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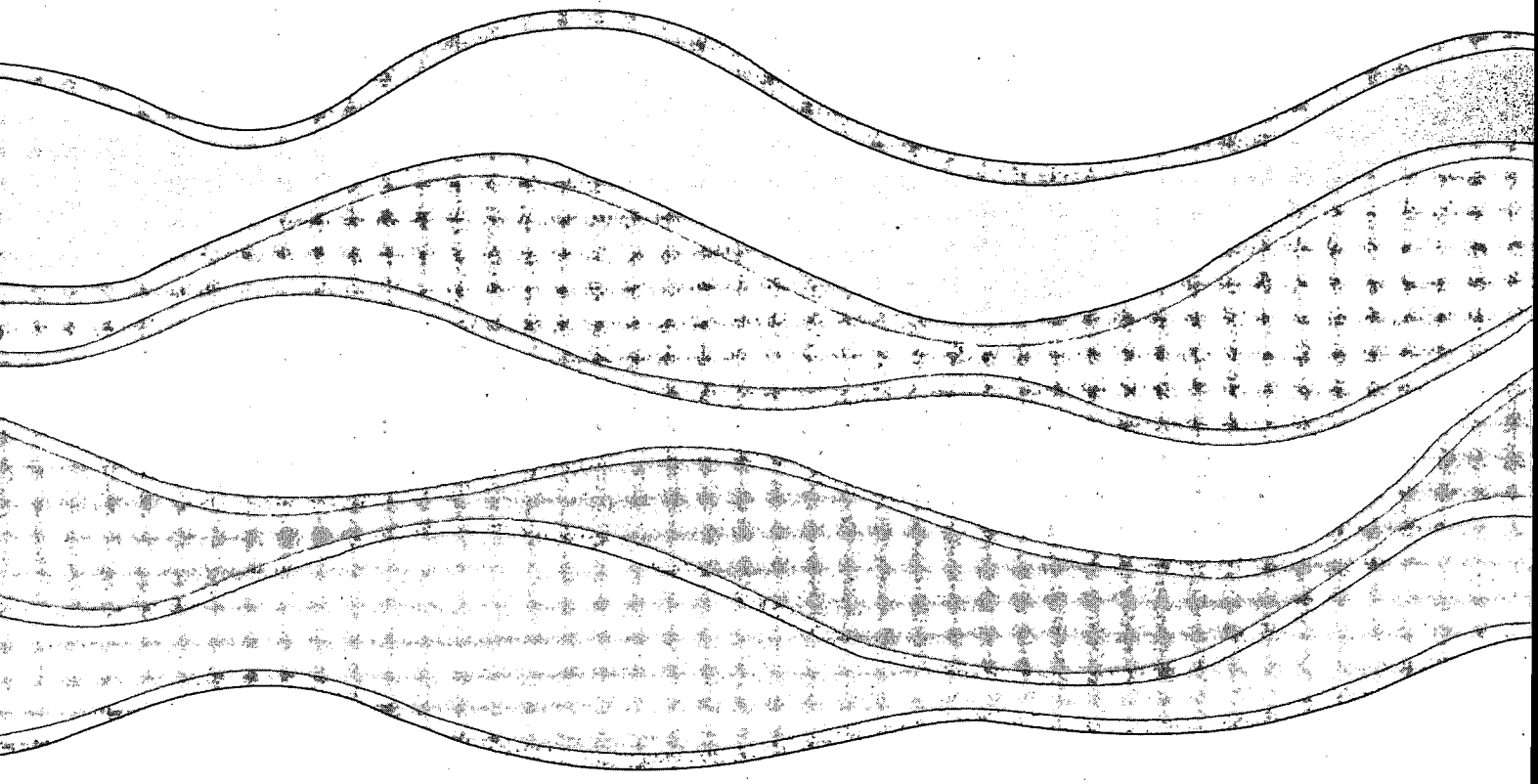

LITERATURE SEARCH ON THE ENVIRONMENTAL IMPACTS OF TAR SANDS OPERATIONS

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by

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MANAGEMENT PERSPECTIVE

This review was commissioned to aid the Rivers Research Branch project on potential impacts of tar sands operations on aquatic environments. It will also be useful in assessments of polynuclear aromatic hydrocarbons under the Canadian Environmental Protection Act.

PERSPECTIVE ADMINISTRATIVE

La présente étude a été commandée pour prêter concours au projet entrepris par la Direction de la recherche sur les cours d'eau portant sur les effets potentiels de l'exploitation des sables bitumineux sur les milieux aquatiques. Elle sera aussi utile pour évaluer les hydrocarbures aromatiques polycycliques en vertu de la Loi canadienne sur la protection de l'environnement.

ABSTRACT

A literature search was performed to assess the chemistry, analytical methods and biological impacts of current and potential effluents and discharges, including effluents from processing plants and tailings ponds leachates, from tar sands plants on the Athabasca River. Some data deficiencies and research needs are noted.

RÉSUMÉ

On a effectué une recherche bibliographique pour évaluer la chimie, les effets biologiques des effluents et rejets courants et potentiels (effluents provenant des usines de traitement, lixiviats de bassins de décantation et de stockage des stériles et des boues et effluents des usines de traitement des sables bitumineux situées sur la rivière Athabasca, entre autres) ainsi que les méthodes d'analyse qui sont appliquées à ces effluents. On note certaines lacunes pour ce qui est des données et certains points qui devront faire l'objet de recherches.

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INTRODUCTION

This literature search was conducted under contract to Environment Canada and arises from federal requirements for regulation of PAHs and other toxic substances associated with heavy oil developments. The work required represents a state-of-the-art assessment of the chemistry, analytical methods and biological impacts of current and potential effluents and discharges including effluents from processing plants and tailings pond leachates.

The terms of reference provided include carrying out a literature search and preparation of a critical analysis of the following groups of compounds to address the following points:

(a) polycyclic aromatic hydrocarbons (PAH), methyl homologues and metabolites including hydroxylated derivatives (HPAH),

(b) polycyclic aromatic sulfur heterocycles (PASH),

(c) polycylic aromatic nitrogen heterocycles (PANH), and

(d) naphthenic acids.

1. What is known of the chemistry of parent compounds and metabolites?

2. What is the state-of-the-art of chemical methods for identifying and quantitating these chemical compounds and their metabolites in concentrations typical of ambient waters, sediments and fish?

3. What is known of the quantities and rates of release of these parent compounds from current tar sands operations including seepage of leachates into the river, and how do these figures compare with natural release rates, if any, into the Athabasca River (e.g. seeps, erosion of geological tar sands materials)?

4. What is known of the toxic nature of these compounds and their metabolites to native fish of the Athabasca system, and to laboratory test organisms commonly used for toxicity assessment at acute and chronic levels?

5. Which of these compounds and their metabolites are known or likely to cause fish tainting in raw or cooked form?

6. What is known of the rates and processes of degradation of parent compounds once they are released into receiving waters?

A list of PAH, PASH, PANH, and HPAH was prepared by (1) searching the literature for compounds associated with the synthetic fuel industry, and (2) reviewing data obtained by

Environment Canada from research conducted on the toxicology and characterization of heavy oil process streams.

Polycyclic aromatic hydrocarbons (PAH) searched along with their Chemical Abstracts Service (CAS) numbers are reported in Table A.

Polycyclic aromatic sulfur heterocycles (PASH) searched along with their CAS numbers are reported in Table B.

Polycyclic aromatic nitrogen heterocycles (PANH) searched along with their CAS numbers are reported in Table C.

Hydroxylated polycyclic aromatic hydrocarbons (HPAH) searched along with their CAS numbers are reported in Table D.

The Chemical Abstracts Database (1967 - September 1989) was searched for the compounds in Tables A - D, along with the keywords: fish, limnology, aquatic organisms, toxicity, tainting, determination and degradation.

The BIOSIS data base was searched from 1969 to September 1989 along with the following keywords: sulfur heterocycles, aromatic amines, polycyclic organic compounds, polycyclic aromatic compounds, organic sulfur compounds, polycyclic aromatic sulfur heterocycles, aliphatic hydrocarbons, neutral polycyclic aromatic

compounds, nitrogen polycyclic aromatic compounds, hydroxyl polycyclic aromatic compounds, polycyclic aromatic oxygen heterocycles, polycyclic aromatic nitrogen heterocycles, and petroleum. These keywords were combined with the following BIOSIS concept codes in order to conduct the search: limnology, oceanography, environmental toxicology, air, water and soil pollution, general toxicology, water research and fish biology, chemical studies and laboratory methods.

The ENVIROLINE data base was searched using the following keywords: sulfur heterocycles, aromatic amines, polycyclic organic compounds, polycyclic aromatic compounds, organic sulfur compounds, polycyclic aromatic sulfur heterocycles, aliphatic hydrocarbons, neutral polycyclic aromatic compounds, nitrogen polycyclic aromatic compounds, hydroxyl polycyclic aromatic compounds, polycyclic aromatic oxygen heterocycles, polycyclic aromatic nitrogen heterocycles, hydrocarbons and petroleum. These keywords were combined with the following concepts to complete the search: fish, limnology, toxicity, tainting, and chemical analysis.

A separate search was conducted using Chemical Abstracts and BIOSIS for naphthenic acids: keywords included toxicity, degradation, determination, fish, aquatic organisms and tainting.

Table A. Polycyclic aromatic hydrocarbons searched

Chemical Name	CAS No.
anthracene	120-12-7
naphthalene	91-20-3
acenaphthene	83-32-9
biphenyl	92-52-4
2-methylbiphenyl	643-58-3
phenanthrene	85-01-8
benzo(a)fluorene	238-84-6
benzo(b)fluorene	243-17-4
benzo(a)anthracene	56-55-3
benzo(k)fluoranthene	207-08-9
benzo(a)pyrene	50-32-8
benzo(e)pyrene	192-97-2
chrysene	218-01-9
pyrene	129-00-0
fluoranthene	206-44-0
9-methyl-9H-fluorene	2523-37-7
1-phenylnaphthalene	605-02-7
2-phenylnaphthalene	612-94-2
1,2,3,4-tetrahydrophenanthrene	1013-08-7
1-methyl-9H-fluorene	1730-37-6
indan	496-11-7
methylindan	29036-25-7
dimethylindan	29348-63-8
methylnaphthalene	90-12-0
dimethylnaphthalene	28804-88-8
3-methyl-9H-fluorene	2523-39-9
4-methyl-9H-fluorene	1556-99-6

Table B. Polycyclic aromatic sulfur heterocycles searched

Chemical Name	CAS No.
dibenzothiophene	132-65-0
naphtho(1,2-b)thiophene	234-41-3
naphtho(1,2-c)thiophene	232-81-5
naphtho(2,1-b)thiophene	233-02-3
naphtho(2,3-b)thiophene	268-77-9
phenanthro(3,2-b)thiophene	224-10-2
phenanthro(4,5-bcd)thiophene	30796-92-0
phenanthro(9,10c)thiophene	235-95-0
dimethyldibenzothiophene	70021-47-5
methyldibenzothiophene	30995-64-3
trimethyldibenzothiophene	70021-48-6
benzothiophene	95-15-8
methylbenzothiophene	31393-23-4
2,7-dimethylbenzothiophene	16587-40-9
benzo(c)thiophene	270-82-6
3,6-dimethylbenzothiophene	16587-50-1

Table C. Polycyclic aromatic nitrogen compounds searched

Chemical Name	CAS No.
1-azafluoranthene	206-56-4
2-azafluoranthene	7148-92-7
3-azafluoranthene	206-55-3
7-azafluoranthene	206-49-5
2-azapyrene	193-98-6
4-azapyrene	194-03-6
2-aminobiphenyl	90-41-5
3-aminobiphenyl	2243-47-2
4-aminobiphenyl	92-67-1
1-aminofluorene	6344-66-7
2-aminofluorene	153-78-6
3-aminofluorene	6344-66-7
4-aminofluorene	7083-63-8
9-aminofluorene	525-03-1
N-methylcarbazole	1484-12-4
N-phenylcarbazole	1150-62-5
1-aminoanthracene	610-49-1
2-aminoanthracene	613-13-8
9-aminoanthracene	779-03-3
acridine	260-94-6
indole	120-72-9
quinoline	91-22-5
naphtho(2,3f)quinoline	224-98-6
naphtho(2,3g)quinoline	257-81-8
naphtho(2,3h)quinoline	84-56-0
1-naphthylamine	134-32-7
2-naphthylamine	91-59-8
phenanthridine	229-87-8
2-methylquinoline	91-63-4
4-methylquinoline	491-35-0
dimethylquinoline	1198-37-4
carbazole	86-74-8
methylcarbazole	27323-29-1
dimethylcarbazole	30642-38-7
1-methylcarbazole	6510-65-2
2-methylcarbazole	3652-91-3
3-methylcarbazole	4630-20-0
4-methylcarbazole	3770-48-7
9-methylcarbazole	1484-12-4

Table D. Hydroxylated polcyclic aromatic hydrocarbons searched

Chemical Name	CAS No.
1-indanol	6351-10-6
2-indanol	4254-29-9
1-naphthalenol	90-15-3
2-naphthalenol	135-19-3
methylnaphthol	28700-83-6
4-indanol	1641-41-4
5-indanol	1470-94-6
hydroxybiphenyl	90-43-7
methylhydroxybiphenyl	38262-85-0

CHAPTER 1 - METABOLISM OF PAH, PASH, PANH AND HPAH BY FISH

Introduction

The notion that fish do not oxidize or conjugate foreign chemicals was popular among students of drug metabolism in the early 1960s. Previous studies had indicated that fish lack the NADPH and oxygen-requiring liver microsomal enzymes for oxidation of drugs (Gaudette et al., 1958; Brodie and Maickel, 1962). It was suggested that fish dispose of foreign lipid-soluble compounds directly by diffusion through gills or skin into the surrounding water without initial metabolism to more polar derivatives (Brodie and Maickel, 1962). Since the early 1960s research into the metabolism of foreign substances (xenobiotics) by fish has accelerated and it is now widely accepted that fish have the capacity to oxidize and conjugate foreign chemicals into more polar derivatives. The two xenobiotic biotransformation systems, namely the cytochrome P_{450}-mediated mixed function oxidase system and the conjugating (mainly glucuronide) system are well established in fish (Chambers and Yarbrough, 1976). A number of reviews on the subject of metabolism of xenobiotics by fish have been published (Tan and Melius, 1986, Bend and James, 1978, Lech and Bend, 1980, and Varanasi and Malins, 1977).

It is a generally accepted view that the metabolism of aromatic hydrocarbons is mediated by the "mixed-function oxidases" present in the endoplasmic reticulum of animal cells. These enzymes, which are NADPH-dependent, catalyze the introduction of oxygen into the aromatic nuclei (Sims and Grover, 1974). The oxygenases participate in electron-transport systems involving the terminal cytochrome P-448 and P-450. The general reaction involved is as follows:

$$RH + NADPH + O_2 + H^+ \longrightarrow NADP^+ + H_2O + ROH$$

The oxygenases, often referred to as aryl hydrocarbon hydroxylases (AHH), are believed to account for the formation of virtually all of the primary metabolic products of aromatic hydrocarbon degradation (Sims and Grover, 1974). It is not certain that the metabolic conversion of aromatic hydrocarbons to oxygen-containing products is a prerequisite for the production of cytotoxicity. Nevertheless, a significant amount of evidence suggests that it is (Magee, 1974, Wood et al., 1976, Yang & Strickhart, 1975, Weinstein et al., 1976, Malaveille et al., 1975, Miyauchi, 1984). Substantial evidence supporting this view has been obtained, and shows that aromatic hydrocarbons become covalently bound to macromolecules (i.e. DNA and RNA) when cofactor requirements for microsomal oxygenases are present (Grover and Sims, 1974, Varanasi et al., 1986, Varanasi and Gmur, 1980, Varanasi et al., 1981, Varanasi et al., 1982, Varanasi et al.,

1980, and Nishimoto and Varanasi, 1985, von Hofe and Puffer, 1986)).

In recent years, electrophilic intermediates (such as the epoxide group) have been viewed with considerable interest. The epoxide group has been implicated in various interactions with genetic materials which result in deleterious alterations in life processes (Varanassi and Malins, 1977, Weinstein et al., 1976, Malaveille et al., 1975).

Figure 1-1 depicts the conversion of benzo(a)anthracene to the epoxide, phenol and glutathione conjugate (Varanassi and Malins, 1977).

Reactions involving the conjugation of epoxides and phenols with water-soluble substances give rise to mercapturic acid derivatives, glucuronides, sulfates, and glycosides in liver and kidney of fish. The glutathione-S-transferases are considered to be important because reactions catalyzed by these enzymes substantially diminish the presumed toxic properties of arene oxides through the conversion of the epoxide group to polar derivatives (Varanasi and Malins, 1977).

Fish possess a microsomal hemoprotein analagous to mammalian cytochrome P_{450}. Thus a similarity between mammalian and fish mixed-function oxidases is indicated (Varanasi and Malins, 1977).

Phytoplankton and certain zooplankton do not possess active AHH enzyme systems (Conover, 1971 and Varanasi and Malins, 1977): however, a number of other marine organisms contain these degradative systems. It has been demonstrated, by isolating metabolic products, that planktonic crustaceans, such as copepods, are able to metabolize both aliphatic and aromatic hydrocarbons (Lee, 1975 and Corner et al., 1976). Other invertebrates such as mussel (M. edulis), scallop (Placopecten sp.), and snail (Littorina littorea) do not possess detectable AHH activity (Varanasi and Malins, 1977). While recent research has shown that mussels (Mytilus edulis) lack P_{450} dependent benzo(a)pyrene monooxygenase, and therefore cannot metabolize polycyclic aromatic hydrocarbons, they do contain FAD-containing monooxygenase and are capable of metabolizing aromatic amines (polycyclic aromatic nitrogen heterocycles, PANH) to genotoxic products or metabolites (Kurelec and Krca, 1987, Kurelec, 1985, Kurelec et al., 1986 and Kurelec et al., 1985). Recent research by other scientists disputes the belief that mussels lack P_{450} dependent benzo(a)pyrene monoxygenase. It has been observed that cytochrome P_{450} mediated MFO is present in the digestive gland of the mussel (Mytilus edulis) (Livingstone and Farrar, 1984, Livingstone, 1985, Livingstone, 1987a, Livingstone, 1987b).

Studies on crab and shrimp larvae showed that marine crustaceans possess enzyme systems for hydroxylation of aromatic hydrocarbons (Lee et al., 1976 and Sanborn and Malins, 1977). It

has also been demonstrated that benzo(a)pyrene, naphthalene and anthracene are actively metabolized by fish (Lee et al., 1972, Goddard et al., 1987, Gmur and Varanasi, 1982, Schoor and Srivastava, 1984, Thornton et al., 1982, Pedersen and Hershberger, 1974, Klotz et al., 1983, Van Veld et al., 1987, Van Veld et al., 1988,Varanasi and Gmur, 1981, Melancon and Lech, 1978, Melancon and Lech, 1979, Malins et al., 1979, Roubal et al., 1977, and Varanasi et al., 1979). Evidence for AHH in aquatic organisms was obtained to a large degree from studies in which metabolic products were isolated from exposed animals. However, an increasing number of reports have been published on the direct measurement of AHH activity (Klotz et al., 1983, Collier et al., 1986, Goddard et al., 1987, Ahokas et al., 1976, Payne, 1976a, Payne, 1976b and Payne and Penrose, 1975). It is evident from the above studies that the livers of fish, possess active microsomal enzyme systems capable of metabolizing aromatic hydrocarbons and a variety of xenobiotics.

PAH Metabolites

Polycyclic aromatic compounds (PAH) are combustion by-products of coal-derived materials and are commonly found in sediment of industrialized areas (Laflamme and Hites, 1978). These compounds reach sediments through atmospheric fallout, erosion, run off, and waste disposal. Since PAH comprise the largest class of known chemical carcinogens their presence in the environment represents

a potential mutagenic threat to aquatic life as well as to man (West et al., 1985).

Recently, research has shown that neoplasms, particularly liver neoplasms, occur in bottom-dwelling fish from areas with highly contaminated sediments. Examples of species and areas in which hepatic neoplasms have been reported include Atlantic tomcod (Microgadus tomcod) from the Hudson River estuary (Smith et al., 1979), and English sole (Parophrys vetulus), staghorn sculpin (Leptocottus armatus), and starry flounder (Platichthys stellatus) from urban areas of Puget Sound, Washington (Malins et al., 1984, McCain et al., 1977 and McCain et al., 1982). Liver neoplasms have also been found in the brown bullhead (Ictalurus nebulosus), a bottom-dwelling species inhabiting rivers associated with the Great Lakes (Black, 1983, Black et al., 1982 and Harshbarger, 1981). In another study, it was reported that 33% of the brown bullhead catfish (3 years and older) from an industrialized region of the Ohio Black River, had liver tumors as well as lip and belly tumors and stubbed barbels (Baumann et al., 1982, West et al., 1985). It has also been shown that epidermal hyperplasia and neoplasia could be induced in brown bullheads by repeated skin painting with a PAH-containing extract of sediment from the Buffalo River in New York (Black, 1983).

Associations between toxic chemicals and fish diseases, such as neoplasia, have been studied to only a limited extent. However,

a multi-year study of relationships between toxic chemicals in sediments and diseases in bottom-dwelling fish from Puget Sound, Washington, indicated that hepatic neoplasms (e.g. hepatocellular and cholangiocellular carcinomas) were mainly confined to fish from urban (highly industrialized and/or highly populated areas). These areas included the Duwamish River estuary in Seattle, WA, the Commencement Bay waterways (e.g., the Hylebos) near Tacoma, WA, and the harbor area of Everett, WA (Malins et al., 1984, Malins et al., 1985). These studies revealed positive correlations between liver neoplasms in English sole and concentrations of sediment-associated PAH and metals (Malins et al., 1984, Krahn et al., 1986, and Malins et al., 1983).

In addition to causing hepatic neoplasia in fish, PAH are also known to be reproductive toxins in mammals, causing ovarian toxicity and oocyte destruction after metabolic activation of the PAH (Mattison et al., 1983). Recent research suggests a potential for reproductive toxicity in benthic fish after exposure to sediment-associated contaminants (Collier et al., 1986).

In fish, as in mammals, PAH are both activated and detoxicated by xenobiotic metabolizing enzymes. The most studied of these enzymes in fish is aryl hydrocarbon hydroxylase (AHH), a mixed-function oxidase (MFO) which plays a primary role in activation of carcinogenic PAH (Bend and James, 1978; Stegeman, 1981). It has been reported that the induction of AHH activity in fish is

indicative of their exposure to chemical contaminants and that this enzyme might be useful as a biological monitor of pollution in the marine environment (Payne, 1984). Recent work suggests that the level of hepatic AHH activity is inversely correlated with reproductive success in female starry flounder *(Platichthys stellatus)*, a benthic flatfish closely related to English sole (Spies et al., 1984 and Sakamoto, 1984).

In mammalian species, the in vivo binding of many carcinogens to hepatic DNA correlates well with the observed carcinogenicity of that compound (Lutz, 1979). The binding of metabolites to proteins and RNA may, however, also have significant toxicological effects (Shum et al., 1979).

The potential for PAH (such as benzo(a)pyrene) to produce toxic effects other than carcinogenesis is provided by in vivo studies demonstrating developmental abnormalities in freshwater as well as in marine fish embryos following BaP treatment (Hose et al., 1982: Hannah et al., 1982). While binding to DNA is frequently implicated in teratogenesis (Harbison, 1978), binding to proteins may also mediate teratogenic effects (Shum et al., 1979). In addition to developmental effects, PAH metabolites (such as BaP metabolites) may play a role in decreasing fecundity as indicated by the finding that PAH metabolites (such as BaP metabolites) bind in vivo to DNA and proteins in the gonads of English sole (Varanasi et al., 1982).

In addition to macromolecular binding, other consequences of PAH metabolism such as quinone and free radical formation should be considered before predicting toxicological implications (Pryor, 1976).

Chemical carcinogenesis as a result of PAH metabolism may not be the most damaging effect of hydrocarbon pollution. Embryototoxicity and/or effects of pollution on fecundity, which may be mediated by binding of proteins could have much more significant consequences in disrupting the ecology of the aquatic environment (Shum et al., 1979).

From the preceding paragraphs it is apparent that PAH in the environment are rapidly metabolized by fish to more polar products and the products of metabolism may be more toxic than PAH. The metabolites themselves are not readily identifiable by routine analytical techniques therefore it is important to know the chemical identity of metabolites associated with PAH. Analyses of fish for PAH does not provide useful information about exposure of fish to PAH, however, through knowledge of the metabolic processes and the end products, methods can be modified to include metabolites and hence data concerning PAH exposure can be obtained.

Table 1-1 summarizes the common metabolites of PAH in fish. Although phenols, alcohols, quinones, aldehydes, and diols have been reported as the major fish metabolites of PAH, it should be

noted that these first pass metabolites can undergo conjugation to form derivatives of mercapturic acid, glucuronide, sulfate and glycoside. Studies carried out on the conjugating enzyme systems suggest that wide variations exist among individual animals as well as among different species. Much more work is needed to obtain a better understanding of the role played by these enzymes in the conversion of first pass metabolites (e.g. epoxides) to other metabolic products (Varanasi and Malins, 1977). Current studies on the metabolism of PAH by fish reveal that xenobiotics which are conjugated with either glucuronic acid, sulphate or glutathione, accumulate to a greater extent in the bile than compounds which are more slowly metabolized (Lech and Bend, 1980). This observation suggests that the bile of fish may be a convenient sample source for the qualitative determination of the biotransformation products which may be formed in fish.

PASH Metabolites

Although several studies have been published which show that shellfish such as oyster, mussel and clams accumulate polycyclic aromatic sulfur heterocycles (PASH) in their tissue after exposure to oil, no information could be found on the metabolism of PASH. Derivatives of benzothiophene and dibenzothiophene (i.e. alkyl derivatives) have been found to accumulate in the tissue of shellfish and invertebrates after exposure to crude oil (Ogata and

Fujisawa, 1985; Ogata and Miuake, 1980; Vassilaros et al., 1982), however, no metabolites have been reported in the literature.

PANH Metabolites

The occurrence of polycyclic aromatic hydrocarbons (PAH) in the environemnt has been widely studied. Much less attention has been focused on the types and amounts of heterocyclic analogues of PAH, particularly nitrogen-containing aromatic compounds (PANH). Since many of the souces of PANH and PAH are the same (e.g., combustion processes and petroleum products), these heterocycles would be expected to be widespread in areas of concentrated human activities. PANH have been found in such varied materials as cigarette smoke, auto and diesel exhaust, urban air particulate matter, petroleum, shale oil, coal tar and synthetic fuels (Krone et al., 1986).

The presence of PANH in the environment is of concern because many of these compounds are known mutagens and/or carcinogens (Dipple, 1976). For example, quinoline and all of its monomethyl isomers were found to be mutagens in the Ames Salmonella assay (Dong, et al., 1978). Quinoline, 4-methylquinoline, and 8-methylquinoline also initiated skin tumors in SENCAR mice (LaVoie et al., 1984). Carbazole has been shown to induce neoplastic lesions in the livers and forestomachs of mice when included in the diet (Tsuda et al., 1982). 7H-Dibenzo[c,g]carbazole exhibits

strong hepatocarcinogenic activity in XVIInc/z mice (Perin et al., 1981). Numerous isomers of benzacridine and their alkylated forms possess carcinogenic properties toward laboratory animals (Dipple, 1976).

Recent studies have revealed positive correlations between liver neoplasms in English sole and concentrations of sediment-associated PAH and metals (Malins et al., 1984). Subsequent investigations have shown that levels of free radical (N-oxyl) derivatives of certain nitrogen heterocycles (carbazoles) in the liver were significantly higher in English sole, that had hepatic neoplasms and a number of other idiopathic liver lesions, than in sole free of these lesions (Malins et al., 1983, Roubal and Malins, 1985, Malins and Roubal, 1985).

Several PANH and their substituted derivatives are converted enzymatically to free radicals which have received attention in studies of mutagenicity and/or carcinogenicity. For example, N-hydroxy-2-(acetylamino)fluorene is transformed enzymatically to a free radical-lipid adduct which is mutagenic (Floyd, 1980). Whether or not nitroxyl free radicals derived from carbazole and other PANH are involved in the genesis of hepatic lesions in English sole from polluted environments (such as Puget Sound, Washington) is not known, however, interactions of these highly reactive chemical species with macromolecules (e.g. DNA) and other

components of biochemical systems may well lead to a variety of deleterious effects in marine organisms (Malins and Roubal, 1985).

In light of the toxicity associated with PANH and the fact that they may be metobolized to more toxic products (e.g. N-oxides) it is important that studies on the uptake and metabolism of these compounds by aquatic organisms be conducted. Unfortunately, very little published information on the uptake and metabolism of PANH by fish and other aquatic organisms exists.

Kurelec and Krca (1987) found that mussel (Mytilus galloprovincialis) and Carp (Cyprinus carpio) metabolized 2-aminofluorene, 2-acetylaminofluorene and N-hydroxy-acetylaminofluorene to chemical mutagens as determined with the Salmonella typhimurium TA 98 assay. Eight percent of 2-acetylamino[9-C^{14}]fluorene was converted to water soluble glucuronides in mussel. The metabolites liberated from these glucuronides by beta-glucuronidase treatment could be converted to TA 98 mutagens by carp liver postmitochondrial fraction, but not by the mussel's digestive gland preparation (Kurelec et al., 1986). No other metabolites were identified by these researchers.

Guobaitis et al. (1986) found hepatic postmitochondrial fractions from the oyster toadfish and the American eel and the renal postmitochondrial fraction from toadfish metabolized 2-

aminoanthracene to products which were mutagenic as determined with the *Salmonella* preincubation assay. No metabolites were identified.

In a study of the uptake and elimination of quinoline by rainbow trout, Bean et al. (1985) found that rainbow trout *(Salmo gairdneri)* readily absorb and metabolize [^{14}C]quinoline when exposed to 1 mg/L concentration in water. Major metabolites identified include hydroxyquinolines and quinolinethiols. There is evidence that the hydroxy form was present in the gall bladders as the glucuronide. The thiols, predominated over the hydroxy derivative in most tissues examined.

In a study of the uptake and elimination of 6,7-dimethylquinoline and 6,8-dimethylquinoline by rainbow trout *(Salmo gairdneri)* Birkholz et al. (1989) found that these compounds were readily bioconcentrated from water by fish and readily metabolized. Metabolites were concentrated in the bile three orders of magnitude above the exposure level (1 mg/L in water) even after depuration for 63-96 h with feeding. The major metabolites identified include glucuronide and/or sulfate conjugates of 7-hydroxymethyl-6-methylquinoline, 6-hydroxymethyl-7-methylquinoline, 6,8-dimethyl-5-hydroxyquinoline, 6,8-dimethyl-7-hydroxyquinoline, 6,8-dimethyl-3-hydroxyquinoline and 6-hydroxymethyl-8-methylquinoline.

In a study of the uptake and elimination of acridine by the fathead minnow (Pimephales promelas), Southworth et al. (1979)

found that this PANH was rapidly accumulated and metabolized by this fish. The degree of bioaccumulation of acridine by fathead minnows was not particularly high in comparison with some chlorinated pesticides and PCBs which exhibit concentration factors of 10^5 or greater. However, high levels of acridine were found to accumulate in the eyes of the exposed fish. No metabolites were identified in this research.

HPAH Metabolites

No literature could be found on the metabolism of hydroxypolycyclic aromatic hydrocarbons (HPAH). However, since many of the primary metabolites (Phase I metabolic reaction) associated with PAH are HPAH it is reasonable to expect that these compounds would be readily metabolized (Phase II metabolic reaction) by conjugation with glucuronide, sulfate, glutathione and mercapturic acid (Varanasi and Malins, 1977; Tan and Melius, 1986). For a general review of metabolism (Phase I metabolic reactions and Phase II metabolic reactions) the reader is referred to Coutts (1986).

This assumption is strengthened by the observation that fish exposed to an accidental spill of pentachlorophenol accumulated high levels of conjugated pentachlorophenol in the bile. The bile to water ratio was as high as 100,000 (Pierce, R.H., Jr., 1978). In a study of the uptake and elimination of xenobiotics by rainbow

trout (Salmo gairdneri) exposed to treated tar-sands tailings pond water, Koning (1987) found that the fish readily concentrated alkylated phenols, in the bile, from the treated tailings pond water. These phenols were conjugated with glucuronide and/or sulfate.

Table 1-1. Phase I (oxygenated) metabolites of PAH in fish

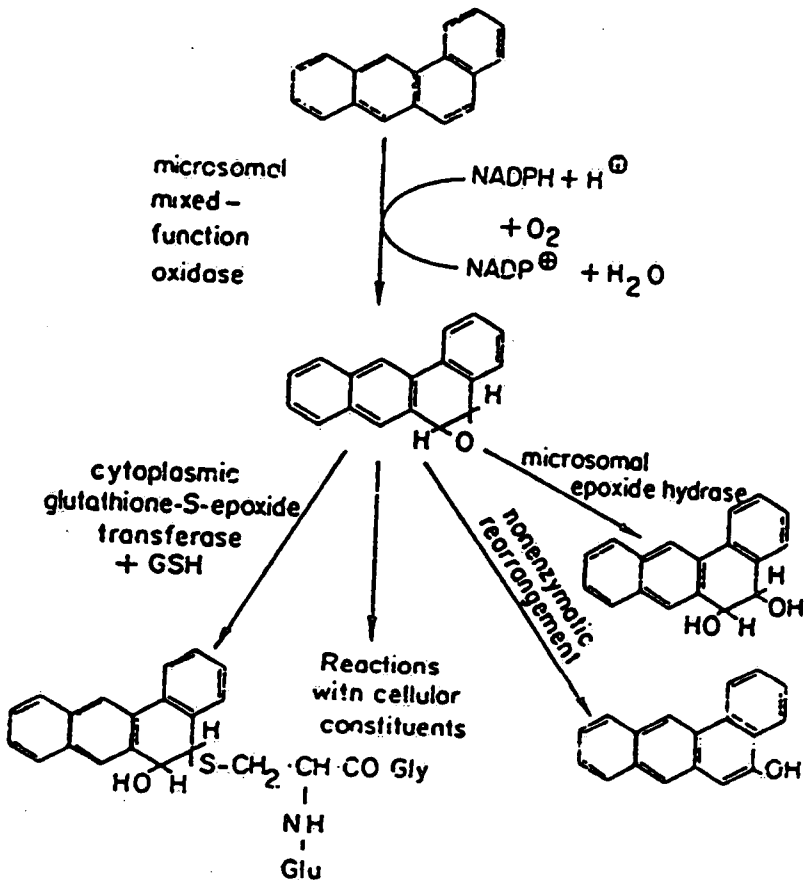
Metabolite	Fish	Reference
Benzo(a)pyrene (BaP)		
BaP 9,10-diol	trout, mullet	Ahokas et al., 1977
BaP 7,8-diol	flounder, sole	Ahokas et al., 1979
BaP 4,5-diol	salmon, scup	Tan et al., 1981
	channel catfish	Varanasi and Gmur, 1981
	sea catfish, fundulus	Stegeman and Woodin, 1980
BaP 1,6-quinone	skate, goldfish	Hinton et al., 1981
BaP 3,6-quinone	bullhead (brown and	Bend et al., 1979
BaP 6,12-quinone	black)	Schoor and Srivastava, 1984
		Melius et al., 1980
BaP 1-phenol		Tan and Melius, 1980
BaP 2-phenol		Varanasi and Gmer, 1980
BaP 3-phenol		Tjessun and Stegaman, 1979
BaP 5-phenol		Gmur and Varanasi, 1982
BaP 7-phenol		
BaP 9-phenol		
Naphthalene		
1-Naphthol	Flounder (liver and	Varanasi et al., 1979
2-Naphthol	muscle), rock sole	Varanasi et al., 1981
1,2-Dihydro-1,2-dihydroxy Naph.	(liver, skin,bile) salmon (brain,liver, gall bladder, heart, flesh), herring (larvae)	Roubal et al., 1977 Malins et al., 1979
2-Methylnaphthalene (MeN)		
3,4-Diol MeN	Trout (bile/liver)	Breger et al., 1981
5,6-Diol MeN		Melancon and Lech, 1984
7,8-Diol MeN		
2-OH MeN		
Dimethylnaphthalene		
C_2-naphthol	English sole	Krahn et al., 1984
Methylnaphthalene-methanol		
Fluorene		
9-Hydroxyfluorene	English sole	Krahn et al., 1984
Hydroxyfluorene		
Anthracene		
9,10-Anthraquinone	English sole	Krahn et al., 1984
Anthracenecarboxaldehyde		

cont'd next page

Table 1-1 cont'd

Metabolite	Fish	Reference
<u>Phenanthrene</u>		
Phenanthrenecarbox-aldehyde Phenanthrol	English sole	Krahn et al., 1984
<u>Biphenyl</u>		
Hydroxybiphenyl	English sole	Krahn et al.,1984

Figure 1-1. Pathways involved in the metabolism of benzo(a)anthracene (Varanassi and Malins, 1977).


microsomal
mixed-
function
oxidase
NADPH + H⊕
+O2
NADP⊕ + H2O
H
H O
cytoplasmic
glutathione-S-epoxide
transferase
+ GSH
microsomal
epoxide hydrase
nonenzymatic
rearrangement
Reactions
with cellular
constituents
H
HO H
S-CH2 CH CO Gly
NH
Glu
H
HO H
OH
OH

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CHAPTER 2 - DETERMINATION OF PAH, PASH, PANH AND HPAH IN FISH, WATER AND SEDIMENT

Determination of PAH in Fish

Grimmer and Bohnke (172) observed that only 30% of the aromatics from a tissue sample was obtained by extraction with boiling methanol, while an additional 60% was recovered upon saponification of the tissue. This observation was emphasized by Howard and Fazio (173) who concluded that a base hydrolysis step was essential for the analysis of hydrocarbons in tissue.

Analysis for PAH after Alkaline Saponification

Methods employed for the saponification of fish tissue include digestion and or reflux with ethanolic KOH (1-11, 23, 24, 26-28, 30, 31), or methanolic KOH (12, 13, 18, 32-39) or sodium hydroxide solution (14, 15, 40).

Extraction of PAH from the caustic digestate was performed using hexane (1-7, 16, 18, 33-35, 38, 41), or isooctane (8, 9, 11, 26, 28, 39), cyclohexane (10, 12, 23, 32), benzene (14), diethyl ether (17, 40), pentane (13, 36), methanol/benzene (36) and dichloromethane (31) as extraction solvents. Following extraction,

with appropriate solvent, the resulting extract is concentrated using a Kuderna-Danish apparatus or rotary evaporator.

Clean-up of the resulting extracts was accomplished using liquid-liquid partition and/or column chromatography. Liquid-liquid partition was accomplished using hexane or cyclohexane (hexane/cyclohexane) and dimethyl sulfoxide (DMSO) (8, 9, 11, 23, 25, 30, 32); or hexane/cyclohexane and dimethyl formamide (DMF): water (9:1) (10, 12, 39). Following removal of the DMSO or DMF solvent by dilution with water, the PAH are back extracted with hexane or cyclohexane.

Column clean-up was performed using the following adsorbents: alumina (11, 15, 25, 31); silica gel (4, 7, 10, 12, 13, 24, 39, 40); silica gel and alumina combinations (1-5, 16, 33, 34, 36, 38); florisil (8, 9, 23, 26-28, 30, 32, 35); and alumina/florisil (18). Solvents similar to those used in the liquid-liquid extraction step are commonly used as column eluants.

Prior to column chromatography it is recommended that biogenic material be separated from PAH. This is accomplished by taking advantage of the molecular size difference between the PAH and the biogenic compounds. Using gels such as Bio Beads (14, 43, 44) or Sephadex LH-20 (10, 12, 26) effective separations can be performed.

Chemical analysis of the cleaned up fish extracts can be accomplished using a variety of analytical techniques including gas chromatography/flame ionization detection (GC/FID), (1-6, 10, 13, 16, 18, 28, 32-34, 36, 38, 40, 41); thin-layer chromatography (TLC) (6, 8, 9, 24, 26, 30); reverse-phase high pressure liquid chormatography with fluorescence detection (HPLC/FLUOR) (12, 23, 32, 39); reverse-phase high pressure liquid chromatography with ultraviolet detection (HPLC/UV) (26, 27); ultraviolet spectrometry (11) and fluorometry (30).

Analyses using GC/FID, HPLC/FLUOR and HPLC/UV rely on retention times for the identification of PAH. This is achieved by comparing observed retention times for samples to retention times obtained for reference chemical standards. Confirmation of identities is achieved by using an alternative chemical method or reanlaysis using gas chromatography/mass spectrometry (GC/CMS). Gas chromatography/mass spectrometry is the method of choice for the analysis of PAH because this method provides both retention time and spectroscopic (i.e. mass spectrum) data which allows for the unambiquous identification of PAH (1-5, 12, 14, 16, 17, 27, 28, 31-34, 40, 45).

Analysis for PAH in Fish without Saponification

The most widely used method of extracting fish without saponification is to homogenize the tissue with excess sodium

sulfate and to soxhlet extract the mixture for 8 to 48 hours. Solvents commonly used include: acetone, pentane, dichloromethane, methanol:benzene (1:1) and hexane:acetone:diethylether: petroleumether(2.5:5.5:1:9). After extraction, the solvent is concentrated and digested with KOH and/or NaOH prior to cleanup or submitted directly to cleanup. (19,34,36,48-50)

Other methods of extraction without prior saponification include reflux of the tissue with methanol:water (1:1)(46) and blending the tissue with excess sodium sulfate using a Virtis homogenizer and pentane (36). The resulting extracts can be saponified prior to cleanup or be submitted directly to cleanup.

Cleanup methods and instrumental methods have been discussed above.

Determination of PAH in Water

The most common method of extraction of PAH from water involves solvent extraction at basic (pH ≥ 12) or neutral pH. Solvents include petroleum ether, dichloromethane, diethylether, cyclohexane and isooctane (24, 65, 69, 74-80, 24). After extraction the solvent is concentrated prior to cleanup and/or analysis.

Reported cleanup of water samples is rare, however; use of adsorbents such as alumina (79) and Sep Paks (81) have been reported.

Analysis of the resultant extracts, before and after cleanup, is conducted using gas chromotography/flame ionization detection (GC/FID) (65, 74, 77); gas chromotography/photoionization detection (GC/PID) (82); high pressure liquid chromatography with fluorescence detection (HPLC/FLUOR) (24, 79, 82) and ultraviolet and spectroluminescence procedures (80).

Confirmatory analysis is best performed using gas chromotography/mass spectrometry (69).

Determination of PAH in sediment

Several methods reviewed employed a saponification step prior to extraction. Typically sediment (1 - 100g) is digested with boiling ethanol/potassium hydroxide; methanol/potassium hydroxide; or benzene/potassium hydroxide. Concentration of the potassium hydroxide is typically 0.5N to 5%. After digestion the PAH is extracted from the digestate using cyclohexane, isooctane, hexane pentane or dichloromethane (23, 30, 34, 66-68).

Another common method employed for the extraction of PAH from sediment involves soxhlet extraction with benzene:methanol (1:1)

for 2 X 24 hours. or with methanol:dichloromethane (2:1) for 2 X 24 hours (67). In a validation of methodology used to isolate PAH from contaminated sediment it was found that dewatering sediment with methanol prior to tumbling (or rolling) with dichloromethane:methanol (2:1) for 16, 6 and 16 hours (change of solvent between extraction) revealed that tumbling was as efficient as soxhlet extraction with benzene:methanol or dichloromethane:methanol and more efficient than direct reflux with alkaline methanol (68). In another interlaboratory study (174) it was found that refluxing sediment with dichloromethane:methanol gave higher recoveries of PAH than soxhlet extraction with benzene:methanol or tumbling with methanol:dichloromenthane. It was concluded that in order to provide consistent data that methods of extraction had to be standardized and that using soxhlet extraction with benzene:methanol or tumbling with methanol:dichloromenthane have results within 50% of each other (174).

Common cleanup techniques employed for sediment analysis include Florisil (23, 30, 66), silica:alumina (2:1) (34); alumina:silica (1:1) (67); silica gel (68-71), alumina (28, 72) and gel permeation chromatography using Sephalex LH-20 (28, 174). Removal of sulfur from the extracts was accomplished using a copper column (28, 67, 70).

Analysis of cleaned up extracts was accomplished using GC/FID (34, 65, 67, 68, 71, 72), HPLC/FLUOR and HPLC/UV (23, 57, 66, 73), and TLC (30). Comfirmatory analysis is best performed using GC/MS (17, 34, 68-70).

Determination of PASH in Water

Of the papers reviewed few methods on PASH extraction in water exist. One method found involved treating the sample with H_2SO_4 and extracting with DCM. The sample was then cleaned up on a silica gel column and injected on a GC/FID/FPD and HPLC (83). Another more complex procedure involved repeated extractions. The sample was acidified and extracted with DCM. The extract was reduced and made up again in hexane which was then neutralized with NaOH. This neutralized extract was then further extracted with hexane. Sodium bicarbonate was added to the extract followed by H_2SO_4, to buffer the extract, and extracted again. The final extract was then hydrolysed by the addition of NaOH and injected on GC/FID and HPLC (84).

Determination of PASH in sediment

One paper was found dealing with extracting PASH from sediment. The procedure involved mixing the sample with H_2SO_4 and tumble extracting with petroleum ether. A 100 mL aliquot was then removed and dried with $MgSO_4$. Silica gel column clean up was

employed prior to analysis of the sample by GC/FID/FPD. Confirmation was done using GC/MS (83).

Determination of PASH in fish

Of the papers reviewed the most often used method of extraction of fish samples for PASH involved saponification with ethanolic KOH, extraction with hexane, a silica gel/alumina column clean up and analysis of the sample by TLC and GC/FID/FPD (6). A few other papers used the same method but employed GC/MS for confirmation of compounds (4, 16, 85). Other methods reviewed involved the same extraction procedure but clean up was performed on a silica gel column and analyzed by GC/FPD (86). As well there were examples of soxhleting with DCM, clean up with GPC followed by a florisil column and analysis by GC/FID (48), and finally a digestion in aqueous NaOH, extracting with ethyl ether replaced by hexane with column clean up done on a silica gel column and analyzing by GC/FID/FPD with confirmation by GC/MS (83).

Determination of PANH in Water

A volume of water was filtered through a C18 Sep Pak extraction cartridge. This is centrifuged to remove the entire sample from the cartridge which is then eluted with acetonitrile. Eluate is membrane filtered through a Swinney appartaus and analyzed by HPLC with fluoroescence (81).

Determination of PANH in Sediment

Sample is tumble extracted with DCM and Na2SO4. Extract is concentrated and exchanged into hexane and passed through a silica/alumina column. Eluate is then analyzed by GC/NPD/FID and confirmed by GC/MS.

Determination of PANH in fish

One method reviewed digested the sample in NaOH and extracted in benzene. The extract was then acid washed with H_2SO_4. GPC column clean up was employed and analysis was done by GC/NPD and confirmed by GC/MS (14).

Another method added NaOH to the sample, and extracted with petroleum ether. The sample was then acidified with H_2SO_4 and extracted with petroleum ether again. The extracts were combined and made basic with NaOH the re-extracted with petroleum ether. Samples were then analyzed by GC/FID and confirmed by GC/MS (74).

A final method involved soxhleting the sample with DCM. GPC clean up was employed and the eluate was extracted with chloroform. An acid-base partition was performed and extracted with HCl. Extracts were combined, made basic with NaOH, and extracted with chloroform. Samples were then put through a 20 g Na_2SO_4 column for

drying and then analyzed by GC/FID. Confirmation was done by GC/MS (48).

Determination of HPAH and Phase I Metabolites in Fish, Sediment and Water

A review of the metabolism of polycyclic aromatic hydrocarbons (PAH) by mammals and fish revealed the major products of Phase I metabolism to be dihydrodiols, phenols and quinones (166, 167).

The general procedure for the isolation and analysis of phenols (i.e. HPAH) and other Phase I metabolites from fish tissue (e.g. bile, liver and muscle) involve extraction with polar solvents such as ethyl acetate, methanol, acetone, ethanol, chloroform-methanol (1:1), and diethyl ether. After concentration of the solvent, analysis for metabolites was obtained using HPLC (fluorescence and UV detection), TLC and GC/MS (33, 55, 118, 141, 151, 164, 168). Extracted metabolites are usually derivatized with BSTFA (to form trimethylsilyl ethers) prior to GC/MS analysis (33).

Analysis of water and sediment samples for phenols (i.e. HPAH) and Phase I metabolites is best accomplished by extraction with a polar solvent such as ethyl acetate. Varanasi and Gmur (168) reported better than 99% recovery of radiolabeled benzo(a)pyrene-associated metabolites from water using ethyl acetate as the extraction solvent.

Analysis of Fish for Conjugated Metabolites

The major Phase II metabolites identified in fish and aquatic organisms include derivatives of glucuronide, sulfate, mercapturic acid and glutathione (169).

The most common method to analyze conjugated metabolites (which are very hydrophilic) is to perform an enzymatic hydrolysis to liberate the Phase I metabolite and then to extract with a polar solvent such as ethyl acetate or chloroform-methanol (1:1). The most common enzymes used include beta-glucuronidase (an enzyme with glucuronidase and sulfatase activity), glucurase (a glucuronidase with very low sulfatase activity), and aryl sulfatase (containing D-saccharic acid-1,4-lactone, 20 mM, to inhibit any glucuronidase activity present in the sulfatase preparation) (33, 109, 168).

Another approach for separating Phase I metabolites from conjugates involves acid hydrolysis. Briefly, tissue is digested in 90% formic acid at room temperature for 24 h. This acid hydrolysis hydrolyzes sulfate, glucuronide, and glucoside. Mercapturic acid conjugates are hydrolyzed by base hydrolysis (170).

Another approach involves a base hydrolysis for 24 h using 4N NaOH (171).

Evaluation of foregoing methods of analysis

(a) Determination of PAH in Fish

Discussions with Mr. William MacLeod, National Marine Fisheries Service, NOAA, Seattle, Washington, revealed that saponification of tissue was not a prerequisite to obtaining good recoveries of PAH from fish tissue. This observation is supported by Farrington and Medeiros (36) in their evaluation of three methods for the analysis of petroleum hydrocarbons in marine organisms. These researchers found that Soxhlet extraction of clam homogenate with benzene:methanol (1:1) gave slightly higher recoveries of hydrocarbons than digestion/extraction with KOH in methanol. In addition to the fact that alkaline digestion is messy (48) the coexistence of alkaline conditions, light and oxygen has been reported to result in poor recoveries for some PAH such as benzo(a)pyrene (7). Furthermore, chemical reactions such as oxidation and chemical rearrangements should be of concern when performing an alkaline saponification. For these reasons it is recommended that fish samples be extracted using Soxhlet extraction and the resulting extracts cleaned up prior to analysis. Soxhlet extraction is currently (1988) used by the National Bureau of Standards (NBS) to analyze fish samples collected and stored under NOAA's (National Oceanic and Atmospheric Administration) National Status and Trends Program (175). Briefly tissue samples are mixed with precleaned sodium

sulfate, surrogates are added (1-butyl pyrene for the determination of PAH, deuterated 4,4'-DDT for the determination of chlorinated pesticides, and PCB #198 for the determination of PCBs) and the mixture Soxhlet extracted with methylene chloride. Another extraction method advocated by NOAA (181) involves mixing 3 g of fish tissue with 25 g of sodium sulfate in a 100 ml centrifuge tube and extracting with 35 mL of dichloromethane using a Tissumizer. After centrifuging the extracted sample for 5 min at 2000 rpm and decanting the solvent into a distillation flask, the extraction is repeated a second time with 35 mL of dichloromethane. Although both extraction methods are satisfactory, the Soxhlet extraction method is preferred because larger sample sizes can be accommodated. Birkholz et al. (48) mixed 20 g of tissue with 80 g of purified sodium sulfate and Soxhlet extracted the mixture with methylene chloride for 6 h. Detection limits in the order of 10-20 ng/g for PAHs were reported using GC/FID and/or GC/MS.

Recommended extract cleanup methods include: alumina chromatography followed by gel permeation chromatography using high pressure liquid chromatography (181), gel permeation chromatography followed by HPLC chromatography using an aminosilane column (175), and gel permeation chromatography followed by Florisil adsorption chromatography (48).

Birkholz et al. (48) reported average recoveries for PAH and PASH of 87% (n=4) and 70% (n=2) from fish fortified at 0.24 - 1.1 and 0.024 - 0.11 ug/g respectively.

(b) Determination of PAH in Sediment

The current method used by NBS involves mixing the wet sediment with precleaned sodium sulfate and Soxhlet extracting with methylene chloride (175). Extraction should be repeated with fresh solvent after 24h, i.e., extract 2 x 24h (67, 68, 174). Another extraction method advocated by NOAA (181) involves mixing 10 g of sediment with 60 g of sodium sulfate and 7.5 g of activated copper prior to extraction with methylene chloride. Extraction is performed using a 250 mL screw cap bottle and rolling the mixture on a soil roller at 100 to 250 rpm for 16h. After decanting the solvent, extraction is repeated twice more with fresh solvent (100 mL) for 6 and 16 h respectively. Recommended cleanup methods include: silica gel followed by HPLC using an aminosilane column (the PAH fraction is collected with a fraction collector, 175), and gel permeation chromatography using two one hundred Angstrom size exclusion columns, an HPLC and associated fraction collector (181). The NBS currently advocates the former cleanup method as part of the National Status and Trends program (175). Recent discussions with W. MacLeod revealed that NOAA was currently recommending the use of liquid chromatography (HPLC) for the cleanup of sediment extracts (181).

The use of HPLC is recommended because precise chromatography can be achieved. Gravity fed systems tend to suffer from varying flow rates and varying deactivation. These problems are overcome using an HPLC system.

(c) Analysis of Conjugated Metabolites in Fish, Sediment and Water

Several methods have been reported for the hydrolysis of conjugated metabolites including: enzymatic hydrolysis, base hydrolysis and acid hydrolysis (33, 109, 168, 170, 171, 182). Since recovery data for the first pass metabolites, formed after hydrolysis, is largely lacking in the literature, it is difficult to compare different hydrolysis methods. The best way to ensure complete hydrolysis is to analyze extracts before and after hydrolysis by reverse phase HPLC/Fluorescence. A large reduction in peaks appearing at the front end of the chromatogram should be observed if hydrolysis is complete. Another method is to fortify samples with naphthyl glucuronide, naphthyl sulfate and naphthyl glucoside prior to hydrolysis and to analyze for naphthol. Recovery of naphthol is directly related to hydrolysis efficiency. These chemicals are readily available from the Sigma Chemical Company.

(d) Instrumental Methods of Analysis

Birkholz et al (48) reported detection limits for PAH in fish of 10-20 ng/g using gas chromatography/flame ionization detection (GC/FID) and gas chromatography/mass spectrometry (GC/MS). These detection limits are based on a 20 g sample size and a final extract volume of 1.0 mL. Lower detection limits by at least one order of magnitude could be achieved using selected ion monitoring (SIM) GC/MS as opposed to scanning GC/MS which was used in the above study.

Lawrence and Das (12) reported detection limits for PAH in fish of 2-90 pg/g using reverse phase high pressure liquid chromatography with fluorescence detection and GC-MS/SIM. These detection limits are based on a 200 g sample size and a final extract volume of 5.0 mL for HPLC/FLUOR and 0.5 mL for GC-MS/SIM.

From these studies is clear that the most sensitive method for analyzing for PAH is HPLC/FLUOR. However, GC-MS/SIM offers the best compromise in terms of excellent sensitivity and specificity.

A recent study by the National Research Council (180) of methods for the detection of PAH in sediment SRM (standard reference material) samples concluded that GC/MS and/or HPLC/MS were the methods of choice. These workers compared data obtained for 14 priority pollutant PAH using the following methods: GC/FID, HPLC/UV, HPLC/FLUOR, GC/MS and HPLC/MS. They concluded

that GC/FID, HPLC/UV and HPLC/FLUOR methods lacked specificity and were prone to interferences which could lead to false positives for specified PAH. For example, alkylated analogs of priority pollutant PAH are usually present in environmental samples at higher levels than the parent PAH and GC/FID and HPLC/UV and HPLC/FLUOR cannot distinguish the two, especially in complex samples. Because both HPLC/MS and GC/MS provide chromatographic data (i.e. retention time) as well as spectroscopic data (i.e. mass spectrum), these methods are recommended for the generation of unambiguous data. The authors suggest the use of GC/MS for the analysis of complex samples because of the higher column efficiencies associated with GC analysis.

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CHAPTER 3 - RELEASE RATES OF PAH, PASH, PANH AND HPAH FROM CURRENT TAR SANDS OPERATIONS

In a study of the Athabasca River, Strosher and Peake (1979) observed that water samples, collected from three locations in the oil sands deposit area upstream of Fort McMurray, consistently contained 9 mg/L total organic matter, the majority of which was in the form of dissolved, unextractable organic carbon. Measurement of the total organic carbon content of extracted waters showed that 1 mg/L of the organic carbon was extracted leaving 8 mg/L in the form of water soluble non-extractable carbon.

Non-extractable carbon compounds were separated into two classes on the basis of retention by ion exchange resins. An average 1.9 mg/L as organic carbon was eluted with water from the resin column while 5 mg/L was characterized as humic-like materials which were retained by the acidic resin and eluted with sodium hydroxide solution. This combined water soluble material which averaged 6.9 mg/L was the largest single component representing 77% of the organic carbon content of the waters.

Contained in the water soluble fraction (8 mg/L) were the naturally occurring tannin and lignin compounds. They accounted for a total of 0.24 mg/L as measured by organic carbon content.

Nitrogen-containing compounds were the next largest group of organic compounds found in waters. The majority of this fraction was determined as extractable amides which occurred at levels ranging from 0.27 to 0.39 mg/L. These nitrogen compounds along with the sulphur- and oxygen-containing compounds which comprise the polar compounds account for an average 3.5% of the total organic carbon or 33% of the extractable organic carbon in water samples.

Asphaltenes, probably contributed by the oil sands deposits, accounted for 0.20 mg/L of the organic carbon in waters. This amount of asphaltenes, although low in terms of total organic carbon content of waters, accounts for 20% of the extractable organic carbon.

Hydrocarbon contribution to the river waters as measured by GC occurs at considerably lower values in terms of organic carbon with 0.005 mg/L found in upstream waters. A 3-fold increase in hydrocarbon content does occur however, between the upstream-100 km and upstream-35 km locations (0.016 mg/L), the majority of which is due to an increase in aliphatic hydrocarbon content. The total hydrocarbon content in upstream-2 km waters was found to be 0.012 mg/L as organic carbon.

Upstream-100 km is approximately 3.5 km downstream from the Livock River confluence and 30 m from S.E. bank. Upstream-55 km

is approximately 1 km downstream of the Algar River confluence and 10 m from the north bank. Upstream-35 km is approximately 4 km upstream of Crooked Rapids point and 20 m from the south bank. Upstream-2 km is approximately 2 km upstream of Fort McMurray bridge on Highway 63 and 50 m from the north bank. Upstream-1 km is approximately 1.3 km upstream of Fort McMurray bridge on Highway 63 and 10 m from the north bank. General sampling locations are depicted in Figure 3-1 (taken from Strosher and Peake, 1979).

Strosher and Peake (1979) found the total organic carbon content of sediments ranged from 20,000 mg/kg in upstream-100 km sediment to 11,000 and 14,000 mg/kg in upstream-55 km sediments, and 16,000 mg/kg in upstream-1 km sediment. Extractable organic carbon content was also determined and the sediment extracts revealed that only 6% of the total organic carbon was in an extractable form. Amounts of this extractable carbon generally increased from the furthest upstream samples with 740 mg/kg in U-100 sediment, 880 to 910 mg/kg in U-55 samples and 1180 mg/kg in U-1. The U-100 sediment, located on the outer edge of the oil sands deposit, contained the highest total organic carbon content, indicating a higher percentage of the more naturally occurring organics such as humic acids and kerogen or possibly particulate carbon.

Tannins and lignins were one of the largest groups of compounds detected in the sediments. These compounds were part of the non-extractable organic carbon containing fraction and were found at levels averaging 380 mg/kg or 3% of the non-extractable carbon fractions.

The majority of the investigations conducted by Strosher and Peake (1979) were focused on the extractable organic carbon fractions of sediments in order to measure baseline states of bitumen constituents and their contribution to this segment of the river system. Asphaltenes were the largest single contribution to the extractable fraction, averaging 39% or double the asphaltic content of oil sands bitumen (17-19%). It is apparent that considerable weathering of the bitumen takes place either before or after entering the bottom sediments of the river.

Oily constituents, as measured by weight, comprised an average 33% of the extractable organic carbon in sediments. The components of this oily fraction consist of aliphatic and aromatic hydrocarbons and the O, N, and S containing polar compounds. Because of the nature of these oily compounds that exist in the sediments, gas chromatographic analysis could not detect the majority of these high molecular weight constituents.

Upstream-100 sediment collected at the outer edge of the oil sands deposit area contained 11% aliphatic hydrocarbons, 10% aromatic hydrocarbons, and 79% polar constituents. The remaining sediment deposits which occurred downstream from this sampling point and deeper into the deposit area displayed considerably different compositions of oily components. The U-55 sediments contained 27 to 34% aliphatic hydrocarbons, 25 to 33% aromatic hydrocarbons, and 33 to 48% polar compounds. The U-1 sediment revealed only 14% aliphatic hydrocarbons, 30% aromatic hydrocarbons, and 56% polar compounds. On the basis of the limited data available, it appears that after the river progresses through the bitumen deposit area, hydrocarbon content of the oily constituents (especially aliphatic hydrocarbons) is reduced considerably in sediments closer to Fort McMurray. These results are substantiated by the increased aliphatic hydrocarbon content found in the U-35 and U-2 water samples and reduce the possibility that degradative processes might be occurring on these lighter aliphatic hydrocarbons in the sediments.

The amount of naturally occurring organic material which is extractable in these river waters is low (1 mg/L) in contrast to the wastewaters from the Suncor plant, which was found to contain 13 to 89 mg/L (Strosher and Peake, 1976). This illustrates the fundamental difference in the composition of the natural organic materials occurring in the river system and those which are introduced by oil sands mining and extraction processes.

Occurrences of organic acids, phenolic compounds and sulphur compounds, which are major compound classes of the wastewater effluents, total less than 0.01 mg/L in the undisturbed river waters.

Hydrocarbons presumably derived from the oil sands deposit by natural leaching occur in small quantities in the river waters, 0.005 to 0.016 mg/L. These hydrocarbons are generally higher molecular weight than the hydrocarbons found in the Suncor upgrading plant wastewaters or some components found in the river downstream of the Suncor plant.

In a study of the wastes discharged to the Athabasca River, Stosher and Peake (1978) identified the following sources: tailings pond dike drainage from Suncor, coke and sulfur storage runoff from Suncor, upgrading plant effluent from Suncor and mine depressurization water from Syncrude. Figure 3-2 shows the location of the coke and sulfur storage runoff and the upgrading plant effluent discharges to the Athabasca River. Figure 3-3 shows the location of the mine depressurization wells. These figures were obtained from Strosher and Peake (1978).

Upgrading plant effluents from Suncor contain an average of 36 mg/L of organic carbon when sampled on three occasions between September 1976 and February 1977 (Strosher and Peake, 1978). This value is identical to that obtained in the previous study

conducted during November and December of 1975. The extractable organic carbon content of these wastewaters (which are discharged to the Athabasca River) averaged 17 mg/L, or 46% of the total organic carbon, which is comparable to the extractable content of the 1975 wastewaters (42%). The extractable carbon likely exists as dissolved or dispersed liquid hydrocarbons and other compounds of low polarity which are readily extractable with benzene from water. The remaining total organic carbon may exist in one or more forms: as dissolved polar compounds (possibly including humic and fulvic acids), as compounds adsorbed on the surface of clay particles or occluded within particles, or as discrete carbon particles.

The source of the extractable carbon is mainly the upgrading plant whereas the source of the non-extractable carbon in the upgrading plant effluent is the river water. During the study period, Athabasca River waters upstream of the plant contained 9 to 15 mg/L organic carbon, of which 3 mg/L was extractable. The upgrading plant effluents of Suncor (which are discharged to the Athabasca River) contained 31 to 41 mg/L organic carbon, of which 12 to 19 mg/L was extractable. Thus, the upgrading plant contributed 16 to 26 mg/L organic carbon to the water, of which the majority, 9 to 16 mg/L is extractable (Strosher and Peake, 1978).

Organic sulphur compounds were one of the major contributors to the upgrading plant effluents, representing 24% of the extractable organic matter, or 4 mg/L. These compounds, which are composed largely of substituted benzothiophenes, contain approximately 17% sulphur, thus making the sulphur content of the extractable organic matter roughly 4%. This value is in good agreement with the 4.2% sulphur content of the Athabasca bitumen (Berkowitz and Speight, 1975).

The second major group of extractable organic compounds was the oxygen-containing compounds, including organic acids, organic acid esters, phenols, aldehydes, ketones, and quinones. These compounds account for 17% of the extractable organic matter and occur at concentrations averaging 3 mg/L. When the amides (which contain both oxygen and nitrogen atoms) are included in the oxygenated values, they increase the percentage to 24% of the extractable material. The estimated content of oxygenated compounds in bitumen is 10% (Strosher and Peake, 1978). It is therefore indicated that a large percentage of the oxygenated compounds found in upgrading plant effluents is likely formed during processing of the extracted bitumen.

Hydrocarbons and weakly polar compounds comprise an average of 16% of the extractable carbon, or 2.8 mg/L. The main source of these compounds is the upgrading plant. Fifty-eight per cent were aliphatic hydrocarbons, 30% were aromatic hydrocarbons and

12% were polar compounds. The composition is more similar to that of synthetic crude oil, which is 79% aliphatics, than that of raw bitumen which contains only 19% aliphatics (Strosher and Peake, 1978). It is greatly different from that of the upstream river water which average only 4% aliphatics, confirming the upgrading plant as the source of these hydrocarbons.

The remaining constituents examined in the wastewaters were asphaltenes, which account for 10% of the extractable organics, and the nitrogen-containing compounds, which account for 7%. As the synthetic crude produced by the upgrading plant is devoid of asphaltenes, and the raw bitumen contains 17 to 19% asphaltenes (Strosher and Peake, 1976; Berkowitz and Speight, 1975), the asphaltenes in the upgrading plant effluents likely originated in the oil sands bitumen.

The average daily discharge of organic carbon to the Athabasca River from the upgrading plant effluent (Suncor) is 1460 kg per day (Strosher and Peake, 1976).

Mining operations in Syncrude lease 17 require the lowering of the water table by pumping groundwater from wells in the mine area. The mine waters contain an average of 30 mg/L of total organic carbon, of which 62%, or 19 mg/L is extractable. Oxygen-containing compounds were the most abundant group of compounds in the mine water composites, averaging 24% of the extractable

organics (4.6 mg/L). The majority of these compounds were in the form of organic acids (39%) and organic acid esters (28%). Phenols, aldehydes, and quinones comprised the remaining 33% of the oxygenated compounds. Organic sulphur compounds represented 15% of the extractable organic matter (3 mg/L). Asphaltenes were the next in abundance, comprising 14% of the extractables (2.6 mg/L). Both nitrogen-containing compounds (2%) and hydrocarbons (1%) were very minor contributors to the extractable organic matter.

Meteoric water percolating through the coke storage pile and sand tailings, used to contain the coke (i.e. coke runoff), contained 25 mg/L total organic carbon, of which 60% (15 mg/L) was in the form of extractable organic carbon. Asphaltenes were found to be the major component of the extractable organics in the coke storage waters (18% - 2.7 mg/L). Oxygen-containing compounds accounted for 13% of the extractable organic matter and organic sulphur compounds for 11%.

The remaining groups of measured compounds in the extractable organic matter were the nitrogen-containing compounds at 2% and the hydrocarbons at 1%.

Tailings pond dike drainage waters contained an average 108 mg/L total organic carbon of which 69% (75 mg/L) was in the form of extractable organic carbon. Most of the extractable organic

matter consisted of oxygenated compounds (89%) the majority of which were in the form of organic acids (79%). Thus by far the most abundant compounds in the tailings pond dike drainage waters were the organic acids which averaged 59 mg/L. These acids were multi-ring aromatic and not aliphatic (Strosher and Peake, 1976). Other oxygenated-compounds included phenols (4.5%), ketones (2.7%), aldehydes (1.9%), organic acid esters (1.5%), amides (1.0%), and quinones (0.2%). Organic sulphur compounds averaged 5.3%, organic nitrogen compounds 1.1% and hydrocarbons 0.04%.

The daily organic carbon release to the Athabasca River from the tailings pond dike filter system is 198 kg. The daily organic carbon discharged from the upgrading plant effluent is 1460 kg. Using a measured flow of the Athabasca River of 2.03 x 10^6 m^3 per day and a total organic carbon content of the river of 10 mg/L, Strosher and Peake (1978) calculated that tailings pond drainage and upgrading plant effluent contributed 0.8% of the organic load of the Athabasca river.

Water samples taken upstream of the Athabasca River were found to contain an average of 9 mg/L organic carbon, the majority of which was determined to be dissolved organic carbon. Water soluble organics, which include the humic acids, averaged 6.9 mg/L and were the largest single organic component of the river water. Also contained in this water soluble fraction were the naturally occurring tannin and lignins at 0.24 mg/L. The

extractable carbon fraction contained 20% asphaltenes, 33% polar constituents, and 10% hydrocarbons (Strosher and Peake, 1979).

Figure 3-1. Sampling locations in Athabasca River basin.

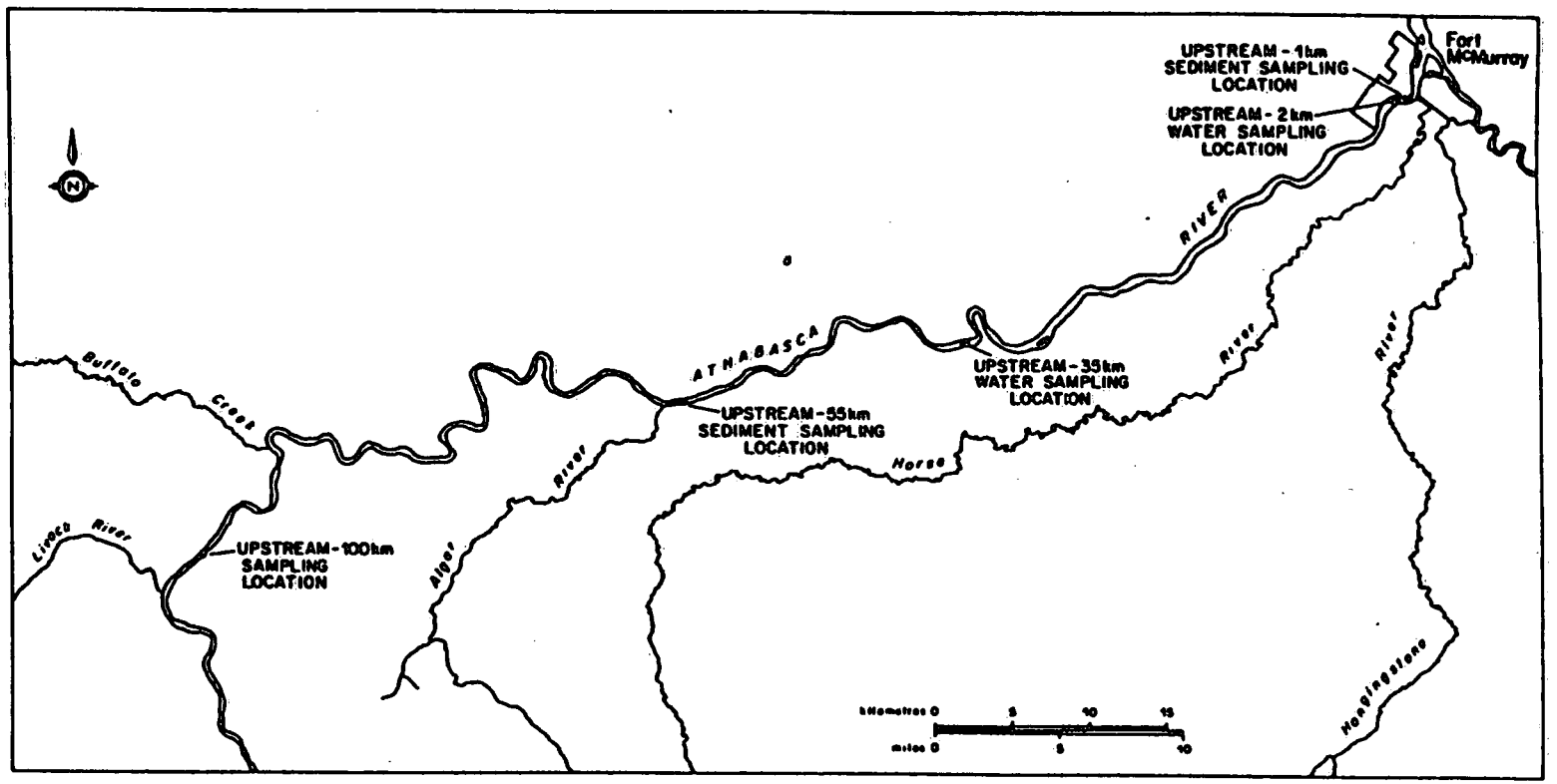

UPSTREAM - 1km
SEDIMENT SAMPLING
LOCATION
UPSTREAM - 2km
WATER SAMPLING
LOCATION
Fort
McMurray
RIVER
ATHABASCA
UPSTREAM - 35km
WATER SAMPLING
LOCATION
UPSTREAM - 55km
SEDIMENT SAMPLING
LOCATION
Buffalo
Creek
Horse
River
River
River
Liard River
UPSTREAM - 100km
SAMPLING
LOCATION
Algar
River
Hangingstone
kilometres 0 5 10 15
miles 0 5 10

Figure 3-2. Sampling locations in the Athabasca River in the vicinity of tar sands plants near Fort McMurray.

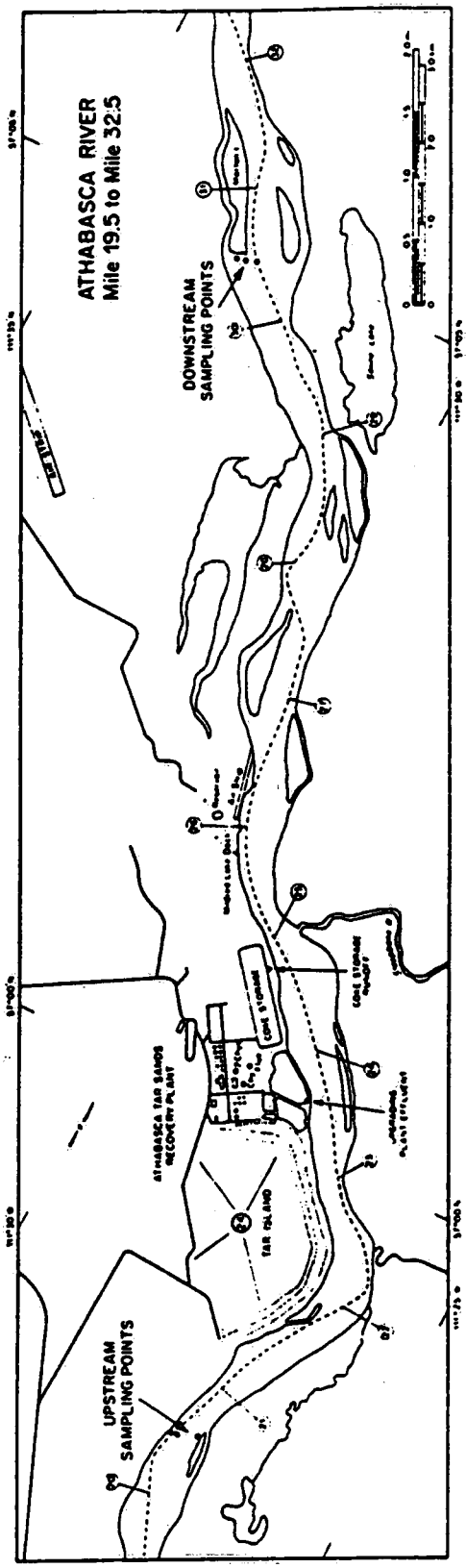
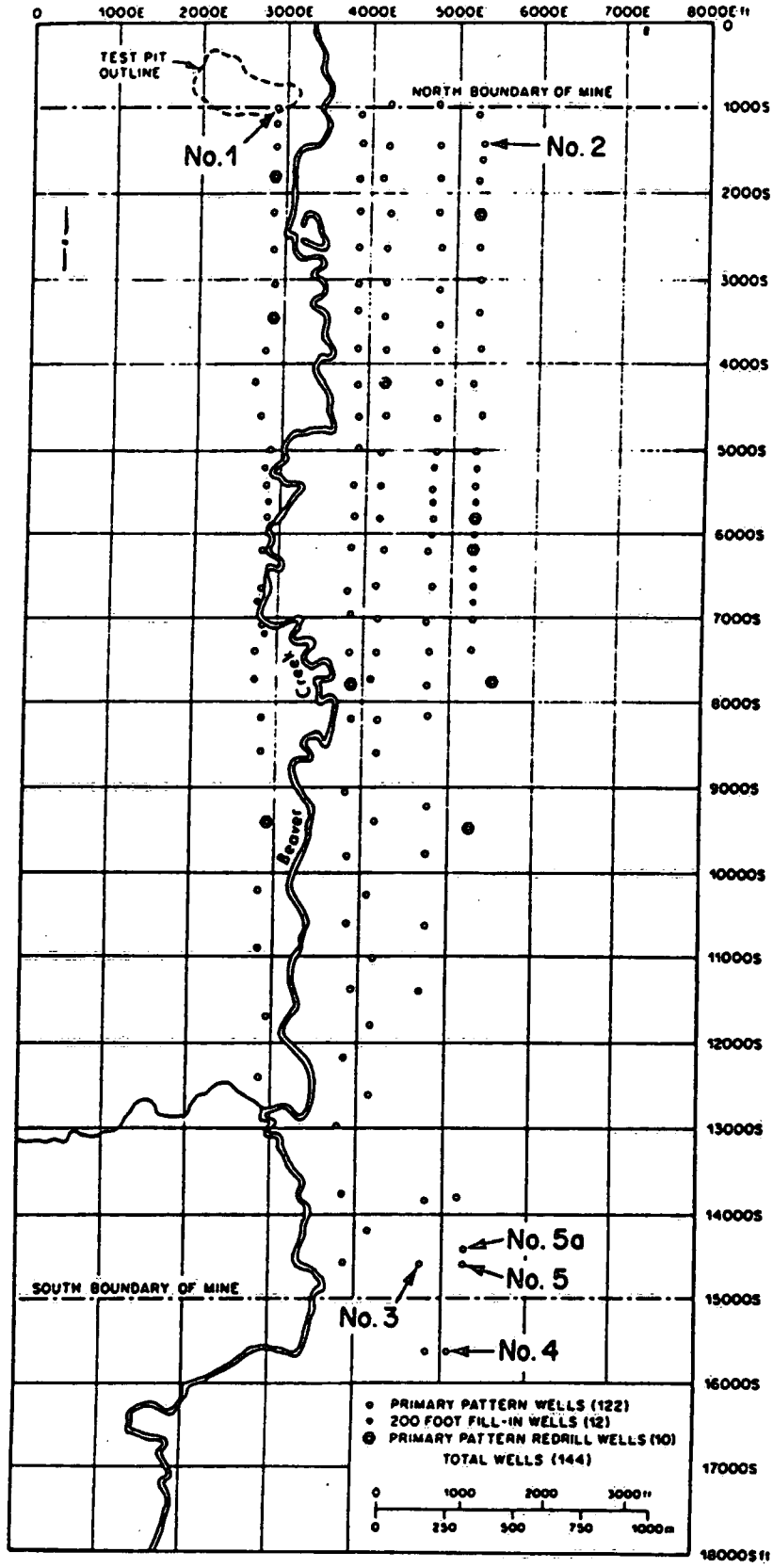

ATHABASCA RIVER
Mile 19.5 to Mile 32.5
DOWNSTREAM
SAMPLING POINTS
UPSTREAM
SAMPLING POINTS
TAR ISLAND

Figure 3-3. Sampling locations in Syncrude Canada Ltd. mine development field near Fort McMurray.



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CHAPTER 4 - ACUTE AND CHRONIC TOXICITY OF PAH, PASH, PANH AND HPAH

Acute Toxicity

Data summarizing the acute toxicity for PAH, PANH and HPAH are presented in Tables 4-1 to 4-3, respectively.

Physiological Effects of PAH, PASH, PANH, HPAH and Petroleum Products

(a) suppression of heart beat

Tests done on sheepshead minnows *(Cyprinodon variegatus)* *(Fundulus heteroclitus)* and *(Fundulus similus)* showed that levels of water soluble fractions of a No 2 fuel oil that significantly suppressed heart beat were the same as those resulting in a 50% or greater reduction in hatching success (i.e. 2.5 ppm total hydrocarbons and 0.5 ppm total naphthalenes) (44).

(b) increase in breathing rate

Pink salmon (Onchorhynchus gorbuscha) tested with crude oil and fuel oil showed that highest breathing rates occurred between three and six hours of exposure. Increase in breathing rates was

linear with increasing oil concentrations. The suspicion is that an increase in oxygen consumption is needed to support the increase in physiological activities required for the metabolism and excretion of hydrocarbons. Reduced but still elevated breathing rates that continue after the initial response suggest that greater than normal quantities of energy are still needed to maintain the enzyme synthesis and oxidation of hydrocarbons (45).

(c) disorientation

An exposure to concentrations of 1/2 to 1/8 the mortality levels of crude oil reduced the swimming capacity of squawfish *(Ptychorcheilus lucius)* thereby significantly impairing their ability to capture prey (18).

A common detritivore *(Asellus aquaticus)* upon a one hour exposure to crude oil showed that the organisms surviving had limited mobility and were mainly turned upside down (46).

(d) other effects noted

An increase in predation-induced mortality was associated with a loss of schooling behavior among fathead minnows *(Pimephalus promelas)* that were exposed to crude oil. Fluorene was shown to impair feeding efficiency as well as impair consumption of prey in bluegills *(Lepomis macrochirus)* and to

inhibit consumption of food in coho salmon *(Onchorhynchus kisutch)*. Cutthroat trout *(Salmo clarki)* exposed to Wyoming Crude Oil showed gill damage, fin erosion and impairment of their swimming capacity. Organisms such as *(Chironomidae)*, *(Baetis)* and *(Isoperla)*, commonly found in one study, were shown to be sensitive to low level concentrations of shale oil. Their absence from a system could provide an early warning of shale oil contamination as low as 0.5-1.0 mg/L (18).

English sole *(Parophrys vetulus)* upon exposure to crude oil were shown to take up substantial amounts of petroleum hydrocarbons and thereby developed abnormalities of the liver, weight loss, and had a higher rate of mortality (30).

Sea urchin eggs *(Strongycentrotus droebachiensis)* and cod fish eggs *(Gadus morhua L.)* showed tolerance limits to naphthalene and naphthalene derivatives from 0.6 to 1.8 ppm (47).

Embryo and larval stages of fathead minnows *(Pimephalus promelas)* showed impaired growth in phenol concentrations of 2.5 mg/L. Early life stages of rainbow trout *(Salmo gairdneri)* proved to be more sensitive to phenol than some warm water species studied (13).

Studies done on fathead minnows *(Pimephalus promelas)* and rainbow trout *(Salmo gairdneri)* showed that concentrations of

naphthalene as low as 0.85 mg/L reduced growth in fathead minnows, reduced egg hatchability and weight and length of fry of both species at 30 days in the same concentration (4).

Anthracene was shown to be phototoxic with anthraquinone being a photoproduct of anthracene. A study done on bluegills (Pimephalus promelas) held in anthracene-contaminated water in a shaded channel showed that they died only after being placed in clean water and exposed to sunlight. The study suggested this mortality was due to direct sunlight exposure of the anthracene-contaminated fish rather than the toxic effects of anthracene photoproducts in the water. Since light is known to increase fish activity levels and trigger complex physiological processes this could then contribute indirectly to the mortality under stress conditions of anthracene-contaminated fish (48).

Studies done on the effects of HPAH show sublethal effects including lethargy, loss of equilibrium and body deformities which could lead to increased susceptibility to predation and/or inability to obtain food (49).

Effects of Temperature

A study done on naphthalene in fish showed that concentrations in tissue were 1.6 to 15 times higher in fish held at 4 degrees C than fish held in water at 12 degrees C. At 4

degrees C greater than 30% of an administered dose of naphthalene was found in the gastrointestinal tract compared with less than 3% in gastrointestinal tracts at 12 degrees C. Results showed that a decrease in water temperature led to substantially higher concentrations of naphthalene being retained in tissue of starry flounder *(Platichthys stellatus)* exposed to naphthalene (50).

Chronic Toxicity

The following studies show the correlation between the presence of aromatic hydorcarbons in water habitats and the increased incidence of genetic damage (usually seen as neoplastic growths) or abnormal physiological behavior in fish and other aquatic animals.

Studies on brown bullhead catfish showed a relationship between the presence of polycyclic aromatic hydrocarbons (PAH) in sediment and the inducement of tumor formation over a two to four year period. Approximately 33% of the fish from the study area had incidences of liver, lip and belly tumors as well as stubbed barbels. Consistent with the mutagenic effects of PAH, sediment extracts were shown to cause epidermal hyperplasia and neoplasia when painted on fish skin (23).

In a contaminated area of Puget Sound abnormal tissue morphologies were seen in various species. For example,

observations were all made on lesions and hyperplasia in fish gills, lesions in fish kidneys, skin, fin, heart, gastrointestinal tract, spleen, gonad and gall bladder, necroses and melanized nodules and granulomas in shrimp gills, various necrotic and abnormal conditions in shrimp and crab hepatopancreas, antennal gland, bladder and midgut (24). Another study of Puget Sound showed incidences of liver, kidney and gill lesions in bottom dwelling fish (25).

Test studies done on Spot (Leistomus xanthurus), a bottom feeder, revealed penetrating integumental lesions, severe fin and gill erosion, significant reduction in hematocrits and no weight gain. Pancreatic and liver alterations also resulted (26).

Sea urchin larva (Strongylocentrotus droebachiensis) produced skeletal abnormalities in the presence of 1- and 2-methylnaphthalene. It has been postulated that metabolic conversion of PAH may result in potentially damaging intermediates that may interact with DNA (27).

Incubation time in Japanese medaka (Oruzias latipes) eggs has been shown to be shortened upon exposure to petroleum hydrocarbons as compared with killifish (Fundulus heteroclitus) and cod (Gadus morhua) eggs in which hatching time is delayed. It has been shown in at least one study that contaminants in the surface such as petroleum hydrocarbons are capable of causing not

only mortality but also malformations in developing fish eggs and larvae (28).

Studies done on the edible portion of carp (Cyprinus carpio) and their hybrids, in this case carp-goldfish hybrids, showed concentrations of PAH compounds. Since these fish are for the most part, bottom feeders and are exposed to PAH contaminants in sediment, it is interesting to note from this study that the hybrid forms frequently showed gonadal tumors whereas the parental types did not (29).

Dover sole (Microstomus pacificus) which were in contact with contaminated sediments showed early signs of fin erosion. Approximately 50% of the flat fish (family Pleuronectidae) exposed to Prudhoe Bay Crude Oil contaminated sediment exhibited severe hepatocellular lipid vacuolization and weight loss. Spot shrimp (Pandalus platyceros) exposed to similar sediment showed a decrease in search type feeding activities (30).

Sand sole eggs (Psettichtys melanostichus) treated with benzo(a)pyrene showed a significant decline in hatching success and a higher incidence of developmental anomalies (31).

Some effects of petroleum hydrocarbon exposure include damage to the olfactory organs including hyperplasia and attenuation of the chemosensory cilia, changes in the amount of

lipid storage in the hepatocytes, effects on eye tissue involving hydration of lens fibre cells and cataract formation, and abnormal subcellular inclusions in the intestine (32).

Studies done on a polluted area of Southern California reported incidences of fin and tail erosion, tumors and abnormal coloration of organisms (33).

Tests done on striped mullet (Mugil cephalus L.) with analogs of fluorene showed a toxic action on brain stores of catecholamines and depletion of indolamines. Uncontrolled muscle twitches were also observed. This same study also showed that brain levels of neurotransmitters can be affected by exposure to PAH (34).

Evidence of neoplasms and lesions were found in various species tested in the Great Lakes. The sediments from the areas tested were found to contain various concentrations of PAH. Of particular interest is the information in Table 3-4 involving the species of white sucker as they are also a common species in the Athabasca River (35).

Ames testing and DNA synthesis showed that 2- and 3-aminodibenz-thiophenes demonstrated significant genotoxicities (36).

Induction of benzo(a)pyrene monooxygenase activity occurred in (Blennius pavo) in response to a Diesel 2 oil (37).

Testing of water soluble fractions from a fresh and a water-leached, solvent-refined coal showed toxicity in the form of suppression of growth, reproduction and survival of invertebrates (38). The study suggested that fractions that were absorbed on particulate matter and accumulated in sediment were responsible for sublethal effects with the greatest impact resulting on benthic communities. Studies showed that benthic insects may survive acute exposure but that sublethal effects on growth and development could result in significant delays in emergence timing. An upset in the life cycles could result in a decrease in population size which could lead to exclusion of bottom-dwelling species of the benthic drift did not colonize in a polluted area (38).

Aryl Hydrocarbon Hydroxylase (AHH) Activity

Studies on brown trout (Salmo trutta) and capelin (Mallotus villosus) both showed significant increases in the specific activity of aryl hydrocarbon hydroxylase (AHH). Such activity may also be found in the kidney and/or adrenal gland. Contaminants such as PCBs and aromatic hydrocarbons could well be inducers of AHH (39, 40).

Benzopyrene hydroxylase is induced in fish by exposure to petroleum. Studies done on cunner (Tauogolabrus adsperus) showed liver hydroxylase activity to be higher in fish from petroleum contaminated sites whereas the same activity was generally low in gill tissue (41).

Studies done on killifish (Fundulus heteroclitus) embryos suggests that they have the capacity to activate benzopyrene and related compounds presumably via AHH activity. A single one hour exposure of rainbow trout (Salmo gairdneri) embryos to benzopyrene induced a significant number of liver tumors in these fish nine to twelve months later. Killifish (Fundulus heteroclitus) eggs exposed again to PCBs or whole No. 2 Fuel oil showed increased AHH activity near hatching. PCBs induced AHH activity in the liver and extrahepatic tissures of similarly exposed yolk sac larvae (39).

Studies on rainbow trout (Salmo gairdneri) gonad cells showed these cells capable of converting pro-mutagens to their genotoxic forms. Some of the tests done in this study were with benzo(a)pyrene (42).

Rainbow trout (Salmo gairdneri) gonads, bluegill (Pimephales promelas) fry and steelhead embryo cells were tested against several mutagens including benzo(a)pyrene. Results of the study showed that the number of viable cells continued to decline in

direct proportion to the amount of chemical in the medium (i.e. increasing toxicity as the concentration of the mutagen was increased). Mutagen treated cells were also impaired in their ability to proliferate (43).

Summary

(a) Acute Toxicity

Freshwater organisms vary considerably in their sensitivity to petroleum hydrocarbons. There are a number of reasons for this variability including: life cycle stage and the sex of the experimental organism, the nutritional status of the organism, whether it was fed or starved, and its exposure history prior to the exposure to the hydrocarbons. Toxicity is also affected by the chemical composition of the product, the exposure conditions (temperature, exposure duration), the condition of the oil or hydrocarbons (whether emulsion, slick or water-soluble fraction), and the general water quality. An added problem in assessing toxicity is the variation found in the literature in the method of reporting toxicity thresholds (LC_{50}s). This problem is then compounded by differences in the test method, whether static or flow-through and by the method of preparation of the oil/chemical-in-water mixture (for example, shaking or stirring). Finally the test duration varies widely, from as short as 5 min (for the bacterium *Photobacterium phosphorium*) to 70 days.

As noted above, organisms are not constant in their sensitivity to toxic materials, but vary somewhat unpredictably with differences both between genera and species as well as within any one species between the different life cycle stages. This is demonstrated in Table 4-5, showing the results of toxicity tests involving a range of aromatic hydrocarbons to three freshwater teleosts.

Despite species differences for specified chemicals, some interesting patterns emerge. An obvious relationship apparent for all species is that increased ring size results in higher toxicities. For example, in terms of 96h LC_{50} for rainbow trout, toxicity can be ranked phenanthrene > naphthalene > benzene. The same applies for polycyclic aromatic hydrocarbons containing heteroatoms. For example, acridine is more toxic than quinoline to fathead minnow and β-naphthol is more toxic than phenol for all three species. This finding is similar to that of Trucco and co-workers (51) who determined that the toxicities of five aromatic hydrocarbons, to the freshwater zooplankton *Daphnia pulex*, to be in direct relationship to their increasing ring size, i.e. benzo(a)pyrene > benzo(a)anthracene > phenanthrene > naphthalene > benzene. The toxicity range (96h LC_{50}) extended from 5 μg/L, for benzo(a)pyrene, to 15 mg/L for benzene.

Somewhat similar findings were obtained in recent investigations on aromatic hydrocarbons found in coal-derived

synthetic fuels (13). Despite differences between the various individual tests (related mainly to the length of exposure) a similar pattern of increasing toxicity corresponding to increasing aromaticity (ring number) emerged, with β-naphthol being 2 to 45 times more toxic than phenol; acridine 7 to 27 times more toxic than quinoline; and phenanthrene 3 to 9 times more toxic than naphthalene (13). Test organisms included: green algae (Selenastrum capricornutum), diatoms (Nitzschia palea), adult snails (Physa gyrina), juvenile cladocerans (Daphnia magna), larval midges (Chironomus tentans), adult amphipods (Gammarus minus), juvenile fathead minnows (Pimephales promelas), and embryo-larva stages of rainbow trout (Salmo gairdneri) and largemouth bass (Micropterus salmoides).

Another interesting observation is that the introduction of a heteroatom into the PAH nucleus generally results in decreased toxicity. For example quinoline is less toxic than naphthalene to rainbow trout (10); phenanthrene is more toxic than acridine to green algae, diatoms, Cladoceran, amphipods, midge, largemouth bass (eggs and larvae) and rainbow trout (eggs and larvae); and naphthalene is more toxic than quinoline to green algae, diatoms, snails, cladoceran, amphipods, midge, largemouth bass (eggs and larvae) and rainbow trout (eggs and larvae).

A similar observation can be made for the introduction of a hydroxy group into the PAH nucleus. For example, β-naphthol is

less toxic than naphthalene for green algae, diatoms, snails, cladoceran, midge, fathead minnow, and large mouth bass (13).

Another important observation is that PAH such as pyrene, fluoranthene and anthracene become more toxic after irradiation with sunlight. Research conducted by Kagan and co-workers (9) revealed that 1 mg/L solutions of anthracene, fluoranthene and pyrene were not toxic to Daphnia magna, Aedes aegypti, Artemia salina, Rana pipiens, and *Pimephales promelas* in the dark. However, after irradiation with sunlight for 1 h these chemicals became very toxic. This observation may explain some of the discrepancies in the literature concerning acute lethality testing of PAH.

(b) sublethal effects

The range of sublethal effects is both wide and varied. The effects are found at all stages in the development of aquatic organisms, impacting on egg hatchability, larval development, adult metabolism and behaviour, growth and reproduction. They frequently result in pathological abnormalities involving eyes, gill membranes, liver and spinal deformations.

The significance of these sublethal effects in terms of the population or at the community level is as yet poorly understood. Undoubtedly, at the individual organism level, any negative

alteration in normal functioning lessens its chances for survival in the continuous predator-prey cycle.

(c) genotoxicity/mutagenicity

The information presented in Chapter 1 suggests that exposure of fish to PAH can lead to neoplasms, tumors, reproductive toxicity, and developmental abnormalities. More and more research is lending support to the hypothesis that PAH metabolites and not PAH are responsible for observed genetic damage. It has been observed in animal studies that PAH metabolites may play a role in decreasing fecundity as indicated by the finding that PAH metabolites bind in vivo to DNA and proteins.

Conclusions

From information presented in this chapter and Chapter 1 it should be clear that PAH and associated homologs can have a number of effects on the aquatic system including death, sublethal effects and mutagenic/genotoxic effects. These effects are complicated by the knowledge that PAH and associated derivatives can be transformed into more toxic end products in the environment by sunlight and metabolism. In order to more fully understand the impact of industrial discharges of PAH and associated homologs to the aquatic environment it is imperative

that researchers study the impact on the aquatic community. Furthermore, analytical methods designed to isolate PAH will have to be modified to incorporate photoreduction products as well as metabolites.

Table 4-1. Acute toxicity of PAH compounds.

TEST LENGTH	COMPOUND	CONCENTRATION	SPECIES	SCIENTIFIC NAME	REF
24h	anthracene	LC50 26.8 ug/l	mosquito larvae	Aedes aegypti	2
96h	anthracene	LC50 11.9 ug/l	Bluegill juvenile	Lepomis macrochirus	2
5min	benzene	EC50 214 mg/l	bacteria	Photobacterium phosphoreum	3
15min	benzene	EC50 238 mg/l	bacteria	Photobacterium phosphoreum	3
96h	benzene	LC50 >15 mg/l	Fathead minnow	Pimephales promelas	4
96h	benzene	LC50 5.3 mg/l	Rainbow Trout	Juvenile Rainbow Trout	4
96h	benzene	LC50 22 mg/l	Bluegill juvenile	Lepomis macrochirus	4
96h	benzene	LC50 32 mg/l	Goldfish	Crassius auratus	4
96h	benzene	LC50 32 mg/l	Guppy	Lebistes reticulatus	4
96h	benzene	LC50 12.0 ug/l	Dolly varden juvenile	Salvelinus malma	5
96h	benzene	LC50 11.9 ug/l	Dolly varden smolt	Salvelinus malma	5
96h	benzene	LC50 11.7 ug/l	Chinook salmon	Oncorhynchus tshawytscha	5
96h	benzene	LC50 14.1 ug/l	Coho salmon juvenile	Oncorhynchus kisutch	5
96h	benzene	LC50 542 ug/l	Coho salmon embryo	Oncorhynchus kisutch	5
96h	benzene	LC50 31 mg/l	Fathead minnow	Pimephales Promelas	52
96h	benzene	LC50 50 ug/l	Coho salmon alevin	Oncorhynchus kisutch	5
96h	benzene	LC50 9.8 ug/l	Coho salmon emergent fry	Oncorhynchus kisutch	5

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Table 4-1 cont'd

TEST LENGTH	COMPOUND	CONCENTRATION	SPECIES	SCIENTIFIC NAME	REF
96h	benzene	LC50 10.8 ug/l	Sockeye salmon smolt	Oncorhynchus nerka	5
96h	benzene	LC50 14.7 ug/l	Arctic grayling juvenile	Thymallus arcticus	5
96h	benzene	LC50 15.4 ug/l	Slimy sculpin	Cottus cognatus	5
96h	benzene	LC50 24.8 ug/l	Threespine Stickleback	Gasterosteus aculeatus	5
96h	benzene	LC50 339 ug/l	Pink salmon embryo	Oncorhynchus gorbuscha	5
96h	benzene	LC50 50 ug/l	Pink salmon alevin	Oncorhynchus gorbuscha	5
96h	benzene	LC50 5.3 ug/l	Pink salmon emergent fry	Oncorhynchus gorbuscha	5
96h	benzene	LC50 17.1 ug/l	Pink salmon outmigrant fry	Oncorhynchus gorbuscha	5
	benzo-anthrene	LT50 0.83 h	Fathead minnow	Pimephales promelas	6
	benzo(a) anthracene	LT50 65.09 h	Fathead minnow	Pimephales promelas	6
24h	benzo(a) pyrene	LC50 3.75 mg/l	Topminnow	Poeciliopsis monacha and P. lucida	7
24h	biphenyl	LC50 1.5-<10mg/l	Fathead minnow	Pimephales promelas	8
			Rainbow Trout	Salmo gairdneri	
			Sheepshead minnow	Cyprinodon variegatus	
			Bluegill	Lepomis macrochirus	
			Golden Shiner		
			Catfish		
24h	fluor-anthracene	LC50 4 ug/l	Waterflea	Daphnia magna	9

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Table 4-1 cont'd

TEST LENGTH	COMPOUND	CONCENTRATION	SPECIES	SCIENTIFIC NAME	REF
24h	fluor-anthracene	LC50 40 ug/l	Brine shrimp	Artemia salina	9
24h	fluor-anthracene	LC50 0.2 mg/l	Fathead minnow	Pimephales promelas	9
96h	napthalene	LC50 150 mg/l	Mosquito fish	Gambusia affinis	4, 13
96h	naphthalene	LC50 4-5 mg/l	Sunfish	Lepomis humilus	4
96h	naphthalene	LC50 1.6 mg/l	Rainbow trout juvenile	Salmo gairdneri	4
96h	naphthalene	LC50 0.11 mg/l	Rainbow trout embryo-larvae stage	Salmo gairdneri	10
96h	naphthalene	LC50 0.51 mg/l	Largemouth bass embryro-larvae stage	Micropterus salmoides	10
96h	naphthalene	LC50 6.1	Fathead minnow 31-35 days old	Pimephales promelas	49
96h	phen-anthrene	LC50 40 ug/l	Rainbow trout embryo-larvae stage	Salmo gairdneri	10
96h	phen-anthrene	LC50 180 ug/l	Largemouth bass embryo-larvae stage	Micropterus salmoides	10
96h	styrene	LC50 32 mg/l	Fathead minnow	Pimephales promelas	14
96h	toluene	LC50 313 mg/l	Waterflea	Daphnia magna	11
96h	toluene	LC50 20-36 mg/l	Fathead minnow	Pimephales promelas	11
96h	toluene	LC50 >433 mg/l	Algae	Selenastrum capricornutum	12
96h	Naphtha-lene	LC50 7.9 mg/l	Fathead Minnow		4
96h	toluene	LC50 >433 mg/l	Algae	Skeletoneima costatum	12

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Table 4-1 cont'd

TEST LENGTH	COMPOUND	CONCENTRATION	SPECIES	SCIENTIFIC NAME	REF
96h	toluene	LC50 12.7 mg/l	Bluegill sunfish	Lepomis macrochirus	12
96h	toluene	LC50 366 mg/l	Sheepshead minnow	Cyprinodon variegatus	12
96h	xylene	LC50 42 mg/l	Fathead minnow	Pimephales promelas	14
24h	crude oil	LC50 2-4 mg/l	Crab larvae	Paralithodes camtschatica	17
24h	crude oil	LC50 1-3 mg/l	Shrimp larvae	Evalus suckleyi	17
96h	kerosene	Mort 8980 mg/l	Fathead minnow	Pimephales promelas	5
96h	crude oil	LC50 3.7 mg/l	Coho salmon juvenile	Oncorhynchus *kisutch*	5
96h	crude oil	LC50 >12.0 mg/l	Coho salmon embryo	*Oncorhynchus* kisutch	5
96h	crude oil	LC50 >12.0 mg/l	Coho salmon alevin	Oncorhynchus kisutch	5
96h	crude oil	LC50 8.0 mg/l	Coho salmon emergent fry	Oncorhynchus kisutch	5
96h	crude oil	LC50 1.79 mg/l	Sockeye salmon juvenile	Oncorhynchus nerka	5
96h	crude oil	LC50 2.2 mg/l	Sockeye salmon smolt	Oncorhynchus nerka	5
96h	crude oil	LC50 4.4 mg/l	Arctic grayling juvenile	Thymallus arcticus	5
96h	crude oil	LC50 2.2 mg/l	Arctic char juvenile		5
96h	crude oil	LC50 6.4 mg/l	Slimy sculpin	Cottus cognatus	5
96h	crude oil	LC50 >10 mg/l	Threespine stickleback	*Gasterosteus* aculeatus	5
96h	crude oil	LC50 >12.0 mg/l	Pink salmon embryo	Oncorhynchus gorbuscha	5

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Table 4-1 cont'd

TEST LENGTH	COMPOUND	CONCENTRATION	SPECIES	SCIENTIFIC NAME	REF
96h	crude oil	LC50 >12.0 mg/l	Pink salmon alevin	Oncorhynchus gorbuscha	5
96h	crude oil	LC50 8.0 mg/l	Pink salmon emergent fry	Oncorhynchus gorbuscha	5
96h	crude oil	LC50 8.0 mg/l	Pink salmon outmigrant fry	Oncorhynchus gorbuscha	5
96h	crude oil	LC50 2.8 mg/l	Dolly varden juvenile	Salvelinus malma	5
96h	crude oil	LC50 2.7 mg/l	Dolly varden smolt	Salvelinus malma	5
96h	crude oil	LC50 3.6 mg/l	Chinook salmon	Oncorhynchus tshawytscha	5
96h	shale oil	LC50 1.3-2.1 mg/l	Cutthroat trout	Salmo clarki	18
96h	shale oil	LC50 2.1 mg/l	Fathead minnow	Pimephales promelas	18
96h	dissolved petroleum	LC50 100 ug/l	bivalve	Cardium lamarki	20
96h	dissolved petroleum	LC50 100 ug/l	bivalve	Didacna trigonoides	20
96h	dissolved petroleum	LC50 100 ug/l	bivalve	Monodacna caspies	20
60-70 days	dissolved petroleum	LD50 10-20 ug/g	Eggs	Salmotrutta caspius	20
60-70 days	dissolved petroleum	LD50 22-83 ug/g	Eggs	Hypophthalmichthys molitrix	20
60-70 days	dissolved petroleum	LD50 40-100 ug/g	carp		20

(a) Maximum acceptable toxicant concentration

Abbreviations:

LC50 - Lethal concentration for 50 percent kill.
EC50 - Effective concentration causing 50% reduction in light using Microtox.
LT50 - Median-lethal-time for acute photo-induced toxicity.
Mort - Total mortality
LD50 - Lethal dose 50 percent kill

Table 4-2. Acute toxicity of PANH Compounds.

TEST LENGTH	COMPOUND	CONCENTRATION	SPECIES	SCIENTIFIC NAME	REF
96h	acridine	LC50 2.24 mg/l	Fathead minnow juvenile	Pimephales promelas	13
96h	acridine	LC50 2.3 mg/l	Fathead minnow	Pimephales promelas	1
96h	acridine	LC50 3.1 mg/l	Daphnids	Daphnia magna	1
	diphenyl-amine	LC50(a) 1.11 mg/l	Red killifish	Orizias latipes	19
32	diphenyl-amine	BCF(b) 30	Fathead minnow	Pimephales promelas	5
	N-methyl-aniline	LC50(a) 2.55 mg/l	Red killifish	Orizias latipes	19
96h	quinoline	LC50 46 mg/l	Fathead minnow juvenile	Pimephalus promelas	14
96h	quinoline	LC50 0.44 mg/l	Fathead minnow juvenile	Pimephalus promelas	13
96h	quinoline	LC50 11.0 mg/l	Rainbow trout embryo-larval stage	Salmo gairdneri	10
96h	quinoline	LC50 7.5 mg/l	Largemouth bass embryo-larval stage	Micropterus salmoides	10
5min	3,6-di-methyl quinoline	EC50 0.30 mg/l	Bacteria	Photobacterium phosphorium	21
5min	5,6-di-methylquinoline	EC50 0.74 mg/l	Bacteria	Photobacterium phosphorium	21
5min	5-methyl-quinoline	EC50 0.95 mg/l	Bacteria	Photobacterium phosphorium	21
5min	3,5-di-methylquinoline	EC50 1.0 mg/l	Bacteria	Photobacterium phosphorium	21
5min	6,7-di-methylquinoline	EC50 1.3 mg/l	Bacteria	Photobacterium phosphorium	21
48h	6,7-di-methylquinoline	LC50 7.6 mg/l	Rainbow	Salmo gairdneri phosphorium	21

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Table 4-2 cont'd

TEST LENGTH	COMPOUND	CONCENTRATION		SPECIES	SCIENTIFIC NAME	REF
5min	4,8-di-methylquinoline	EC50 1.7	mg/l	Bacteria	Photobacterium phosphorium	21
5min	isoquinoline	EC50 1.7	mg/l	Bacteria	Photobacterium phosphorium	21
5min	6,8-di-methylquinoline	EC50 2.2	mg/l	Bacteria	Photobacterium phosphorium	21
48h	6,8-di-methylquinoline	LC50 2.6	mg/l	Rainbow trout	Salmo gairdneri	21
5min	6-methyl-quinoline	EC50 2.2	mg/l	Bacteria	Photobacterium phosphorium	21
5min	4,6-di-methylquinoline	EC50 4.0	mg/l	Bacteria	Photobacterium phosphorium	21
5min	3,7-di-methylquinoline	EC50 4.5	mg/l	Bacteria	Photobacterium phosphorium	21
5min	3,8-di-methylquinoline	EC50 4.6	mg/l	Bacteria	Photobacterium phosphorium	21
5min	2,6-di-methylquinoline	EC50 5.7	mg/l	Bacteria	Photobacterium phosphorium	21
48h	2,6,di-methylquinoline	LC50 6.2	mg/l	Rainbow trout	Salmo gairdneri	21
5min	3-ethyl-quinoline	EC50 6.3	mg/l	Bacteria	Photobacterium phosphorium	21
5min	7,8-di-methylquinoline	EC50 7.0	mg/l	Bacteria	Photobacterium phosphorium	21
5min	2,7-di-methylquinoline	EC50 9.6	mg/l	Bacteria	Photobacterium phosphorium	21
5min	8-methyl-quinoline	EC50 8.8	mg/l	Bacteria	Photobacterium phosphorium	21
5min	3-iso-propylquinoline	EC50 10.0	mg/l	Bacteria	Photobacterium phosphorium	21
5min	2-methyl-8-ethylquinoline	EC50 14.0	mg/l	Bacteria	Photobacterium phosphorium	21
5min	2,8-di-methylquinoline	EC50 14.0	mg/l	Bacteria	Photobacterium phosphorium	21
5min	2,4-di-methylquinoline	EC50 30.0	mg/l	Bacteria	Photobacterium phosphorium	21

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Table 4-2 cont'd

(a) Indicates estimation of LC50 by logP

(b) Bioconcentration factor
Abbreviations:

LC50 - Lethal concentration for 50 percent kill.
EC50 - Effective concentration causing 50% reduction in light using Microtox.
LT50 - Median-lethal-time for acute photo-induced toxicity.
Mort - Total mortality
LD50 - Lethal dose 50 percent kill

Table 4-3. Acute toxicity of HPAH Compounds.

TEST LENGTH	COMPOUND	CONCENTRATION	SPECIES	SCIENTIFIC NAME	REF
5min	bi-phenylol	EC50 1.72 mg/l	Rainbow trout	Salmo gairdneri	15
96h	B-naphthol	LC50 0.07 mg/l	Rainbow trout embryo-larval stage	Salmo gairdneri	10
96h	B-naphthol	LC50 1.77 mg/l	Largemouth bass embryo-larval stage	Micropterus salmoides	10
96h	B-naphthol	LC50 3.46 mg/l	Fathead minnow juvenile	Pimephales promelas	13
	1-naphthol	LC50(a) 1.56mg/l	Red killifish	Orizias latipes	19
	2-naphthol	LC50(a) 1.39mg/l	Red killifish	Orizias latipes	19
96h	phenol	LC50 0.15 mg/l	Rainbow trout embryo-larval stage	Salmo gairdneri	10
96h	phenol	LC50 2.80 mg/l	Largemouth bass embryo-larval stage	Micropterus salmoides	10
96h	phenol	LC50 25.6 mg/l	Fathead minnow	Pimephales promelas	13
96h	phenol	LC50 0.34-2.42 mg/l	Goldfish eggs & young	Crassius auratus	13
96h	phenol	LC50 0.34-2.42	Bluegill	Lepomis macrochirus	13
96h	phenol	EC50 25 mg/l	Bacteria	Photobacterium fischeri	16
96h	phenol	LC50 8.9 mg/l	Rainbow trout juvenile	Salmo gairdneri	16
96h	phenol	LC50 68 mg/l	Fathead minnow	Pimephales promelas	16
96h	resorcinol (m-dihydroxybenzene)	EC50 310 mg/l	Bacteria	Photobacterium fischeri	16

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Table 4-3 cont'd

TEST LENGTH	COMPOUND	CONCENTRATION		SPECIES	SCIENTIFIC NAME	REF
96h	resorcinol (m-dihydroxybenzene)	LC50 >100	mg/l	Rainbow trout	Salmo gairdneri	16
96h	resorcinol (m-dihydroxybenzene)	LC50 100	mg/l	Fathead minnow	Pimephales promelas	16
96h	resorcinol (m-dihydroxybenzene)	LC50 53.4	mg/l	Fathead minnow juvenile	Pimephales promelas	5
96h	3,5-xylenol	LC50 22.0	mg/l	Goldfish	Crassius auratus	5
96h	2,6-di-tert-butyl-p-cresol	LC50 3.0	mg/l	Rainbow	Salmo gairdneri	22

(a) Indicates estimation of LC50 by logP

Abbreviations:

LC50 - Lethal concentration for 50 percent kill.
EC50 - Effective concentration causing 50% reduction in light using Microtox.
LT50 - Median-lethal-time for acute photo-induced toxicity.
Mort.- Total mortality
LD50 - Lethal dose 50 percent kill

Table 4-4. Some Neoplasms and Pre-neoplastic Lesions observed in fish from the Niagara River and Eastern Lake Erie.*

SPECIES	NEOPLASMS/LESIONS	ANATOMIC LOCATION
Goldfish	Fibro-sarcoma	Peritoneal cavity
White Sucker	Ora papilloma	Lips
	Basophilic nodule	Liver
Redhorse Sucker	Scirrhous carcinoma	Intestine
	Adenocarcinoma	Stomach
	Epidermal papilloma	Flank, fins
	Cholangioma	Liver
	Basophilic nodule	Liver
Brown Bullhead	Epidermal papilloma	Head, mouth
	Epidermal carcinoma	Head
	Cholangioma	Liver
	Odontoma	Mouth
	Epidermal hyperplasia	Fins, lips, barbels
Freshwater Drum	Chromatophoroma/Neurolemmoma	Skin-random sites
	Carcinoma	Liver
Walleye Pike	Ossifying fibroma	Skin-random sites
Carp Goldfish Hybrid	Gonadal fibroma/Dysgenesis	Gonads

*Ref. 35.

Table 4-5. Comparison of toxicities to three freshwater teleosts

Test Chemical	Test Organism					
	fathead minnow 96h LC50	life stage	rainbow trout 96h LC50	life stage	l. mouth bass 96h LC50	life stage
benzene	31 mg/L > 15 mg/L	juvenile	5.3 mg/L	juvenile	NA	
naphthalene	6.1 mg/L 1.99 mg/L	juvenile	0.11 mg/L 1.6 mg/L	embryo/larv. juvenile	0.51 mg/L	emb./larv.
phenanthrene	NA		0.04 mg/L	emb./larv/	0.18 mg/L	emb./larv.
quinoline	0.44 mg/L 46.0 mg/L	juvenile juvenile	11.0 mg/L	emb./larv.	7.5 mg/L	emb./larv.
acridine	2.24 mg/L	juvenile	NA		NA	
phenol	25.6 mg/L	juvenile	0.15 mg/L 8.9 mg/L	emb./larv. juvenile	2.80 mg/L	emb./larv.
β-naphthol	3.46 mg/L		0.07 mg/L		1.77 mg/L	emb./larv.

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CHAPTER 5 - FISH TAINTING BY PAH, PASH, PANH AND HPAH

Ogata et al. (1979) reported offensive odours present in fish caught in waters close to petroleum industries or from waters where oil spills had occurred. These authors suggested the offensive odors were due the presence of toluene and xylene which are readily taken up by fish.

Further work conducted by Ogata et al. (1978, 1980, 1983) revealed that eels, short necked clams and fish readily concentrate alkyl derivatives of benzothiophene and dibenzothiophene from crude oil suspensions and these compounds were consequently implicated in fish tainting.

In addition to toluene, other compounds which have been implicated in tainting include phenols, naphthenic compounds and dimethyl sulphide (Corner et al. 1976).

Koning (1988) found that rainbow trout exposed to treated tailings pond water were significantly tainted compared to control fish. Tainted fish were found upon analysis to contain alkylated phenols in the muscle. Analysis of the bile from the tainted fish revealed the presence of phenolic metabolites (i.e. glucuronide conjugates of alkylated phenols. This finding is

consistent with Dietz and Traud (1978) and Jardine (1987) who reported low taste thresholds for phenolic compounds.

In light of the information presented in Chapter 1 on metabolism, it should be emphasized that compounds which taint may be metabolites of other compounds and that analysis of tainted flesh should not ignore this possibility.

Jardine and Hrudey (1988) determined threshold detection values of potential fish tainting substances from oil sands wastewaters. Samples of walleye muscle (Stizostedium vitreum) were fortified with the compounds listed in Table 5-1 and subjected to sensory evaluation using a prescreened panel of eleven individuals. The absolute detection threshold was determined by 50% of the judgements using the procedure described by A.S.T.M. (1968).

Threshold detection limits for alkylated benzenes, alkylated PASH, alkylated PAH and for an alkylated phenol are presented in Table 5-1. Also included in Table 5-1 are associated molecular weights and vapor pressures for the compounds.

From Table 5-1 it is apparent that naphthalene, benzothiophene, and 2,5-dimethylphenol have the lowest detection thresholds, and thus have the strongest capability of the compounds tested for causing taint in fish.

The sensory sensation experienced on consuming petroleum hydrocarbons is primarily due to the volatile components present (Connell and Miller, 1981). The factors that control volatilization from water are the molecular weight, vapor pressure and the water solubility of the chemical (Jardine and Hrudey, 1988).

In general, the characteristic odour of a chemical compound decreases with molecular weight in a homologous series (Amerine et al., 1965). This is evident in the increased detection threshold level with the addition of one or two methyl groups to naphthalene (Table 5-1). However, the authors point out that the detection thresholds for 2,6-dimethylnaphthalene and 2,3,5-trimethylnaphthalene are not significantly different.

Vapor pressures indicate the tendency of the compound to evaporate. Compounds with an extremely high vapor pressure, such as thiophene, 2-methylthiophene, toluene, p-xylene and mesitylene evaporate very quickly on being exposed to air. Their odour would dissipate very quickly, possibly explaining why these compounds could not be readily detected by the panelists (Jardine and Hrudey, 1988). At the other extreme, compounds with a relatively low vapor pressure, such a 2,6-dimethylnaphthalene, 2,3,5-trimethylnaphthalene, and dibenzothiophene, tend to remain in the tissue longer, and thus have a high detection value. Compounds between these extremes, such as naphthalene,

benzothiophene, and 2,5-dimethylphenol, were detected at very low levels.

Dietz and Traud (1978) also found phenols to be highly odorous. Odor threshold concentrations of 126 phenolic compounds ranged from 10 to over 10,000 ppb. Monochlorophenols and methylphenols were determined to have the strongest odor.

Phenols are common components of petroleum wastewaters, and have been implicated in many tainting literature reports (Alabaster and Lloyd, 1982; Cote, 1976).

It is now known that taint in fish can be one of the most sensitive water-quality criteria for some chemicals. This is illustrated by work conducted by Branson et al (1979). These researchers reported that common criteria employed for recommending an environmentally safe concentration of a chemical in receiving waters were the rate of degradation of the compound and its toxicity to fish. Applying an arbitrary one-tenth of a 96h LC_{50} toxicity as a standard, a safe concentration for a chemical such as diphenyl oxide (DPO) would be 81 ug/L.

Relative fish taint from fish exposed to 32 to 21 ug/L DPO indicated that the fish were tainted and defined as different from those exposed to 10, 3, or 0 ug/L DPO at the 99 percent confidence level.

Trout flesh containing 7.5 ± 1.2 ug/g DPO was identified by a panel as being at the breakpoint between tainted and untainted taste. Trout continuously exposed to 16 ug/L of DPO in the water for one week show flesh concentrations that corresponded to this fish-taint threshold. Accordingly, a guideline based on the aesthetics of fish flavour would be 16 ug/L in the receiving waters. This number represents a value 1/50 of the 96h LC_{50}.

This study illustrates that fish tainting must be considered when developing licence requirements for industrial discharges and that tainting could be the most sensitive water quality criterion and the most difficult criterion to meet by current industrial treatment methods.

The presence of trace contaminants in the Athabasca River could exert an adverse effect by causing an unacceptable taste or odour in fish or in the water itself. Threshold taste or odour levels for oily contaminants in fish have not been rigorously reported; however, several papers report threshold odour concentrations for specific contaminants in water. These are summarized in Table 5-2 for the contaminants relevant to this study. An important aspect of odour detection is the relative sensitivity of different individuals which varies over a wide range. Cees et al. (1974) showed that for a series of chemicals including trimethylbenzene, 5% of observers may be expected to

detect odours at 0.01 of the group mean threshold odour concentration.

Another concern is whether the source of off-odours or tastes can be determined. Because some of the old literature concerning naturally caused oily taints is continually cited in recent reviews (i.e. Connel and Miller, 1981; Motohiro 1983), an impression exists that oily taints may easily be confused with other taint sources. Experienced researchers who have tested a wide variety of off-odours or flavours do not agree. Specifically, Kuusi and Suihko (1983) state: "The taste of oil is so distinct that it is usually identifiable even when present simultaneously with another off-flavour. The panelists were able to recognize it unambiguously."

Table 5-1. Summary of Molecular Weight, Vapor Pressure and Detection Threshold for Each Compound Tested.*

Compound	Molecular Weight	Vapor Pressure (torr) at 110°C	Detection Threshold (mg/kg)
Naphthalene	128.17	28.51	0.33
1-Methylnaphthalene	142.20	11.12	1.38
2,6-Dimethylnaphthalene	156.23	5.05	12.2
2,3,5-Trimethylnaphthalene	170.25	4.68	6.4
Toluene	92.14	746.63	NE
p-Xylene	106.17	333.16	9.0
Mesitylene	120.19	145.48	NE
Thiophene	84.14	1580.53	NE
2-Methylthiophene	98.16	706.01	NE
Benzothiophene	134.20	24.80	0.09
Dibenzothiophene	184.26	0.59	4.67
2,5-Dimethylphenol	122.17	21.51	0.21

*Jardine and Hrudey (1988). NE means not evaluated because of poor detectability by panelists in preliminary tests.

Table 5-2. Threshold odour levels for oily contaminants in water.

Substance	Threshold Odour Level (ppm)	Reference
n-alkanes C7-C_{12}	10 to 50	Zoeteman et al. (1971)
branched alkanes	10 to 500	Zoeteman et al. (1971)
benzene	10	Zoeteman et al. (1971)
	0.072	Alexander et al. (1982)
toluene	1.0	Zoeteman et al. (1971)
	0.024	Alexander et al. (1982)
ethylbenzene	0.10	Zoeteman et al. (1971)
	0.14	Rosen et al. (1963)
	0.0024	Alexander et al. (1982)
n-butylbenzene	0.10	Zoeteman et al. (1971)
t-butylbenzene	0.05	Zoeteman et al. (1971)
xylenes	1.0	Zoeteman et al. (1971)
diethylbenzene	0.0012	Alexander et al. (1982)
cumene	0.10	Zoeteman et al. (1971)
trimethylbenzenes	0.50	Zoeteman et al. (1971)
p-cymene	0.10	Zoeteman et al. (1971)
tetralin	0.01	Zoeteman et al. (1971)
	0.018	Rosen et al. (1963)
indene	0.001	Zoeteman et al. (1971)
naphthalene	0.005	Zoeteman et al. (1971)
	0.007	Zoeteman et al. (1971)
1-methylnaphthalene	0.02	Lillard and Powers (1975)
2-methylnaphthalene	0.01	Lillard and Powers (1975)
2,6-dimethylnaphthalene	0.01	Brady (1968) as cited in Zoeteman et al. (1971)
2,6-dimethylanthracene	0.50	Brady (1968) as cited in Zoeteman et al. (1971)
acenaphthene	0.08	Lillard and Powers (1975)

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Table 5-2 cont'd

Substance	Threshold Odour Level (ppm)	Reference
dibenzofuran	0.12	Lillard and Powers (1975)
	0.003	Alexander et al. (1982)
2-benzothiazole	0.08	Lillard and Powers (1975)
2-mercaptobenzothiazole	1.76	Lillard and Powers (1975)
various crude oils	0.0005 - 0.01	Zoeteman et al. (1971)
gasolines	0.01	Zoeteman et al. (1971)
diesel	0.0005	Zoeteman et al. (1971)
fuel oil (400 Shell)	0.002	Zoeteman et al. (1971)
#2 fuel oil	0.0008	Alexander et al. (1982)
naphtha	0.016	Alexander et al. (1982)

References - Chapter 5

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CHAPTER 6 - DEGRADATION OF PAH, PASH, PANH AND HPAH IN THE ATHABASCA RIVER

Utilizing laboratory incubations of Athabasca River water or water-sediment Nix and co-workers (1981) determined the extent of microbial degradation of trace amounts (100 ug/L) of m-cresol and camphor. Sites both above the oil sands region and downstream from the area of mining activity had a noticeably high level of activity regarding the degradation of these compounds. In addition, the incubation of samples taken along a transect of the river just downstream from the oil sands plants showed higher rates of microbial degradation on the west bank where effluents and drainage would likely be concentrated.

Analysis of ^{14}C-labelled substrates indicated that natural compounds such as amino acids and starch were degraded more quickly than hydrocarbons and that significant degradation of hydrocarbons occurred only after nutrient supplementation. Hydrocarbons studied include hexadecane and anthracene.

These findings should be compared to those of Fedorak and Westlake (1981) who found that aromatic hydrocarbons (derived from Prudhoe Bay crude oil) are more readily degraded than saturated hydrocarbons by marine microorganisms at 8°C. Time course studies showed that simple aromatics (e.g. naphthalene and

2-methylnaphthalene) are more readily degraded than the n-alkanes. The aromatic degradation continues from lower molecular weight, less complex molecules to larger, more complex molecules in the approximate series C_2 naphthalenes; phenanthrene and dibenzothiophene; C_3 naphthalenes and methylphenanthrenes; C_2 phenanthrenes. This study suggests that the choice of substrates, namely hexadecane and anthracene (Nix et al., 1981) may have been poor. A more appropriate substrate may have been an alkylated naphthalene such as ethyl naphthalene.

In a later study Fedorak and Westlake (1982) found that like PAH many sulfur heterocycles (derived from Prudhoe Bay crude oil) are readily degraded by microorganisms without nutrient addition. The order of susceptibility of the sulfur heterocycles was found to be: C_2 benzothiphene > C_3 benzothiophene; dibenzothiophene > C_1 dibenzothiophene > C_2 dibenzothiophene. This study was also conducted at 8°C.

From these studies conducted by Fedorak and Westlake it is possible to infer that PAH and PASH, discharged to the Athabasca River from current heavy-oil mining and upgrading operations, are degraded by microorganisms in the river. However, further research is required to prove or disprove this inference.

Aquatic Persistence and Fate of Oil Sands Contaminants

(a) benzene

Volatilization appears to be the major transport process of benzene from the water column to the atmosphere. The atmospheric photoxidation of volatilized benzene probably subordinates all other fate processes. The reported rates of oxidation are atmospheric photoxidation rates based on smog chamber data. One half-life value and rate reported for the atmospheric photoxidation of benzene is based on the assumptions that benzene depletion is due solely to attack by hydroxyl radicals and that even high concentrations of ozone present in ambient atmospheres will not contribute significantly to the photoxidation of alkanes and aromatics in general. Since benzene is relatively soluble in water, some benzene is expected to persist in the water column. That portion of benzene which persists in the water column would be expected to eventually biodegrade at a slow rate. The biodegradation of benzene would probably be enhanced by the presence of other hydrocarbons. Table 6-1 summarizes the aquatic fate information found for benzene. For further information the reader is referred to the review by Callahan et al. (1979).

(b) ethylbenzene

The data obtained for ethylbenzene are summarized in Table 6-2. Volatilization appears to be the major route of removal of this chemical from aquatic environments. The atmospheric

reactions of ethylbenzene probably overshadow all other fate processes. The estimated photoxidation half-life in Table 6-2 is based on smog chamber data and is, therefore, an approximation applicable only to a metropolitan environment. For further information the reader is referred to Callahan et al. (1979).

(c) toluene

The data obtained for toluene are summarized in Table 6-3. Volatilization appears to be the major route of removal of this chemical from aquatic environments. The atmospheric reactions of toluene probably subordinate all other fate processes. The precipitation of the atmospheric oxidation products could introduce benzaldehyde into the water. The estimated photoxidation half-life in Table 6-3 is based on smog chamber data and is, therefore, an approximation applicable only to a metropolitan environment. For further information the reader is referred to Callahan et al. (1979).

(d) phenol

Table 6-4 summarizes the aquatic fate data for phenol. Photooxidation, metal-catalyzed oxidation and biodegradation probably all contribute to the aquatic destruction of this pollutant. There is a possibility that some volatilization into the atmosphere can occur. Any phenol that passes into the

atmosphere would be rapidly destroyed by oxidation in the troposphere. Neither sorption nor bioaccumulation appear to be important processes in the aquatic fate of phenol. For further information the reader is referred to Callahan et al. (1979).

(e) 2,4-dimethylphenol

Table 6-5 summarizes the aquatic fate date for 2,4-dimethylphenol. Photooxidation appears to be a likely degradative pathway in the aquatic environment but the data regarding biodegradation are somewhat conflicting and inconclusive. Metal-catalyzed oxidation may be important in some localized situations. There may be some absorption by lipophilic materials but sorption by clay minerals appears unlikely. Volatilization and bioaccumulation are probably not important processes in this pollutant's fate. For further information the reader is referred to Callahan et al. (1979).

(f) polycyclic aromatic hydrocarbons: acenaphthene, acenaphthylene, fluorene and naphthalene

The results of the data review indicate that, under most environmental conditions, the dominant aquatic transport process for the polycyclic aromatic hydrocarbons will be adsorption onto suspended particulates. The role of volatilization is unknown but under certain conditions (e.g. water agitation) it could be

competitive with adsorption. The ultimate fate of naphthalene and possibly other polycyclic aromatics appears to biodegradation by microorganisms and metabolism (biotransformation) by multicellular organisms. The summary statements presented in Tables 6-6 and 6-7 are, for the most part, taken from fate data which apply to polycyclic aromatic hydrocarbons in general. For further information the reader is referred to Callahan et al. (1979).

(g) polycyclic aromatic hydrocarbons: anthracene, fluoranthene and phenanthrene

A summary of the data is presented in Tables 6-8, 6-9 and 6-10. For further information the reader is referred to Callahan et al. (1979).

(h) polycyclic aromatic hydrocarbons: benzo(a)anthracene, benzo(b)fluoranthene, benzo(k)fluoranthene, chrysene and pyrene

The results of the data review indicate that these polycyclic aromatic hydrocarbons will accumulate in the sediment and biota and will be transported with the suspended sediment. Some amount will be dissolved in water and will probably be degraded by direct photolysis. The ultimate fate of the adsorbed compounds is believed to be biodegradation and transformation by

benthic organisms, microbes, and vertebrate organisms in the food chain. The data are summarized in Tables 6-11, 6-12 and 6-13. For further information the reader is referred to Callahan et al. (1979).

(i) polycyclic aromatic hydrocarbons: benzo(g,h,i)perylene, benzo(a)pyrene, dibenzo(a,h)anthracene and indeno(1,2,3-cd)pyrene

The results of the data review indicate that benzo(a)pyrene and probably benzo(g,h,i)perylene, dibenzo(a,h)anthracene and indeno(1,2,3-cd)pyrene will accumulate in the sediment and biota and will be transported with the suspended sediment. Some amount will be dissolved and be degraded by direct photolysis. The ultimate fate of the dissolved polycyclic aromatic hydrocarbons however, is believed to be biodegradation and transformation by benthic organisms, microbes, and vertebrate organisms in the food chain. The data are summarized in Tables 6-14 and 6-15. For further information the reader is referred to Callahan et al. (1979).

TABLE 6-1 SUMMARY OF AQUATIC FATE OF BENZENE

ENVIRONMENTAL PROCESS	SUMMARY STATEMENT	RATE	HALF-LIFE ($t_{1/2}$)	CONFIDENCE OF DATA
Photolysis	Since the ozone layer in the upper atmosphere effectively filters out wavelengths of light less than 290nm, direct excitation of benzene in the aquatic or atmospheric environment is unlikely unless a substantial wavelength shift is caused by the media.	-	-	Low
Oxidation[a] Medium	Direct oxidation of benzene in environmental waters is unlikely. Smog chambers data, however, indicate that benzene is photooxidized at a rapid rate in the atmosphere.	0.85×10^{9} l. mol^{-1} [d]	20 to 30 hours[b] 2.4 to 24 hours[c]	
Hydrolysis	Probably not a significant fate process.	-	-	Medium
Volatilization	Probably the primary transport process.	-	4.81 hours[e]	Medium
Sorption	No specific information. The log P value for benzene indicates that sorption may occur.	-	-	Low
Bioaccumulation	The log P value of benzene indicates a low bioaccumulation potential for benzene.	-	-	Low
Biotransformation/ Biodegradation	Benzene can be utilized as the sole source of carbon by several microorganisms and is probably biodegradable at a slow rate.	-	-	Medium

a. The predominant environmental process which is thought to determine the fate of the compound.

b. This half-life is calculated from the half-conversion time for benzene bases on smog chamber data by Altshuller et al. (1962) and the table of relative reactivities given by Laity et al. (1973).

c. This half-life is the estimated half-life value proposed by Darnall et al. (1976) and is based on the assumption that benzene depletion is due solely to attack by hydroxyl radical.

d. This second-order rate of reaction of benzene with hydroxyl radicals has been obtained by Darnall et al. (1976) by averaging rates from smog chamber data by Hansen et al. (1975) and Davis et al. (1975).

e. This is the half-life estimated by Mackay and Leinonen (1975) for volatilization of benzene from a water column one meter thick at 25°C. This rate of volatilization varies with the environmental situation encountered.

TABLE 6-2 SUMMARY OF AQUATIC FATE OF ETHYLBENZENE

ENVIRONMENTAL PROCESS	SUMMARY STATEMENT	RATE	HALF-LIFE ($t_{1/2}$)	CONFIDENCE OF DATA
Photolysis	No information found.	-	-	Low
Oxidation[a]	Probably not important as an aquatic fate; however, atmospheric photooxidation is probably the main fate process.	-	-	Low
Hydrolysis	Not aquatically significant.	-	-	High
Volatilization	Significant transport process responsible for removal of ethylbenzene from water.	-	5-6 hr.	Low
Sorption	Relative importance cannot be determined.	-	-	Low
Bioaccumulation	Probably not important.	-	-	Low
Biotransformation/ Biodegradation	Relative importance cannot be determined.	-	-	Low

a. The predominant environmental process which is thought to determine the fate of the compound.

TABLE 6-3 SUMMARY OF THE AQUATIC FATE OF TOLUENE

ENVIRONMENTAL PROCESS	SUMMARY STATEMENT	RATE	HALF-LIFE ($t_{1/2}$)	CONFIDENCE OF DATA
Photolysis	Direct photolytic cleavage is energetically improbable in the troposphere.	-	-	Medium
Oxidation[a]	Probably not important as an aquatic fate: however, atmospheric photooxidation subordinates all other fate processes.	-	15 hrs.	Medium
Hydrolysis	Not aquatically significant.	-	-	High
Volatilization	Significant transport process responsible for removal of toluene from water.	0.193 hr^{-1}	5.18 hrs.	Medium
Sorption	Relative importance cannot be determined.	-	-	Low
Bioaccumulation	Probably not important.	-	-	Low
Biotransformation/ Biodegradation	Relative importance cannot be determined.	-	-	Low

a. The predominant environmental process which is thought to determine the fate of the compound.

TABLE 6-4 SUMMARY OF AQUATIC FATE OF PHENOL

ENVIRONMENTAL PROCESS	SUMMARY STATEMENT	RATE	HALF-LIFE ($t_{1/2}$)	CONFIDENCE OF DATA
Photolysis[a]	Photooxidation may be an important degradative process in aerated clear surface waters.	-	-	Medium
Oxidation[a]	Metal-catalyzed oxidation may be relevant in some aerated surface waters.	-	-	Medium
Hydrolysis	Not a relevant environmental process.	-	-	High
Volatilization	There is a possibility of some phenol passing into the atmosphere.	-	-	Medium
Sorption	Not a significant process in the aquatic environment.	-	-	Medium
Bioaccumulation	Not a significant process in the aquatic environment.	-	-	Medium
Biotransformation/ Biodegradation	A very significant fate pathway in aqueous media with a sufficient concentration of microorganisms.	-	-	High

a. The predominant environmental process which is thought to determine the fate of the compound.

TABLE 6-5 SUMMARY OF THE AQUATIC FATE OF 2,4-DIMETHYLPHENOL

ENVIRONMENTAL PROCESS[a]	SUMMARY STATEMENT	RATE	HALF-LIFE ($t_{1/2}$)	CONFIDENCE OF DATA
Photolysis	Photooxidation may be an important degradative process in aerated clear surface waters.	-	-	Low
Oxidation	Metal-catalyzed oxidation may be relevant in some aerated surface waters.	-	-	Low
Hydrolysis	Not a relevant environmental process.	-	-	High
Volatilization	Not a significant process in the aquatic environment.	-	-	Low
Sorption	Probably not a significant process in the aquatic environment.	-	-	Low
Bioaccumulation	Probably not a significant process in the aquatic environment.	-	-	Low
Biotransformation/ Biodegradation	Available information is inconclusive with regard to degradation in natural surface waters.	-	-	Low

a. There is insufficient information in the reviewed literature to permit assessment of a most probable fate.

TABLE 6-6 SUMMARY OF AQUATIC FATE OF ACENAPHTHENE, ACENAPHTHYLENE AND FLUORENE

ENVIRONMENTAL PROCESS[a]	SUMMARY STATEMENT[b]	RATE	HALF-LIFE ($t_{1/2}$)	CONFIDENCE OF DATA[c]
Photolysis	Disolved portion may undergo rapid photolysis.			
Oxidation	Oxidation of PAH by RO_2. radical is a slow process; not a significant process.			
Hydrolysis	PAHs do not contain groups amenable to hydrolysis.			
Volatilization	Is probably not as important as adsorption as a transport process.			
Sorption[d]	Measured adsorption coefficients for PAH and suspended solids are high; movement via sediment is considered an important transport process for PAH.			
Bioaccumulation	A short-term process; PAHs with less than 4 rings are readily metabolized and long-term partitioning into biota is not a significant fate process.			
Biotransformation/ Biodegradation	PAHs with less than 4 rings are degraded by microbes and are readily metabolized by multicellular organisms; biodegradation is considered the ultimate fate process.			

a. There is insufficient information in the reviewed literature to permit assessment of a most probable fate.

b. Very little environmental fate data specific to acenaphthene, acenaphthylene, and fluorene were found; the summary is made from data reviewed for PAHs as a group.

c. Data on acenaphthene, acenaphthylene and fluorene are not sufficient to permit confidence ranking. The confidence of the data reviewed for PAHs in general ranges from low to high.

d. Because the solubility of these compounds is relatively high, 50 percent or more may exist in true solution under conditions of normal sediment loading (Southworth 1979).

TABLE 6-7 SUMMARY OF AQUATIC FATE OF NAPHTHALENE

ENVIRONMENTAL PROCESS[a]	SUMMARY STATEMENT	RATE	HALF-LIFE ($t_{1/2}$)	CONFIDENCE OF DATA
Photolysis	Dissolved portion may undergo raped photolysis.	-	-	Medium
Oxidation	Oxidation of naphthalene by RO_2 radical is slow; not a significant process.	-	-	Medium
Hydrolysis	Naphthalene does not contain groups amenable to hydrolysis.	-	-	High
Volatilization	Role is unknown; could be competitive with adsorption under highly stirred conditions.	-	-	Low
Sorption	Measured adsorption coefficients for PAH and suspended solids are high; movement via sediment is considered an important transport process for PAH.	-	-	Medium
Bioaccumulation	A short-term process; naphthalene is readily metabolized and long-term partictioning into biota is not a significant fate process.	-	-	High
Biotransformation/ Biodegradation	Naphthalene is degraded by microbes and readily metabolized by multi-cellular organisms; biodegradation is probably the ultimate fate process.	0.04-3 $\mu g\ l^{-1} day^{-1}$	~1 day	Medium

a. There is insufficient information in the reviewed literature to permit assessment of a most probable fate.

TABLE 6-8 SUMMARY OF AQUATIC FATE OF ANTHRACENE

ENVIRONMENTAL PROCESS[a]	SUMMARY STATEMENT	RATE	HALF-LIFE ($t_{1/2}$)	CONFIDENCE OF DATA
Photolysis	Dissolved portion may undergo rapid photolysis.	-	~35 minutes	Medium
Oxidation	Oxidation of anthracene by RO_2 radical is slow; not a significant process.	50 liter mol^{-1} sec^{-1}	1600 days	Medium
Hydrolysis	Anthracene does not contain groups amenable to hydrolysis.	0	-	Medium
Volatilization	May be competitive with adsorption.	0.002 to 0.179^{-1}	18-300 hours	Low
Sorption[b]	Measured adsorption coefficients for anthracene and suspended solids are high; movement via sediment considered to be an important transport process.	-	-	Medium
Bioaccumulation	A short-term process; anthracene is readily metabolized and long-term partitioning into biota is not a significant fate process.	-	-	Medium
Biotransformation/ Biodegradation	Anthracene can be degraded by microbes and is readily metabolized by multicellular organisms; biodegradation is probably the ultimate fate process.	<0.0612 hr^{-1}	>11.3 hours	Medium

a. There is insufficient information in the reviewed literature to permit assessment of a most probable fate.

b. Because the solubility of this compound is relatively high, 50 percent or more may exist in true solution under conditions of normal sediment loading. Southworth (1979).

TABLE 6-9 SUMMARY OF AQUATIC FATE OF FLUORANTHENE

ENVIRONMENTAL PROCESS[a]	SUMMARY STATEMENT[b]	RATE	HALF-LIFE ($t_{1/2}$)	CONFIDENCE OF DATA[c]
Photolysis	Dissolved portion may undergo rapid photolysis.	-	-	
Oxidation	Oxidation of PAH by RO_2 radical is a slow process; not a significant process.			
Hydrolysis	PAHs do not contain groups amenable to hydrolysis.	-	-	
Volatilization	Is probably not as important as adsorption as a transport process.	-	-	
Sorption	Measured adsorption coefficients for PAH and suspended solids are high; movement via sediment considered to be an important transport process.	-	-	
Bioaccumulation	A short-term process; PAHs with 4 or less aromatic rings are readily metabolized and long-term partitioning into biota is not a significant fate process.	-	-	
Biotransformation/ Biodegradation	PAHs with 4 or more aromatic rings are degraded by microbes and are metabolized by multicellular organisms; biodegradation is probably the ultimate fate process.	2.2×10^{-3} μmol hr^{-1} mg^{-1} bacterial protein[d]	-	

a. There is insufficient information in the reviewed literature to permit assessment of a most probable fate.

b. Very little environmental fate data specific to fluoranthene were found; the summary statement is made from data reviewed for PAHs as a group.

c. Data on fluoranthene are not sufficient to permit confidence ranking. The confidence of the data reviewed for PAHs in general ranges from low to high.

d. Barnsley (1975).

TABLE 6-10 SUMMARY OF AQUATIC FATE OF PHENANTHRENE

ENVIRONMENTAL PROCESS[a]	SUMMARY STATEMENT[b]	RATE	HALF-LIFE ($t_{1/2}$)	CONFIDENCE OF DATA[c]
Photolysis	Dissolved portion may undergo rapid photolysis.	-	-	-
Oxidation Medium	Oxidation of PAH by RO_2 radical is slow; not a significant process.	0.01 1 $mol^{-1}sec^{-1}$	8×10^6 days	
Hydrolysis	PAHs do not contain groups amenable to hydrolysis.	-	-	-
Volatilization	Is probably not as important as adsorption as a transport process.	-	-	-
Sorption	Measured adsorption coefficients for PAH and suspended solids are high; movement via sediment considered to be an important transport process.	-	-	-
Bioaccumulation	A short-term process; PAHs with 4 or less aromatic rings are readily metabolized and long-term partitioning into biota is not a significant fate process.	-	-	-
Biotransformation/ Biodegradation	PAHs with 4 or more aromatic rings are degraded by microbes and are readily metabolized by multi-cellular organisms; biodegradation is probably the ultimate fate process.	-	-	-

a. There is insufficient information in the reviewed literature to permit assessment of a most probable fate.

b. Very little environmental fate data specific to phenanthrene were found; the summary statement is made from data reviewed for PAHs as a group.

c. Data on phenanthrene are not sufficient to permit confidence ranking in most dases. The confidence of the data reviewed for PAHs in general ranges from low to high.

TABLE 6-11 SUMMARY OF AQUATIC FATE OF BENZO(A)ANTHRACENE

ENVIRONMENTAL PROCESS[a]	SUMMARY STATEMENT	RATE	HALF-LIFE ($t_{1/2}$)	CONFIDENCE OF DATA
Photolysis	Dissolved portion may undergo rapid photolysis.	$\sim 6 \times 10^{-6}$ sec^{-1}	10-50 hrs	Medium
Oxidation	Oxidation of PAH by RO_2 radicals is slow; not a significant process.	$5 \times 10^{3} m^{-1}$ sec^{-1}	38 hrs	Medium
Hydrolysis	PAHs do not contain groups amenable to hydrolysis.	0	-	High
Volatilization	Is probably not as important as adsorption as a transport process.	8×10^{3} hr^{-1}	~90 hrs	Medium
Sorption	Measured adsorption coefficients for PAH and suspended solids are high; movement in sediment considered to be an important transport process.	Kp=26,200	- -	Medium
Bioaccumulation	A short-term process; PAHs with 4 or less aromatic rings are readily metabolized and long-term partitioning into biota is not a significant fate process.	-	-	Low
Biotransformation/ Biodegradation	PAHs with 4 or more aromatic rings are degraded by microbes and are readily metabolized by multi-cellular organisms; biodegradation is probably the ultimate fate process.	-	-	Low

a. There is insufficient information in the reviewed literature to permit assessment of a most probable fate.

TABLE 6-12 SUMMARY OF AQUATIC FATE OF BENZO(B)FLUORANTHENE, BENZO(K)FLUORANTHENE, AND CHRYSENE

ENVIRONMENTAL PROCESS[a]	SUMMARY STATEMENT[b]	RATE	HALF-LIFE ($t_{1/2}$)	CONFIDENCE OF DATA[c]
Photolysis	Dissolved portion may undergo rapid photolysis.			
Oxidation	Oxidation of PAH by RO_2 radical is slow; not a significant process.			
Hydrolysis	PAHs do not contain groups amenable to hydrolysis.			
Volatilization	Is probably not as important as adsorption as a transport process.			
Sorption	Measured adsorption coefficients for PAH and suspended solids are high; movement in sediment considered to be an important transport process for PAH.			
Bioaccumulation	A short-term process; PAHs with 4 or less aromatic rings are readily metabolized and long-term partitioning into biota is not a significant fate process.			
Biotransformation/ Biodegradation	PAHs with 4 or more aromatic rings are degraded by microbes and are readily metabolized by multicellular organisms; biodegradation is probably the ultimate fate process.			

a. There is insufficient information in the reviewed literature to permit assessment of a most probable fate.

b. Very little environmental fate data specific to benzo(b)fluoranthene, benzo(k)fluoranthene and chrysene were found; the summary statement is made from data reviewed for PAHs as a group

c. Data on benzo(b)fluoranthene, benzo(k)fluoranthene and chrysene are not sufficient to permit confidence ranking. The confidence of the data reviewed for PAHs in general ranges from low to high.

TABLE 6-13 SUMMARY OF AQUATIC FATE OF PYRENE

ENVIRONMENTAL PROCESS[a]	SUMMARY STATEMENT[b]	RATE	HALF-LIFE ($t_{1/2}$)	CONFIDENCE OF DATA[c]
Photolysis	Dissolved portion may undergo rapid photolysis.	-	-	
Oxidation	Oxidation of PAH by RO_2 radical is slow; not a significant process.	-	1,000 days	
Hydrolysis	PAHs do not contain groups amenable to hydrolysis.	-	-	
Volatilization	Is probably not as important as adsorption as a transport process.	-	-	
Sorption	Measured adsorption coefficients for PAH and suspended solids are high; movement in suspended sediment is considered to be an important transport process.	-	-	
Bioaccumulation	A short-term process; PAHs with 4 or less aromatic rings are readily metabolized and long-term partitioning into biota is not a significant fate process.	-	-	
Biotransformation/ Biodegradation	PAHs with 4 or fewer aromatic rings are degraded by microbes and are readily metabolized by multicellular organisms; biodegradation is probably the ultimate fate process.	-	-	

a. There is insufficient information in the reviewed literature to permit assessment of a most probable fate.

b. Very little environmental fate data specific to pyrene were found; the summary statement is made from data reviewed for PAHs as a group

c. Data on pyrene are not sufficient to permit confidence ranking. The confidence of the data reviewed for PAHs in general ranges from low to high.

TABLE 6-14 SUMMARY OF AQUATIC FATE OF BENZO(G,H,I)PERYLENE, DIBENZO(A,H)ANTHRACENE, AND INDENO(1,2,3-CD)PYRENE

ENVIRONMENTAL PROCESS[a]	SUMMARY STATEMENT[b]	RATE	HALF-LIFE ($t_{1/2}$)	CONFIDENCE OF DATA[c]
Photolysis	Dissolved portion may undergo rapid photolysis.			
Oxidation	Oxidation of PAH by RO_2 radical is slow; not a significant process.			
Hydrolysis	PAHs do not contain groups amenable to hydrolysis.			
Volatilization	Is probably not as important as adsorption as a transport process.			
Sorption	Measured adsorption coefficients for PAH and suspended solids are high; movement in sediment is considered to be the primary transport process.			
Bioaccumulation	A short-term process; PAHs with 4 or more aromatic rings are slowly metabolized and long-term partitioning into biota is not a significant fate process.			
Biotransformation/ Biodegradation	PAHs with 4 or more aromatic rings are slowly degraded by microbes and are metabolized by multi-cellular organisms; degradation is probably the ultimate fate process.			

a. There is insufficient information in the reviewed literature to permit assessment of a most probable fate.

b. Very little environmental fate data specific to benzo(a)perylene, dibenzo(a,h)anthracene and indeno(1,2,3-cd)pyrene were found; the statement is made from data reviewed for PAHs as a group.

c. Data on benzo(a)perylene, dibenzo(a,h)anthracene and indeno(1,2,3-cd)pyrene are not sufficient to permit confidence ranking. The confidence of the data reviewed for PAHs in general ranges from low to high.

TABLE 6-15 SUMMARY OF AQUATIC FATE OF BENZO(A)PYRENE

ENVIRONMENTAL PROCESS[a]	SUMMARY STATEMENT	RATE	HALF-LIFE ($t_{1/2}$)	CONFIDENCE OF DATA
Photolysis	Dissolved portion may undergo rapid photolysis.	$2.8 \times 10^{-4} sec^{-1}$	1-2 hrs.	Medium
Oxidation	Oxidation of PAH by RO radicals is slow; not a significant process.	$1.68 \times 10^{3} M^{-1} sec^{-1}$	96 hrs.	Medium
Hydrolysis	PAHs do not contain groups amenable to hydrolysis.	0	-	High
Volatilization	Is probably not as important as adsorption as a transport process.	$3 \times 10^{2} hr^{-1}$	22 hrs.	Medium
Sorption	Measured adsorption coefficients for PAH and suspended solids are high; movement in sediment is considered to be the most important transport process.	150,000	-	Medium
Bioaccumulation	A short-term process; PAHs with 4 or more aromatic rings are slowly metabolized and long-term partitioning into biota is not a significant fate process.	-	-	Medium
Biotransformation/ Biodegradation	PAHs with 4 or more aromatic rings are slowly degraded by microbes and are slowly metabolized by multi-cellular organisms; biodegradation is probably the ultimate fate process.	$0.2\text{-}0.9 \mu mol^{-1} mg^{-1}$ bacterial protein		Medium

a. There is insufficient information in the reviewed literature to permit assessment of a most probable fate.

References - Chapter 6

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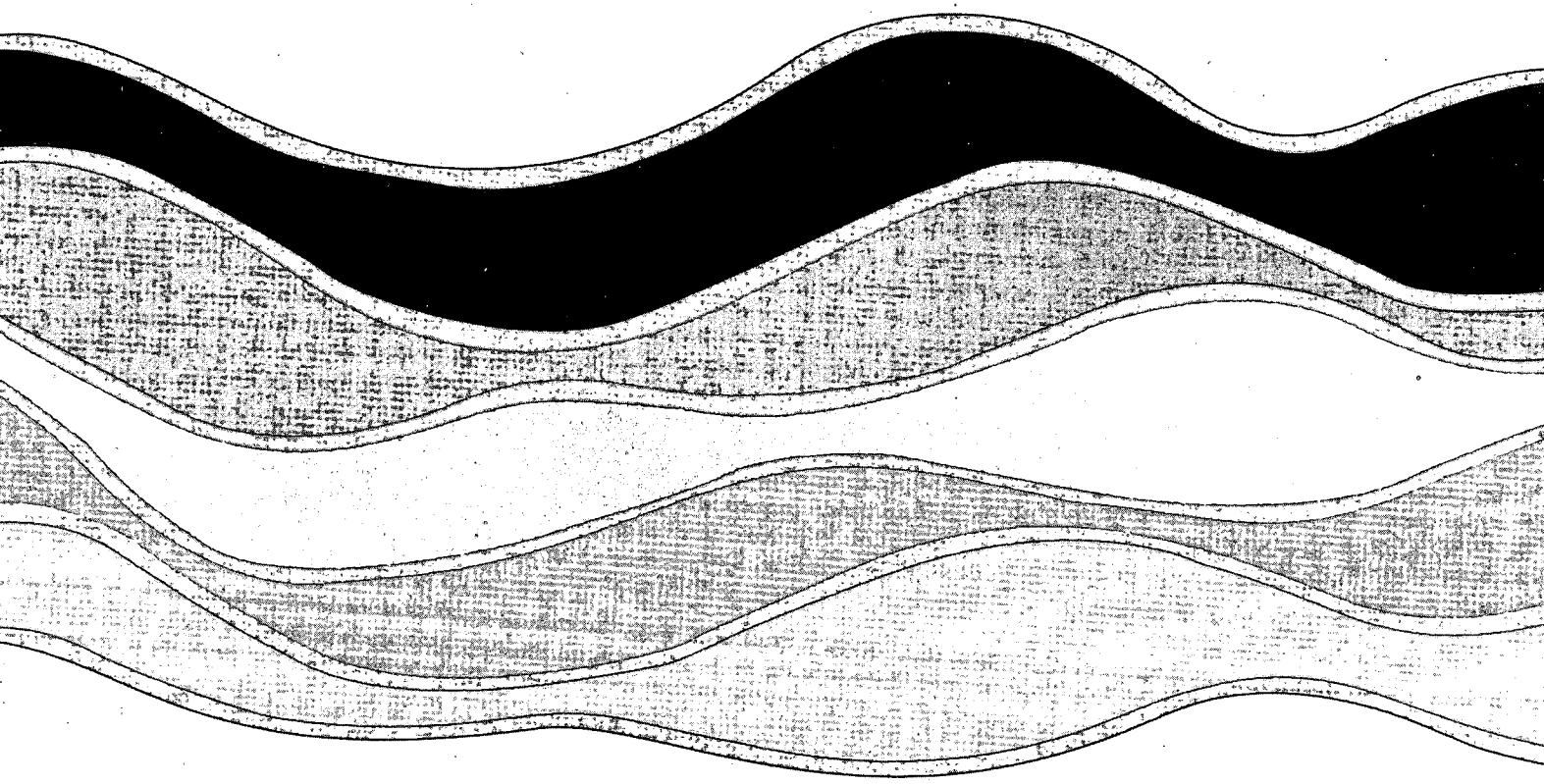

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