1981) have shown that it is possible to predict quantitatively the partitioning of many non-polar compounds between particulate and aqueous phases by the use of linear free energy equations, e.g. (Schwarzenbach & Westall, 1981).
where $K_p$ is the non-dimensional linear partition coefficient between particles and water, $K_{ow}$ is the n-octanol–water partition coefficient and $f_{oc}$ is the organic carbon fraction (weight/weight) of the particulates. Using an average of 4.0% C for the Tamar particulates and the $K_{ow}$ data reported in the literature (Table 1), we have calculated the corresponding $K_p$ values for each PAH studied. These clearly show the trend of increasing particulate affinity (higher log $K_p$) with molecular weight. For example, benzo(a)pyrene shows approximately one-hundredfold greater affinity for the Tamar particulates than naphthalene. This agrees qualitatively with Lee's (1977) results which show adsorption of only 0.7% of naphthalene and 53 and 71% for benz(a)anthracene and benzo(a)pyrene respectively. However, there is no distinct break in $K_p$ values, indicating that adsorption alone is unlikely to account for the sharp differences observed in the environmental behaviour of Groups 1 and 2 PAH. If, however, the competitive partitioning of PAH between air and water as controlled by their vapour pressures (Table 1) is also taken into account, then it is possible to explain these differences. The vapour pressures of Group 1 PAH are at least three orders of magnitude greater than those of Group 2 (Table 1). The segregation of PAH into Groups 1 and 2 based entirely on our environmental data is in agreement with laboratory-based volatilization experiments reported by Southworth (1979b). We therefore conclude that air–water exchange is an important pathway for Group 1 PAH in the Tamar Estuary.

The role of microheterotrophic degradation of Group 1 PAH will be discussed in 'Microbial Degradation'. Other processes such as photo-chemical oxidation (Southworth, 1979a; Lee et al., 1978) were not investigated. Correlations between individual Group 1 PAH were not significant ($-0.14 < r < -0.43$), and only phenanthrene exhibited a cross correlation with Group 2 compounds as illustrated in the benz(a)anthracene/phenanthrene plot ($r = 0.84$) shown in Figure 5.

Group 2 PAH (fluoranthene, pyrene, chrysene, benz(a)anthracene, benzo(b)fluoranthene, benzo(k)fluoranthene, benzo(a)pyrene)

The axial distribution of Group 2 PAH generally correlates with suspended particulate distribution throughout the estuary (Figure 3) exhibiting highest concentrations at the turbidity maximum. Superimposed on this trend is a secondary PAH concentration maximum located off Plymouth and is associated with urban discharges including sewage outfalls, an oil jetty terminal and the dockyards. This secondary PAH maximum does not appear to be entirely associated with particulates but may be present as transient soluble or colloidal forms. In these samples additional peaks were evident in the chromatograms suggesting the presence of increased numbers of alkylated derivatives which would be indicative of 'oil' pollution (Hites et al., 1980).

Correlations with respect to salinity are all negative ($-0.68 < r < -0.45$) pointing to a non-causal correlation via suspended particulates (salinity/total suspended particulates, $r = -0.84$). As with Group 1 there is no evidence of salting out of Group 2 compounds. Highly significant correlations exist between the Group 2 PAH and suspended particulates (typically $r = 0.70$) even though the concentrations in the estuary are less than 1% saturated. As previously indicated this relationship is strengthened further by excluding samples associated with the localized Plymouth inputs ($r = 0.87$) (Table 1). Figure 4 shows the almost linear plots of benzo(k)fluoranthene ($r = 0.97$) and benz(a)anthracene ($r = 0.97$) against total suspended particulate load. Plymouth input samples exhibit positive concentration (enrichment) anomalies. Extrapolation of the Group 2 PAH plots against suspended
particulates all have negative ordinate intercepts, inferring the absence of soluble forms of these PAH in the Tamar Estuary. These plots may also indicate the presence of a proportion of particulates that are apparently depleted of PAH. The filtration of water samples into ‘dissolved’ and ‘particulate’ forms of the PAH was not attempted as these operations might lead to errors as a result of contamination, adsorption and photo-degradation (Lee et al., 1978; Means et al., 1979). Although, as will be shown later ('PAH in Tamar Estuary Sediments'), there is significant correlation between carbon content and PAH concentrations in the sediment, this was not observed to the same degree in the organic suspended solids. The overall correlation coefficient of Group 2 PAH with organic suspended particulates was 0.53 which is not significantly different from that obtained for total suspended solids ($r = 0.65$) (Table 1), and may have arisen from the imprecision inherent in the organic suspended solids determination.

It should be noted that although photo-chemical oxidation is also likely to be important in the fate of Group 2 PAH (Lee et al., 1978), it was not investigated in this survey.

Significant correlations existed between some members of the Group 2 PAH (Figure 5) for example benzo(a)pyrene and chrysene ($r = 0.92$). With others e.g. benzo(k)fluoranthene and benzo(a)anthracene, bimodal distributions were apparent (Figure 5), showing that the Plymouth inputs were particularly rich in benzo(a)anthracene (see also Figure 3). These compositional differences can be exploited (e.g. through benzo(a)anthracene : chrysene ratio) to derive source indices (Figure 3) capable of resolving the Plymouth specific inputs of PAH from those PAH associated with the estuarine particulates. Without further studies it is not possible to characterize the nature of the inputs of PAH from Plymouth.

Irrespective of location pyrene correlated best with Total PAH ($r = 0.91$) (Figure 5) indicating that pyrene might be utilized as the most representative constituent of the total PAH in the Tamar Estuary.

Figure 5 Scatter plots between concentrations (ng l$^{-1}$) of selected PAH indicates sample sites 16-20 in the Plymouth region of the estuary.
Polycyclic aromatic hydrocarbons in the Tamar Estuary

Individual PAH concentrations were found to vary typically between 30 to 1500 ng g\(^{-1}\) dry sediment. These values are comparable with published data, e.g. for the Severn Estuary (John et al., 1979; Thompson & Eglinton, 1978).

In general, suspended particulates in the water column contain levels of Group 2 PAH (expressed as ng g\(^{-1}\) dry suspended particulates) similar to those recorded in the surface sediments (Table 2). Furthermore, as shown in Table 2, Group 1 PAH are significantly enriched in the water column relative to the sediments confirming the presence of non-sedimentary sinks. (See ‘Group 1 PAH in the water column’ and ‘Degradation rates’.) Benz(a)anthracene appears enriched in the water owing to the high concentrations encountered at the Plymouth input.

Figure 6 shows the axial distribution of PAH throughout the surface sediments of the Tamar Estuary. High concentrations are apparent at the riverine end and in particular in the region of Calstock (Figure 1). Riverine particulates at the freshwater input contain high levels of PAH that may originate from atmospheric fallout, runoff, moorland fires or smelting processes from the past mining industry and domestic inputs. Microscopic examination of suspended particulates collected during a recent survey of the Tamar has shown increasing numbers of coal-like particles towards the riverine end of the estuary. It is likely that subsequent dilution with less polluted marine sediments together with degradation are the main reasons for the seaward trend of decreasing concentrations of Group 2 PAH. Associated with the urbanized portion of the estuary are again elevated levels originating from chronic anthropogenic sources such as sewage outfalls, dockyards, oil jetty and road runoff. Although highest Plymouth concentrations were found beneath the Tamar Road Bridge, it is not yet possible to completely resolve the contribution of the various sources to the sedimentary PAH. There is a general uniformity in the PAH composition of all the sediments (Figure 6). This suggests that either compositionally similar inputs occur (not supported by water data, Figure 3) or the sedimentary reservoir of PAH is so large as to buffer any compositional changes in recent inputs of PAH.

The seaward decrease in the concentrations of PAH in the sediments is mirrored by decreasing carbon content. The relatively elevated carbon values off Plymouth reflect

### Table 2. Average concentrations of PAH and comparative enrichments in water and surface sediments of the Tamar Estuary

<table>
<thead>
<tr>
<th>Compound</th>
<th>Water concentration (ng l(^{-1}) suspended solids)</th>
<th>Sediments (ng g(^{-1}) dry weight)</th>
<th>PAH enrichment relative to surface sediments</th>
</tr>
</thead>
<tbody>
<tr>
<td>Naphthalene</td>
<td>13.9</td>
<td>1080(^{a})</td>
<td>245</td>
</tr>
<tr>
<td>Phenanthrene</td>
<td>8.8</td>
<td>1042(^{a})</td>
<td>368</td>
</tr>
<tr>
<td>Anthracene</td>
<td>4.9</td>
<td>497(^{a})</td>
<td>120</td>
</tr>
<tr>
<td>Fluoranthene</td>
<td>10.4</td>
<td>760</td>
<td>802</td>
</tr>
<tr>
<td>Pyrene</td>
<td>18.0</td>
<td>1597</td>
<td>878</td>
</tr>
<tr>
<td>Chrysene</td>
<td>3.5</td>
<td>283</td>
<td>345</td>
</tr>
<tr>
<td>Benz(a)anthracene</td>
<td>15.2</td>
<td>2710</td>
<td>601</td>
</tr>
<tr>
<td>Benzo(b)fluoranthene</td>
<td>9.3</td>
<td>871</td>
<td>475</td>
</tr>
<tr>
<td>Benzo(k)fluoranthene</td>
<td>4.2</td>
<td>382</td>
<td>336</td>
</tr>
<tr>
<td>Benzo(a)pyrene</td>
<td>9.1</td>
<td>741</td>
<td>724</td>
</tr>
</tbody>
</table>

\(^{a}\)Calculated to illustrate that even if these PAH were particle associated they are significantly enriched in the water column compared with the sediment.
Figure 6. Axial profile of concentrations of PAH and percentage carbon content in the surface 5 cm of sediment cores in the Tamar Estuary. Distances from the 'narrors' (at the mouth of the estuary) are indicated at the bottom of the diagram. The position of Plymouth is shown. Symbols: O, naphthalene; □, phenanthrene; +, anthracene; ▽, pyrene; ▼, fluoranthene; x, chrysene; △, benz(a)anthracene; ●, benzo(b)fluoranthene; Δ, benzo(k)fluoranthene; ■, benzo(a)pyrene.
mainly the input of sewage whereas the corresponding PAH peak, which is axially more confined, points to the importance of non-sewage inputs of PAH in Plymouth, e.g. road run-off, dockyard and oil jetty. The relatively carbon-poor sample (S5, Figure 6) located below the Tamar Bridge may have arisen as a consequence of particles size selection of carbon-poor coarse particles associated with the high current velocities in this narrow part of the estuary.

**Microbial degradation**

An indication of the relative microbial degradation rates of spiked PAH may be derived from the radiochemical data by calculating the percentage degradation (mineralization) per day or by calculating the 'turnover times' as shown by Lee (1977). The axial distribution

![Diagram](image.png)

**Figure 7.** Axial profiles of naphthalene and benzo(a)pyrene concentrations and microbial heterotrophic degradation rates in the Tamar Estuary water column. 95% confidence interval bars are indicated for triplicate concentration samples (7, 8 and 9) and for each triplicate degradation sub-sample. The profile for total suspended solids is also shown.
of degradation rates of spiked $^{14}$C-naphthalene and $^{14}$C-benzo(a)pyrene in the Tamar Estuary are plotted in Figure 7. In general, degradation rates in the estuary are considerably higher than those in marine waters with rates for naphthalene far exceeding those for benzo(a)pyrene. For example naphthalene degradation rates off the dockyard (D16) and in Plymouth Sound (D22) were 72 and 3% day$^{-1}$ respectively, whereas the corresponding rates for benzo(a)pyrene were only 0.035 and 0.011% day$^{-1}$ respectively.

Naphthalene degradation rates were independent of both naphthalene concentrations in the estuary and the suspended particulates (Figure 8). This suggests that free-living bacteria are responsible. However, it is not certain whether the high mid-estuary rates arise from higher microbial biomass or from a microbial population rich in hydrocarbon degraders. The corresponding naphthalene turnover times of 1–2 days, calculated for the mid-estuarine region (D10–D16), are comparable to the flushing times of the estuary 3–5 days. Clearly microbial degradation of naphthalene, at least at the 1-μg l$^{-1}$ level is efficient and rapid and may be the principal fate of naphthalene in the Tamar Estuary.

In contrast, the degradation rates of benzo(a)pyrene peak at the turbidity maximum correlate with suspended particulates (Figure 8) suggesting that particle-associated bacteria are responsible. Thus the slow degradation rates of benzo(a)pyrene (turnover times 2000–9000 days) together with its high particle affinity point to sediment incorporation as the final fate of benzo(a)pyrene in the Tamar Estuary.

![Figure 8](image)

**Figure 8** Plots of (a) naphthalene and (b) benzo(a)pyrene microbial heterotrophic degradation rates against total suspended particulates. Individual points are labelled with sub-sample numbers to indicate their respective positions in the estuary.

**Statistical analysis**

In addition to the paired correlation coefficients referred to in previous sections of this paper the covariability between PAH and environmental variables was evaluated by cluster analysis and multidimensional scaling (MDS) of all 23 estuarine samples. These showed that the four Plymouth samples (17–20) significantly masked the correlations which would otherwise be present throughout the rest of the estuary. An example of this has already been discussed in a previous section ('Group 2 PAH') (see also Table 1). In order to resolve the cross correlations in the bulk of the estuary, cluster analysis and MDS were repeated on all but the four Plymouth samples and the results are shown in Figures 9 and 10. The selection of statistically meaningful clusters in the similarity dendrogram (Figure 9) was
Figure 9. Cluster analysis dendrogram of PAH and environmental variables in the Tamar Estuary (excluding Plymouth samples, 17-26). The dashed line at 83% similarity (similarity = 100 × absolute value of r) was adopted as the criterion for selecting significant correlations (see text). Based on the conservative criterion that correlation coefficients derived from 20 simulations of randomly generated data of identical matrix size to the data set, never exceeded values of r = 0.83. This acceptance criterion is indicated by the dashed line in Figure 9. This clearly identifies a major cluster consisting of all Group 2 PAH (including total PAH), the phthalates and phenyl esters (see ‘Methods’), turbidity, total and mineral suspended solids.
Naphthalene, phenanthrene and anthracene did not display significant correlations with either one another or with Group 2 PAH, confirming their random nature in the estuary. The MDS plot (Figure 10) confirms the results of the cluster analysis but also shows the extent to which naphthalene, phenanthrene and anthracene are disparate and random with respect to other PAH and environmental variables. Aspects of other, less significant correlations shown in Figures 9 and 10 have been previously discussed. Thus, statistical analyses of the covariability of PAH and associated environmental data provide an objective and independent confirmation of conclusions derived from chemical knowledge.

Conclusions

(1) Concentrations of individual PAH in the Tamar Estuary water column and surface sediments ranged from less than 1 to greater than 50 ng l⁻¹ and between 30 and 1500 ng g⁻¹ dry weight respectively.

(2) Based on observed environmental behaviour, physical and chemical properties, microbial degradation rates and mechanisms and statistical analyses of the environmental data, the PAH appear divisible into two groups:

Group 1. Low molecular weight PAH, incorporating naphthalene, phenanthrene and anthracene, are characterized by high relative solubility and vapour pressure and low particulate affinity, exhibit a complex distribution in the Tamar Estuary.
as an example of Group 1 PAH, is subject to rapid microbial degradation apparently by free-living microbes.

**Group 2.** Higher molecular weight PAH (fluoranthene, pyrene, chrysene, benz(a)-anthracene, benzo(b)fluoranthene, benzo(k)fluoranthene, benzo(a)pyrene) are characterized by low solubility and vapour pressure, high particulate affinity and exhibit estuarine distribution primarily controlled by suspended particulate load and inputs at Plymouth. Microbial degradation of benzo(a)pyrene as an example of Group 2 PAH is relatively slow and occurs in association with estuarine particulates.

(3) It is likely that Group 1 PAH which are volatile and subject to relatively high degradation rates have comparatively short residence times in the estuary. In contrast the high particulate affinity and microbial refractivity of Group 2 PAH point to sediment burial as the principal fate of these more persistent PAH.

(4) In general, suspended particulates in the water column contain similar levels of Group 2 PAH to those recorded in the surface sediments whereas Group 1 PAH are significantly enriched in the water. There is a general trend of decreasing PAH concentrations in the surface sediments from the freshwater to marine environments. Elevated levels associated with the urbanized portion of the estuary are superimposed on this trend.

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Use of Stop-flow Ultraviolet Scanning and Variable-wavelength Detection for Enhanced Peak Identification and Sensitivity in High-performance Liquid Chromatography

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Keywords: Stop-flow UV scanning peak identification; variable-wavelength UV detection; high-performance liquid chromatography; polynuclear aromatic hydrocarbons

Polynuclear aromatic hydrocarbons (PAHs) are discussed as an example of the use of stop-flow ultraviolet (UV) scanning for peak identification and in the choice of detection wavelength changes to maximise sensitivity and selectivity. These compounds are potential carcinogens and their monitoring in environmental samples has been proposed. Modern high-performance liquid chromatography (HPLC) offers many advantages for the separation and detection of PAHs.

Experimental

Reagents

All solvents were of Fisons HPLC grade. Water was distilled in glass prior to use.

Acetonitrile. Far-UV grade.
Cyclohexane.
Hexane.
Methanol.
Potassium hydroxide. BDH Chemicals, AnalaR grade.
Kieselgel G silica gel. 60 mesh.
Picric acid polymer. Prepared as in reference 16.
Helium. Air Products.
Nitrogen. White spot.
PAH standards. Standards of high purity were donated by various institutes and were prepared at known concentrations in acetonitrile.

Apparatus

A Perkin-Elmer Series 2 liquid chromatograph with modified check valves, fitted with a Rheodyne 7125 injector, a Perkin-Elmer LC 75 variable-wavelength detector with autocontrol and a Perkin-Elmer 023 recorder were used. A Scientific Glass Engineering 100-/l.1 syringe facilitated injection of the sample on to a Hypersil 5.7-^m octadecylsilane (ODS) column (250 × 5 mm i.d.).

Procedure

Sample preparation

A wet sediment sample (40 g) was extracted with cyclohexane following the digestion procedure of Dunn. The extract was loaded on to a thin-layer chromatographic plate (200 × 200 mm) coated with a 10% charge-transfer polymer-silica gel layer. The plate was developed in hexane, dried and examined under UV light (254 nm). The layer containing PAHs was removed, extracted with acetonitrile, filtered (Whatman GF/F paper), reduced to a suitable volume (100 μl) using a nitrogen flow and stored in darkness at 4 °C prior to analysis.

Elution of excipients from the HPLC column

An acetonitrile-distilled water gradient elution similar to that recommended by Ogan et al. was chosen in preference to other methanol-water, propan-2-ol-water or aceto-
Fig. 1. Analysis of (i) a standard mixture of PAHs and (ii) an estuarine sediment extract utilising stop-flow UV scanning between 210 and 390 nm. Chromatographic conditions as in text; chart speed, 10 cm h⁻¹; wavelength, 254 nm; absorbance scale, (i) 0.08 and (ii) 0.16 a.u.f.s. (100 scale units); injection volume, 20 &. UV scans were performed by initially calibrating the electronic memory at the base of each peak in turn, resuming flow and stopping at a chosen position on the peak, performing the scan between 210 and 390 nm with a chart speed of 10 cm min⁻¹, choosing absorbance ranges to obtain on-scale deflection. Composition of mixture: 1, naphthalene (20 ng µl⁻¹); 2, phenanthrene (3.1 ng µl⁻¹); 3, anthracene (1.6 ng µl⁻¹); 4, fluoranthene (1.35 ng µl⁻¹); 5, pyrene (1.25 ng µl⁻¹); 6, chrysene (0.6 ng µl⁻¹); 7, benzo[a]anthracene (2.5 ng µl⁻¹); 8, benzo[c]pyrene (3.1 ng µl⁻¹); 9, benzo[g+j]pyrene (2.5 ng µl⁻¹); 10, dibenz[a,j]anthracene (5 ng µl⁻¹); 11, benzo[k]fluoranthene (0.25 ng µl⁻¹); 12, unknown contaminant in PAH standard (aromatic in character).
nitrile-water isocratic, step and linear gradients examined. The solvent programme required elution with acetonitrile-distilled water (1 + 4) for 5 min and acetonitrile-distilled water (1 + 1) for 30 min prior to injection and 12 min after injection. The proportion of acetonitrile present was then increased at the rate of 1.5% min$^{-1}$ to 99% and then maintained at that level for 45 min prior to subsequent determinations. A low mobile phase flow-rate (0.5 ml min$^{-1}$) was selected in order to maintain a low back-pressure, enabling the solvent flow through the micro cell to be halted almost instantaneously to aid the stop-flow scanning.

**Ultraviolet detection**

Base-line corrections were performed by stopping the flow at the base of each peak prior to scanning and calibrating the electronic memory between 210 and 390 nm. Flow was recommenced until the selected portion of the peak had been reached, when a scan was performed. On-scale spectra were obtained by selecting suitable absorbance settings. Stop-flow scans were performed on the major peaks (Fig. 1). From the scans, wavelengths of maximum sensitivity and, if required, selectivity, were chosen. Where coincident peaks occur, the UV spectra can be used to choose mutually exclusive wavelengths to allow the determination of one compound with reduced interference from the other.

Identification of the scans was confirmed by comparison with published UV spectra, subject to availability.

Work is to be conducted into the possible quantification of co-eluting compounds utilising a microprocessor-controlled data station.

**Results and Discussion**

Comparison of UV stop-flow scans of extracted sediment and PAH standards (Fig. 1) showed a close correlation between peaks having similar relative retention times. The stop-flow UV scanning of the coincident chrysene (6)/benz[a]anthracene (7) (Fig. 1) peak on the front and back of the peak was able to show differing UV scans that correlate with previously determined scans of each individual standard. The similarity between stop-flow scans of the sediment extract and known PAH standards for certain peaks enhances the possible identification of naphthalene (A,1), phenanthrene (B,2), anthracene (C,3), fluoranthene (E,4), pyrene (F,5), and benz[a]anthracene (I,7). The similarities for standards and sediment sample for benzo[ghi]perylene (N,11) are less marked. The UV scan of the peak for benzo[g]perylene (N,11) closely resembles that of the standard with the exception of additional absorbance at approximately 260 nm. This suggests coincidence with another compound.

Comparisons of peak sensitivity and selectivity for a fixed wavelength of 254 nm and changing UV detection wavelengths for PAH standards and the sediment extract are shown in Fig. 2. From the chromatograms of standard PAHs it can be seen that the sensitivity can be increased by between 2 and 19 times (Fig. 2) when wavelengths other than 254 nm are chosen. From the standard mixture an increase in sensitivity is predicted for benzo[g]perylene (N,11) when detected at 300 rather than 254 nm. The sediment extract, however, showed a reduced sensitivity (Fig. 2), which probably can be accounted for by the reduced interference of the coincident peak(s) as suggested by the UV scan (Fig. 1). The greater sensitivity of 230 as opposed to 254 nm for peak 12 allowed detection of this compound in the sediment extract only when scanned at the shorter wavelength. The structure of peak 12 was not identified other than being aromatic in character and present in the standards. The procedure as described concentrates on sensitivity; however, from Figs. 1 and 2 it can be seen that considerable selectivity can be obtained by changing the wavelength but sometimes at the expense of sensitivity. The determination of benz[a]anthracene (I,7) at 290 nm would greatly reduce the interference of chrysene (6).

Although PAHs appear more amenable to fluorescence analysis, we have demonstrated that the technique of stop-flow UV scanning and variable-wavelength detection can be applied successfully to the analysis of such compounds in environmental samples. UV detection is more applicable to a considerably larger range of compounds than fluorescence. Wells has demonstrated that for Eulan W.A New/U303, gas chromatography-mass spectrometry may on occasions detect thermal degradation products rather than the injected...
Fig. 2. Comparison between fixed- and variable-wavelength UV detection of standard and estuarine sediment PAH separations. (a) Standard mixture with fixed wavelength (254 nm); (b) standard mixture but with variable wavelength; (c) estuarine sediment extract with fixed wavelength (254 nm); and (d) estuarine sediment extract with variable wavelength. Chromatographic conditions as in text; chartspeed, 10 cm h⁻¹; wavelengths as shown; absorbance scale, 0.16 a.u.f.s. (100 scale units); injection volumes, standard mixture 20 µl and sediment extract 10 µl. Composition of standard mixture: 1, naphthalene (40 ng µl⁻¹); 2, phenanthrene (3.1 ng µl⁻¹); 3, anthracene (1.6 ng µl⁻¹); 4, fluoranthene (1.25 ng µl⁻¹); 5, pyrene (1.25 ng µl⁻¹); 6, chrysene (0.6 ng µl⁻¹); 7, benz[a]anthracene (2.5 ng µl⁻¹); 8, benzo[e]pyrene (2.3 ng µl⁻¹); 9, benzo[a]pyrene (2.3 ng µl⁻¹); 10, dibenz[a,j]anthracene (6 ng µl⁻¹); 11, benzo[g,h,i]perylenes (6.25 ng µl⁻¹); 12, unknown contaminant in PAH standard (aromatic in character).
material. The low working temperature used in HPLC allows the analysis of thermally labile compounds. UV detection with stop-flow scanning allows information on peak purity and possible identification to be performed rapidly and routinely using the above instrumentation.

Research is continuing into the distribution and fate of PAHs in the estuarine environment.

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