AN ECOLOGICAL PERSPECTIVE OF THE EFFECTS OF MONOCYCLIC AROMATIC HYDROCARBONS ON FISHES

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INTRODUCTION

Monocyclic aromatic hydrocarbons (MAH) constitute a major class of petrochemicals potentially affecting organisms in the aquatic environment, but they have not received much attention in pollution research. MAH are highly toxic and relatively water-soluble when compared with other classes of petroleum hydrocarbons. However, the assumption has been made for some time that they are so volatile they do not persist in the aquatic environment long enough to affect organisms. We think this assumption is unfounded, based upon inadequate field measurements, and arises from considering these compounds only in relation to oil spills when a single large input occurs. The potential chronic sources of these compounds and their effects on aquatic organisms, particularly in estuaries, have been largely ignored. In addition, most studies of monocyclic aromatics have been conducted in laboratories, testing the effects of high lethal and sublethal levels. The actual occurrence of these compounds in water and organisms in the field, and their potential effects at low chronic levels in relation to other environmental factors, are still relatively unknown.

This paper attempts to: 1) summarize present knowledge of the effects of monocyclic aromatics on fishes, 2) provide an ecological perspective of the effects of these compounds on

fishes, 3) hypothesize modes of action of monocyclic aromatics on fishes and, finally, 4) suggest directions of research needed to determine whether these compounds may represent a threat to our fisheries resources.

Most laboratory studies of the effects of monocyclic aromatics on fishes have been done at the National Marine Fisheries Service laboratories at Tiburon, California, Auke Bay, Alaska and Seattle, Washington and these studies are emphasized in the following discussion. The authors' experience with monocyclic aromatic compounds centers around our laboratory and field studies on fishes of the San Francisco Bay area. We realize that other ecosystems will differ on many points.

Measurements and observations made in our experiments have led us to hypothesize some modes of action of monocyclic aromatics on fishes. The discussion on their effects is placed in the context of these hypotheses. When studies substantiating our hypotheses are available, references are provided. It should be realized, however, that this synthesis is offered primarily to provide a research framework and does not necessarily imply that the hypotheses have been completely validated.

DEFINITION, PROBABLE SOURCES AND FATES OF MONOCYCLIC AROMATIC HYDROCARBONS

We do not intend to provide a comprehensive discussion of the chemistry of the monocyclic aromatic hydrocarbons (MAH), but for the benefit of the reader unfamiliar with these compounds, the following summary of their characteristics, sources and probable fates is provided.

Structure and Chemical Characteristics

Aromatic hydrocarbons are designated aromatic primarily because the earliest known representatives were distinguished by marked aromatic odors. Monocyclic aromatics are substances containing one benzene ring. The benzene ring is characterized by a cyclic arrangement of carbon and hydrogen atoms with a resonant bonding structure: an unsaturated, symmetrical ring of six equavalent CH groups (Gerarde, 1960). The simplest monocyclic compound is benzene itself, consisting of one ring, with no substitutions (Fig. 1). Other common MAH include toluene (one substituted methyl group), ethylbenzene (one substituted ethyl group) and the isomers of

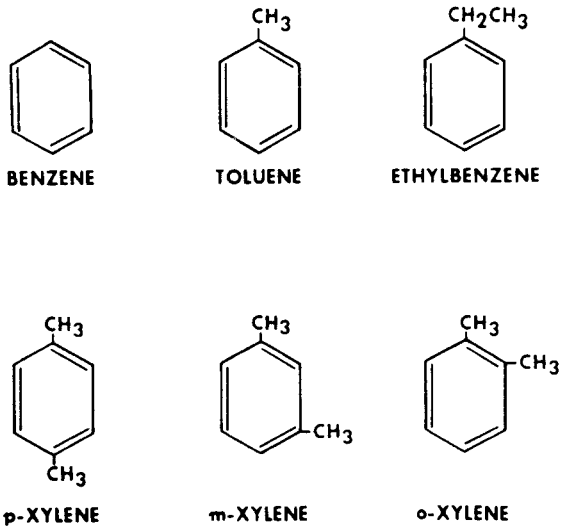


FIGURE 1. Six common monocyclic aromatic hydrocarbons. Unsymmetrical formulas (Kekulé) are used to represent the symmetrical molecules for simplicity.

xylene: *p*-xylene, *m*-xylene and *o*-xylene (three isomeric arrangements of two methyl groups). Some physical and chemical characteristics of these six monocyclics are summarized in Table 1. There are many other possible substituents on the benzene ring; these compounds will be referred to here, for purposes of simplification, as substituted benzenes.

MAH, for example benzene, are relatively soluble in water (Table 1) when compared with other petrochemicals. Since most research on the effects of MAH on fishes has been done with benzene and toluene, these particular compounds are most often discussed in this paper.

Potential Sources

Aromatic hydrocarbons have been used in industry for some time and benzene, toluene and xylene are three of the most important organic chemicals in industry. Recent data on the production of benzene, for example, showed an exponential increase in production in the last twenty years from less than 0.5 billion gallons in 1960 to about 1.5 billion gallons in 1979 (Davis and Magee, 1979).

The aromatic hydrocarbons obtained from petroleum and their derivatives come under the definition of petrochemicals-

TABLE 1. Physical - chemical properties of six prevalent monocyclic aromatic hydrocarbons. Data synthesized from Gerarde (1960) except where otherwise indicated.

Component	Molecular weight	Boiling point (°C) (760 mm Hg)	Melting point (°C)	Vapor pressure (mm Hg)	Index of refraction	Solubilities FW[a] 0 ppt (ppm)	Solubilities SW[b] 25 ppt (ppm)
Benzene	78.11	80.1	+ 5.5	74.6 (20 C)	1.502 (20 C)	2026	1400
Toluene	92.13	110.6	-94.5	36.7 (30 C)	1.489 (24 C)	595	330
Ethyl-benzene	106.16	136.2	-94.9	10.0 (25.9 C)	1.493 (20 C)	175	180
para-Xylene	106.16	138.3	-55.9	10.0 (27.3 C)	1.500 (21 C)		180
meta-Xylene	106.16	139.1	-54.2	10.0 (28.3 C)	1.497 (20 C)		210
ortho-Xylene	106.16	144.4	-13.3	10.0 (32.1 C)	1.506 (20 C)	195	230

[a] Fresh water - McAuliffe, 1966. 25°C and 0 ppt. Converted data to ml/liter.

[b] Sea water (estuarine) - Benville and Korn, 1977. 16°C and 25 ppt.

chemicals derived from petroleum or natural gas. The primary sources of MAH in the aquatic environment are from industrial petrochemicals and from crude oil. Some principal uses of MAH in industry are as: 1) starting materials and intermediates for synthesis of plastics, paints, pesticides, protective coatings, resins, dyes, drugs, flavors, perfumes, vitamins, explosives; 2) solvents for paints, dyes, resins, inks, lacquers, rubber, plastics and pesticides; and 3) constituents of aviation and automotive gasoline.

The MAH may also comprise from about 20 to 50% of the water-soluble fraction (WSF) of crude oil, depending upon the type of crude oil (Anderson *et al.*, 1974b; Clark and MacLeod, 1977). Some crude oils are more aromatic and toxic in that they contain higher proportions of aromatics including monocyclics.

More specifically, we believe a major source of MAH petrochemicals in the San Francisco Bay estuary is from their use in pesticide mixtures as synergists, inhibitors, solvents, emulsifiers, wetting agents, etc. (Wiens, 1977). Petroleum products are also applied directly for control of insects, mites, weeds and fungus. A computer summary of the top 50 pesticides in terms of total pounds (extracted from the 1978 pesticide use reports for California)(State of California, 1978; Jung and Bowes, 1980) shows that aromatic petroleum hydrocarbons are heavily used in the ten counties forming the watershed draining into the San Francisco Bay-Delta. Six of the top ten chemicals listed include categories such as "petroleum hydrocarbons, petroleum oil, petroleum distillates, xylene, aromatic petroleum solvents, xylene-range aromatic solvents and petroleum distilled aromatics", totaling about 5 million pounds applied to 1.3 million acres.

There are other major sources of petrochemicals in the San Francisco Bay-Delta area, including municipal and industrial discharges, particularly in the Carquinez Straits area, which is along a major fish migratory pathway and also an area where fish kills consistently occur in summer (Kohlhorst, 1973). In fact, municipal effluents are probably among the major sources of petroleum input to the Bay, discharging a total of at least 72,400 pounds per day of "oil and grease" (Risebrough *et al.*, 1978). Industrial discharges by refineries are apparently less-a total of 3,510 pounds per day (Risebrough *et al.*, 1978). The total oil and grease measurement does not include, however, a large proportion of the more toxic water-soluble fraction such as the aromatic hydrocarbon components and, thus, inadequately estimates the relative toxicity of the discharges (Wolfe *et al.*, 1979). Data from an API report (American Petroleum Institute, 1978) indicate that total MAH measured in refinery effluents can

range from only traces up to approximately 100 ppb. Data also indicate the concentration in intake water is sometimes higher than in the effluents. The latter fact suggests that there may be chronic water levels of MAH in the 100 ppb range. The refineries listed in this report, however, are not identified, and some San Francisco Bay refineries may have lower or higher effluent concentrations. In addition to refineries, there are many other industrial dischargers of petrochemicals. We need to examine the amounts and effects of discharges more carefully, and further measurements of toxic components in receiving waters are needed, not only in San Francisco Bay, but also in other estuaries.

Other sources of MAH in San Francisco Bay include the increasingly frequent spills associated with oil transport and transfer activities. Petroleum refineries in the Bay currently account for 3 to 4% of the total volume of crude oil transported yearly by tankers throughout the world. The refineries in the northern area of the Bay process on the order of a million barrels of crude oil daily (approximately 136 thousand tons) and the total volume processed yearly is about 50 million tons (Risebrough *et al.*, 1978).

A minor source of MAH is from recreational boating activity, with input of many toxic aromatics through outboard motor gasoline effluent. At times this activity is considerable (Hirsch *et al.*, ms in prep. (a)).

The relative contribution of various sources to the concentration of MAH in the receiving waters of San Francisco Bay has not yet been quantified, but a study has been initiated with the cooperation of the State of California Water Resources Control Board (Jung and Bowes, 1980; Whipple, 1979, 1980). Concentrations of individual MAH from other areas are given below.

Fate of Monocyclic Aromatics in the Aquatic Environment

Little is known about 1) the partitioning of MAH into various compartments of an aquatic ecosystem, 2) the rates of exchange among these compartments, or 3) the relative importance of various pathways. The entire subject needs further study for this class of compounds and will not be discussed in detail here.

Table 2 summarizes some separate studies on various fates of MAH in aquatic systems. There appears to be no study attempting to bring these pathways into a single model for this group of compounds. A comprehensive discussion of the fate of petroleum hydrocarbons, in general, is given in a review by Clark and MacLeod (1977) and in papers by Butler *et al.*, (1976), Gordon *et al.*, (1976) and Karrick (1977).

TABLE 2. Major pathways for the fate of monocyclic aromatics (MAH) in the aquatic environment. Rates probably vary with several environmental factors, including wind and wave action, temperature, and salinity.

Pathway	General conclusions	Selected references
Dissolution	Relatively soluble--152-1780 ppm in fresh water; 180-1400 ppm in seawater (Table 1).	Benville and Korn, 1977 Burwood and Speers, 1974 McAuliffe, 1966, 1977a Wasik and Brown, 1973
Evaporation	Rapid--from single spill a few hours to 10 days.	Gordon et al., 1976 Harrison et al., 1975 McAuliffe, 1977b
Atmospheric Input	Probably considerable.	Wiens, 1977
Concentration at Thermocline	Indication from vertical sampling profiles that volatile aromatics are at higher levels at the position of the thermocline.	Myers and Gunnerson, 1976
Emulsification Colloidal Dispersion	Little known.	Davis and Gibbs, 1975 McAuliffe, 1977a
Agglomeration Sorption Sinking Sedimentation	Monocyclics, including benzene, probably sorb to particulate matter; sorption of benzene to particulate matter in seawater was 4.8-28.4 µg/mg of particulates.	Zsolnay, 1972, 1977
Microbial Modification	In laboratory studies, monocyclics are biodegraded; including benzene, toluene, xylenes, tri- and tetra-methylbenzenes, alkylbenzenes, cyclo-alkyl benzenes. In the marine environment microbial degradation may be slower.	Gibson and Gibson et al., 1968 to 1977 (11 papers) Lee and Ryan, 1976 Marr and Stone, 1961
Photochemical Modification	There is preferential decomposition of aromatics.	Burwood and Speers, 1974 Hansen, 1977
Biological Ingestion and Excretion	Monocyclics are rapidly bioaccumulated from water to relatively high levels in adult fish and usually depurated rapidly. (Larval fish do not readily depurate, with high bioaccumulation resulting). Monocyclics are probably not bioaccumulated from food by fish.	See next few sections for references.

As previously discussed, MAH when compared to other petroleum hydrocarbons are relatively soluble in water. They also have high boiling points and vapor pressures (Table 1) and would be expected to volatilize from water relatively rapidly. This pathway has been assumed to predominate, with aromatics volatilizing too rapidly to affect aquatic biota. Monocyclics also undergo photooxidation and microbial modification. However, there is some evidence that MAH, e.g., benzene, are sorbed to sediments and possibly to organic aggregates. They may also be concentrated at thermoclines and haloclines. Reversing loss through evaporation, MAH may return to the water through atmospheric input. Finally, and most significantly, MAH do occur in the water and are taken up by aquatic organisms, including fish. The latter aspect of the fate of monocyclic aromatics is emphasized in this paper and discussed more fully below.

CONCENTRATIONS OF MONOCYCLIC AROMATICS IN WATER AND TISSUES OF FISH SAMPLED IN THE FIELD

In lakes, rivers and estuaries, such as San Francisco Bay, with continuous input of pollutants, there is some evidence that MAH, including benzene, are present (e.g., Brown *et al.*, 1979; Whipple, 1979) in both water and fish tissues. Table 3 summarizes some measurements of MAH in water samples taken from the field. As can be seen from these data, few measurements of individual monocyclic compounds have actually been made. Many measurements of aromatic hydrocarbons found in the literature, usually designated "total aromatics", do not include benzene and toluene, and usually, by most analytical methods, exclude ethylbenzene and xylenes.

One reason for the paucity of data on MAH may be the assumption that they are not present in sufficient concentration to be harmful. Another reason is that the measurement of these components requires special analytical techniques which have only recently become feasible for low concentrations. Most previous field measurements of petroleum hydrocarbons have been made of the alkane groups ($>C_7$) and a few aromatics with higher boiling points (polycyclic aromatic hydrocarbons, or PAH; Neff, 1979). The lower boiling point MAH are lost in the concentration step used in the analysis for polycyclic aromatics.

Table 3 includes only data where some estimate or measurement of the MAH is actually made. The range in open ocean waters appears to be from approximately 0.01 to 5 ppb. Closer to shore and in estuaries the range appears to be from

TABLE 3. Concentrations of monocyclic aromatics measured in the water column. Few data are available at the present time.

Location	Water Depth (meters)	No. of Samples	Hydrocarbon type	Hydrocarbon concentration (ppb)	References
Atlantic Ocean					
Skidaway River, GA	Surface	?	Benzene, toluene	3	Lee and Ryan, 1976
Tanker Route New York to Gulf of Mexico	Surface to 10	?	Total volatiles[a]	Med=0.1-0.3 Range=0.08-2.4	Myers and Gunnerson, 1976
Pacific Ocean					
San Francisco Bay	2	8	Total aromatics	≤5-59	DiSalvo and Guard, 1975
San Francisco Bay	Surface	5	Total 6 aromatics[b]	1-50	Benville (unpublished)
Tanker route San Francisco to Cook Inlet	0-10	?	Total volatiles	Med=0.1-0.3 Range=0.01-4.0	Myers and Gunnerson, 1976
GEOSECS					
Tanker routes	0-10	223	Total volatiles	Mean=0.22 Range=0.01-4.32	Brown and Huffman, 1976
Offshore oil seep Coal Oil Point, CA	Surface	10	Benzene Toluene	ND-50 ND-80	Koons and Brandon, 1975
Baltic Sea and approaches					
Open water	1-200	6	Saturateds and monoaromatics	48-64	Zsolnay, 1972
Open water	1-200	40	Saturateds and monoaromatics	0-50	Zsolnay, 1977
Refineries[c]					
Refinery intake water	--	40			
			Benzene	Trace-40	American Petroleum Institute, 1978
			Toluene	Trace-15	
			Ethylbenzene	Trace-20	
			Total	Trace-65+	
Refinery effluents	--	36			
			Benzene	Trace-30	American Petroleum Institute, 1978
			Toluene	Trace-60	
			Ethylbenzene	Trace-40	
			Total	Trace-90+	
Fresh water lakes and rivers					
Fox River, Illinois (Industrial river)	?	?	Benzene Toluene	100-200 100	Brown et al., 1979
Lake Chetek (Resort lake)	?	?	Benzene Toluene	8-9 90-100	Brown et al., 1979
Lake of the Woods, Canada (Pristine)	?	?	Benzene Toluene	ND Trace	Brown et al., 1979

[a] Total volatiles measured are comprised of 75% (benzene + toluene + xylenes); 25% other.
[b] Total six monocyclics: benzene, toluene, ethylbenzene and p-, m-, and o-xylenes.
[c] Unidentified: some presumably on estuaries.
ND = Not detected.

1 to 100 ppb, and in fresh water up to 200 ppb. Measurement units used for concentrations of MAH reported throughout this paper are as follows:

ppm=µl/L, ppb=nl/L in water; ppm=nl/g in tissues.

Tables 4 and 5 summarize types and concentrations of low-boiling point petrochemicals, including MAH, found in tissues of striped bass collected from the Carquinez Straits area of the San Francisco Bay-Delta (Whipple, 1979) during their upward spawning migration. As far as we know, there are no equivalent data for low-boiling point MAH for other fishes. Although traces of naphthalenes were present, few fish tissues examined contained significant levels of dicyclic aromatics. Other polycyclic aromatics (PAH) may be present, but tissue samples have not yet been analyzed for PAH. Our measurements of MAH in tissues of striped bass show surprisingly high concentrations (maximum in liver tissue=approx. 5.7 ppm; in ovary tissue=approx. 1.3 ppm; Table 5). The predominant monocyclic aromatic found is benzene. These concentrations closely approximate those resulting in tissues of fish exposed in the laboratory to 100 ppb of benzene (refer to Table 10). The initial data from field-captured fish are highly suggestive of a potential problem resulting from the occurrence of these compounds in a chronically polluted estuary, particularly in view of the effects observed at similar levels in laboratory studies.

RELATIONSHIP OF MONOCYCLIC AROMATIC HYDROCARBONS TO OTHER FACTORS IN THE AQUATIC ENVIRONMENT

As shown above, analyses of tissue samples from striped bass collected in the San Francisco Bay - Delta show the presence of MAH in relatively high concentrations (Whipple, 1979, 1980; Whipple *et al.*, 1979; Jung and Bowes, 1980). Although studies are still underway, we find that there are high correlations between the presence of these compounds and poorer condition of fish and their gametes. However, the cause and effect relationships between MAH and certain deleterious effects in the field-captured fish are as yet unclear, and laboratory tests are being performed in an attempt to clarify them.

Traditionally, much of the work on effects of petroleum hydrocarbons (including MAH) on aquatic organisms has been restricted to controlled laboratory studies, testing single compounds. This is primarily because of the difficulties in

TABLE 4. Low-boiling point petrochemicals scanned for in striped bass (*Morone saxatilis*) from San Francisco Bay; n=70 fish. Those compounds identified in striped bass liver tissue (total of 14 compounds) indicated with an asterisk. (Whipple, 1979, 1980; Whipple et al., 1979).

Aromatics	Alkyl cyclohexanes
*Benzene	*Methylcyclohexane
*Toluene	*1,4- and/or 1,1-Dimethylcyclohexanes
*Ethylbenzene	*1,2-Dimethylcyclohexane
*para-Xylene	
*meta-Xylene	
*ortho-Xylene	
Isopropylbenzene	
n-Propylbenzene	
*1, 3, 5-Trimethylbenzene	
1, 2, 4-Trimethylbenzene, tert-Butylbenzene	
1, 2, 3-Trimethylbenzene, sec-Butlybenzene	
Isobutylbenzene	
n-Butylbenzene, 1-Phenylbutene-2	
tert-Pentylbenzene	
1, 2, 4, 5-Tetramethylbenzene	
1, 2, 3, 5-Tetramethylbenzene	
1, 2, 3, 4-Tetramethylbenzene	
*Naphthalene (Trace only)	
Hexylbenzene	
Pentamethylbenzene	
2-Methylnaphthalene	
*1-Methylnaphthalene (Trace only)	
n-Heptylbenzene	
Hexamethylbenzene	
n-Octylbenzene, n-Nonylbenzene	
Fluorene	
n-Decylbenzene	

TABLE 5. Summary of low-boiling point petroleum hydrocarbons in liver and gonads[a] in striped bass (*Morone saxatilis*). Prespawning adults; n=70; gonads maturing (Whipple, 1979; Whipple et al., 1979)

	Mean samples w/hydrocarbons (ppm-WW)	No. fish w/hydrocarbons detectable	No. fish without hydrocarbons (ND)	Total no. fish analyzed	Range in mean Low	Range in mean High (ppm-WW)
Liver tissue						
Females						
Total monocyclic aromatics[b]	0.430	46	10	56	0.020	3.285
Total alkyl cyclohexanes[c]	0.202	40	16	56	0.020	1.188
Total both	0.606	48	8	56	0.020	3.481
Males						
Total monocyclic aromatics	2.338	9	5	14	0.020	5.735
Total alkyl cyclohexanes	0.648	7	7	14	0.020	1.567
Total both	2.894	10	4	14	0.020	7.302
Gonadal tissue						
Females - Ovaries						
Total monocyclic aromatics	0.088	26	30	56	0.020	1.311
Total alkyl cyclohexanes	0.113	10	46	56	0.020	0.202
Total both	0.131	30	26	56	0.020	1.408
Males - Testes						
Total monocyclic aromatics	0.576	3	11	14	0.020	1.687
Total alkyl cyclohexanes	Trace	2	12	14	(Trace only)	
Total both	0.589	3	11	14	0.020	1.707

ND = not detectable

[a] Data from 1978; fish from 1979 and 1980 being analyzed.

[b] Total monocyclic aromatics = Benzene + toluene + ethylbenzene + m-xylene + o-xylene; p-xylene undetectable.

[c] Total alkyl cyclohexanes = 1,4-dimethylcyclohexane + 1,1-dimethylcyclohexane + 1,2-dimethylcyclohexane.

testing complex mixtures of compounds such as occur in crude oil and petroleum fractions. It has been pointed out by Sprague (1971) and many others that laboratory studies are not necessarily indicative of what actually happens in the natural environment. The effect of any pollutant, or class of pollutants such as the MAH, on a fishery's population must ultimately be considered in relation to many other interacting variables before we can estimate its relative contribution to declines in the fishery. These variables may be naturally occurring (e.g., salinity) and/or man-introduced (e.g., pesticides). Fishes often migrate into or through estuaries with chronic levels of several different pollutants, probably interacting additively and/or synergistically with MAH and with natural factors.

An example of the probable interactions of various environmental factors affecting fish is shown in the conceptual model of Figure 2. This figure shows a portion of a qualitative model for the striped bass population in the San Francisco Bay - Delta, and postulates the effective variables and

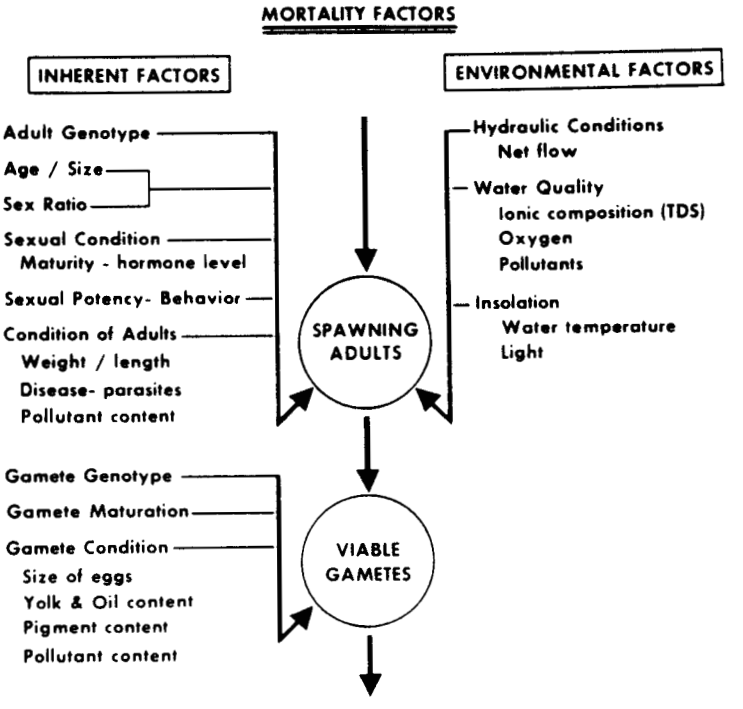


*FIGURE 2. Conceptual model of factors affecting mortality in striped bass (*Morone saxatilis*) during the spawning adult and gamete life history stages (from Whipple, 1979, 1980; Whipple* et al., *1979).*

their interactions affecting survival (or alternatively growth or reproduction) of a given life history stage (Whipple, 1979, 1980; Whipple *et. al.*, 1979). The stages shown in the figure are the spawning adults and their gametes. We believe these stages are among the most sensitive to the effects of pollutants. For this population, we are hypothesizing that pollutants, including MAH, are interacting with other environmental variables to affect adults and their gametes. Our ultimate goal is to make this model quantitative and predictive, determining the relative contribution of pollutants to reducing survival, growth and reproduction in the population considered. Very little has been done to quantify pollutant effects on a population in the actual environment where pollutants may be a probable factor in population persistence.

Although we would like ultimately to be able to predict effects of MAH on fishes in the aquatic environment, until further field and laboratory work has been done on their interaction with other factors, such prediction will be difficult. Data are available, however, from several studies on bioaccumulation and effects of MAH in fishes (primarily benzene and toluene). These studies enable us to make some preliminary predictions on the effects of MAH on fishes in the natural environment.

One of the difficulties in using data available from laboratory experiments to predict effects in the field, is that studies have been done under variable conditions. For example, we believe that fishes possess considerable interspecific and intraspecific inherent variability in their responses to pollutants, and environmental variability complicates this situation further. Table 6 lists some selected variables which we hypothesize will influence bioaccumulation of MAH in both field and laboratory studies and, subsequently, effects of these compounds on fishes. The point of listing these sources of variability is to provide a framework for future laboratory studies, where variation can be controlled or eliminated.

Further discussion of the influence of the variable listed in Table 6 on uptake, retention and depuration of MAH follows.

UPTAKE AND ACCUMULATION OF MONOCYCLIC AROMATIC HYDROCARBONS

The major route of uptake for MAH in fishes is probably through the water column, although this is not the only potential source of exposure to MAH (Table 6). Few studies exist comparing the routes of uptake and their relative importance in the bioaccumulation of MAH in fishes. We have done studies comparing the bioaccumulation of benzene in Pacific herring

TABLE 6. Some inherent and environmental variables affecting uptake, metabolism, retention and effects of MAH on fishes. Some factors (*) are still hypothetical, although currently being tested (Whipple, 1979, 1980; Whipple et al., 1979).

Major Inherent Factors	Major Environmental Factors
Interspecific variability (Table 9)	Source of exposure (Tables 7, 8) Water, food, particulates, or sediments, or combinations of these
Sex (Table 5)	Type of component or component mixture (Table 11)
Life history stage (Tables 7, 8, 9, 11)	Concentration, length of exposure (Tables 9, 11)
*Intraspecific genotypic variability Metabolism - functional Structural	
	Temperature (Table 11)
*Condition when exposed Degree of disease or parasitism Previous feeding regime - amount of fat Existing pollutant load	Salinity (Table 11)
	Alkalinity - pH (Table 11)
	*Presence of other pollutants Chlorinated hydrocarbons Heavy metals Others

Laboratory experiments can reduce this variability to a considerable degree by controlling (fixing) the variability and thus maximizing the variation due to effects of test (treatment) components.

In field experiments, more variables are uncontrollable and sample sizes must be larger, to reveal differences between exposed and unexposed populations.

larvae exposed from the water only,, food only (contaminated rotifers) and water and food together (Eldridge and Echeverria, 1977). We have also compared the uptake and bioaccumulation of the components in the WSF of Cook Inlet crude oil in starry flounder exposed through water only, food only (contaminated clams) and both water and food (Whipple *et al.*, 1978a; also Yocom *et al.*, ms in prep.). Summaries of concentrations accumulated from different exposure sources are shown in Tables 7 and 8. Our general conclusion from studies done to date is that only trace amounts of MAH are taken up from contaminated food and that, in fish, bioaccumulation of these compounds probably does not occur from food. There are no studies of the potential bioaccumulation from other sources (e.g., particulates).

A number of studies (discussed below), however, show that MAH are readily and rapidly bioaccumulated from water. We believe that this is the major route of uptake in the field, and that the source of exposure is most likely to be a low concentration (1-100 ppb, occasionally higher) of MAH occurring chronically in polluted estuaries.

The rates of uptake of MAH, particularly benzene, are very rapid. Figure 3 shows uptake of benzene in the blood of striped bass exposed to approximately 1 ppm benzene (Benville *et al.*, 1980). Other studies show that uptake to maximum equilibrium levels in most tissues is reached within 2-24 hrs (Korn *et al.*, 1976a, 1977). Maximum levels in organs involved with metabolism of MAH are reached later (48-72 hrs). In longer exposures, such as in starry flounder exposed to the WSF of Cook Inlet crude oil in the laboratory, the order of magnitude of accumulation remained the same over the entire exposure period once equilibrium levels were reached (Figure 4). There was some indication of increased accumulation at about five weeks.

Tables 9 and 10 summarize the concentrations and bioaccumulation of benzene and toluene in fishes. Data are summarized to compare species, life history stages and tissues. Most data are from laboratory experiments (L); however, some field data (F) are also included for comparison.

Test concentrations of single components (benzene and toluene) varied from 1 ppb to approximately 10,000 ppb (0.001 - 10.000 ppm). The 10,000 ppb (10 ppm) level was near lethal for some species. Most test concentrations of both single components and total MAH, however, were approximately 100 ppb and were chosen to approximate field chronic levels. Data from field-captured striped bass (F) show that maximum accumulation of benzene is comparable to that in striped bass exposed to approximately 100 ppb in the laboratory. Except at the lowest and highest test concentrations (1 ppb and 10,000 ppb), the order of magnitude of accumulation in juvenile and

TABLE 7. Mean maximum concentrations of benzene and/or metabolites in herring larvae (*Clupea harengus pallasii*) exposed to ^{14}C-benzene in seawater and/or in food (rotifers; *Brachionus plicatilis*) for 72 hours (Eldridge and (Echeverria, 1977).

Initial Mean Benzene Concentration (ppm-µl/L)	Concentrations in Tissues - ppm; nl/g Wet Weight		
	Food • Water Exposure	Water Only	Food Only
0.144 - 2.10		0.49 - 8.16	
1.20	3.98		
1.20 (rotifers)			0.310

TABLE 8. Mean concentrations of different classes of petroleum hydrocarbons in some tissues of starry flounder (*Platichthys stellatus*) exposed to a total of 0.115 ppm MAH in the WSF of Cook Inlet crude oil and/or 8.0 ppm MAH in contaminated clams (*Tapes semidecussata*) for five weeks in the laboratory (Whipple et al., 1978a; also Yocom et al., ms in prep.). Control group (not shown) contained no detectable components.

Component Class Totals	Concentrations in Tissues (ppm; µg/g-WW)					
	Food • Water Exposure		Water Only		Food Only	
	Mean	n	Mean	n	Mean	n
Liver						
Alkyl cyclohexanes	26.45	22	30.38	20	1.22	21
*Monocyclic aromatics	19.53	22	21.44	20	0	21
Higher substituted benzenes	23.89	14	16.17	15	0	13
Dicyclic aromatics	10.15	14	8.37	15	0	13
Muscle						
Alkyl cyclohexanes	0.88	23	0.50	24	0	18
Monocyclic aromatics	1.04	23	0.60	24	0	18
Higher substituted benzenes	0.92	20	2.03	22	trace m-xylene	8
Dicyclic aromatics	0.30	20	0.31	22	0	8

*Monocyclic aromatics = Six most common in WSF of Cook Inlet Crude Oil; benzene, toluene, ethylbenzene, o-xylene, m-xylene, p-xylene.

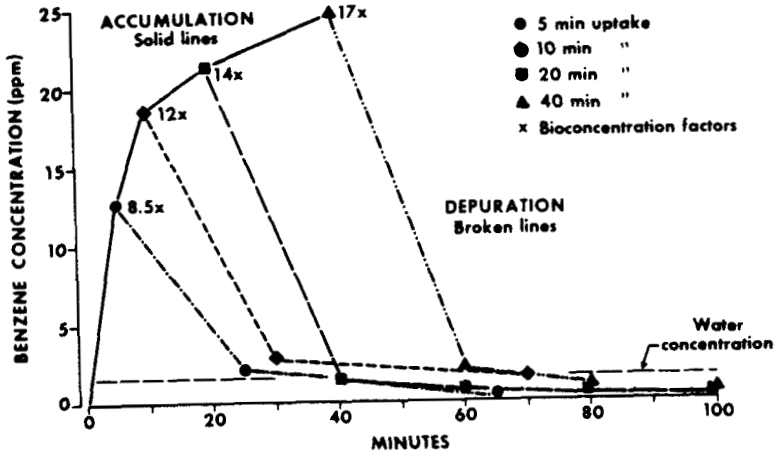


*FIGURE 3. Uptake, accumulation and depuration of benzene in blood of striped bass (*Morone saxatilis*) exposed to approximately 1.0 ppm benzene through the water column for one hour (from Benville* et al., *1980).*

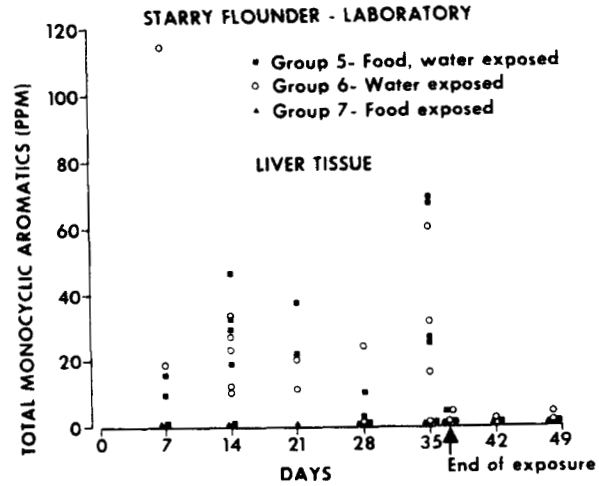


*FIGURE 4. Uptake, accumulation and depuration of six common monocyclic aromatic hydrocarbons in the WSF of Cook Inlet crude oil in the liver tissue of starry flounder (*Platichthys stellatus*). Exposed to 0.100 ppm total MAH in the WSF of Cook Inlet crude oil through the water column for five weeks (from Yocom* et al., *ms in prep.; Whipple* et al., *1978a).*

adult fishes was the same in most species (water concentration from 10 to 5,000 ppb), being about 10 to 100 times the water concentration (8.3 to 140 X). Maximum accumulation of benzene, as a part of the WSF of crude oil, appeared to be of the same order of magnitude as of benzene alone. Maximum accumulation of total MAH in the WAF appeared to be approximately 100 times water concentrations in laboratory studies (1 to 350 X). Toluene accumulated to higher levels than did benzene in most laboratory studies, depending upon tissue.

When tissues and species were compared (Table 10), northern anchovies accumulated the highest concentrations, probably because these fish were more easily stressed by laboratory conditions. Lowest accumulation occurred in Pacific herring. The other species had approximately the same maximum accumulations.

Accumulation of MAH varied with sex and proximity of males and females to spawning (Whipple, 1979). In striped bass during spawning season, benzene and other MAH were approximately equally distributed between liver and ovaries in females. Accumulation in males was higher in liver and levels in testes were usually undetectable, some with a trace of toluene. During the nonspawning season, levels were higher in female tissues, including liver, than in male. The levels in liver and gonads were probably largely determined by the amount of stored lipid, with increasing amounts of lipid being transferred from the liver to the ovaries during vitellogenesis. A corresponding change apparently does not occur in males during the spawning season. The rest of the year, female livers are higher in lipid than male livers. When data are pooled for all species and stages of juveniles and adults, females show higher accumulation (40 X) than males (26 X).

Differences among life history stages for Pacific herring, striped bass, starry flounder and coho salmon are also summarized in Table 9. Accumulation in early life history stages of striped bass was higher than in Pacific herring and coho salmon, particularly in feeding larvae. Maximum accumulation of ^{14}C-benzene and metabolites occurred in feeding larvae of striped bass (1400 X at 384 hrs and still increasing). Gonadal eggs also reached fairly high levels when maturing (14-29 X). Generally, in striped bass, the different stages bioaccumulate in the following, increasing order: yolk-sac larvae, nonspawning adults and juveniles, gonadal eggs, spawned eggs, prespawning adults, nonfeeding post yolk-sac larvae and feeding post yolk-sac larvae. In starry flounder, the order of increasing bioaccumulation is as follows: immature ovaries, juveniles, nonspawning adults,

TABLE 9. Summary of mean concentrations and bioaccumulations of benzene and toluene in different species and life history stages. Most experiments were at an exposure level of approximately 0.100 ppm.

Variable	Water exposure level (ppm)	Benzene: Range - Mean maximum concentrations[a] (ppm)	Benzene: Maximum accumulation factor[b]	Benzene: Hours to maximum accumulation	Toluene: Range - Mean maximum concentrations[a] (ppm)	Toluene: Maximum accumulation factor[b]	Toluene: Hours to maximum accumulation	References
Species and Life history stages								
Starry flounder								
Gonadal eggs								
Immature	L 0.133 B[c]							
	L 0.088 T[c]	ND	No accum.	--	0.200-0.830	9.4x	504	Whipple et al., 1978b
Mature	L 0.047 B[c]							
	L 0.056 T[c]	1.18-1.86	39x	72-96	2.16 - 5.21	93x	72-96	Whipple et al., 1978b
Juveniles	L 0.133 B[c]							
	L 0.088 T[c]	4.61-18.60	140x	504	14.24-34.71	395x	504	Whipple et al., 1978b
Nonspawning adults	L 0.041 B[c]							
	L 0.043 T[c]	0.130-4.19	100x	840	0.420-8.90	205x	840	Yocom et al., ms in prep.; Whipple et al., 1978a
Prespawning adults	L 0.047 B[c]							
	L 0.056 T[c]	1.44-1.57	33x	72-96	3.19-4.61	82x	72-96	Whipple et al., 1978b
Coho salmon								
Spawned eggs	*L 1.80 T				8.10	4.5x	unknown	Korn and Rice, in press
Alevins	*L 1.80 T				2.70	1.5x	unknown	Korn and Rice, in press
Emergent fry	*L 1.80 T				6.70	3.7x	unknown	Korn and Rice, in press
Pacific herring								
Gonadal eggs								
Immature	*L 0.100 B							
	*L 0.100 T	0.240	2.4x	24	0.440	4.4x	6	Korn et al., 1977
Mature	*L 0.100 B	1.40	14.0x	24				Struhsaker, 1977
Spawned eggs	*L 0.100 B	0.600	6.0x	12				Struhsaker, 1977
Feeding larvae								
Fed	*L 0.100 B	0.700	7.0x	not rchd 72				Struhsaker, 1977
Fed	*L 0.140 B	0.250	1.8x	not rchd 72				Eldridge and Echeverria, 1977
Not fed	*L 0.144 B	0.230	1.6x	6				Eldridge and Echeverria, 1977
Nonspawning adults	*L 0.100 B							
	*L 0.100 T	0.830	8.3x	6-48	3.90	39x	6-72	Korn et al., 1977

Variable	Water exposure level (ppm)	Benzene: Range - Mean maximum concentrations[a] (ppm)	Benzene: Maximum accumulation factor[b]	Benzene: Hours to maximum accumulation	Toluene: Range - Mean maximum concentrations[a] (ppm)	Toluene: Maximum accumulation factor	Toluene: Hours to maximum accumulation	References
Striped bass								
Gonadal eggs (mature)	F (?)[d]	Trace only	No. accum.	chronic	Tr.-0.330	(3.3x)[d]	chronic	Whipple, 1979, 1980
Spawned eggs	*L 0.138	4.512	33x	33				Eldridge and Benville, ms in prep.
Yolk-sac larvae	*L 0.415	4.674	11x	120				Eldridge and Benville, ms in prep.
Feeding larvae								
Fed	*L 0.159	226.155	1400x	not rchd 384				Eldridge and Benville, ms in prep.
Not fed	*L 0.159	73.663	460x	360				Eldridge and Benville, ms in prep.
Juveniles	*L 0.088	1.302	15x	6				Korn et al., 1976a
Nonspawning adults	L 0.095	4.35	46x	336				Hirsch et al., ms in prep. (b)
	F(0.100)[d]	Tr.-4.58	(46x)[d]	chronic	0	No toluene, only benzene		Whipple et al., ms in prep.
Prespawning adults	L 0.750	2.0-20.0	27x	336				Hirsch et al., ms in prep. (b)
	F(0.100)[d]	Tr.-2.27	(23x)[d]	chronic	Tr.-0.330	?	chronic	Whipple et al., ms in prep.

*Indicates labeled compounds (accumulation of ^{14}C-benzene or ^{14}C-toluene + their metabolites, if any).
L = accumulation in laboratory-exposed fish. B = benzene.
F = accumulation in field-captured fish. T = toluene.
[a]Measurement of uptake in bile not included in mean maximum concentrations.
[b]Maximum accumulation factor = Mean maximum concentration of MAH in tissues divided by mean concentration in water column.
[c]WSF of Cook Inlet crude oil.
[d]If comparable to laboratory accumulation, approx. 0.100 ppm benzene in field.

TABLE 10. Summary of mean maximum concentrations and bioaccumulation of benzene in different species, tissues and sexes. Most experiments were at an exposure of approximately 100 ppb.

Tissues - Species[a]		Water Exposure Level (ppm)	Mean Maximum Concentration (ppm)	Maximum Accumulation Factors (Increasing order)
Gonads (Immature)				
Pacific Herring (A)	(*)	0.100	0.240	2.4 x
Striped Bass (A)	(-)	0.320	Trace	None
Stomach				
Striped Bass (J)	(*)	0.088	0.24	2.7 x
Testes (Mature)				
Striped Bass (A)	(-)	0.320	1.09	3.4 x
Heart				
Striped Bass (J)	(*)	0.088	0.26	2.9 x
Striped Bass (A)	(-)	0.320	1.82	5.7 x
Kidney				
Pacific Herring (A)	(*)	0.100	0.40	4.0 x
Striped Bass (A)	(-)	0.320	4.16	13 x
Pyloric Cecae				
Pacific Herring (A)	(*)	0.100	0.64	6.4 x
Colon				
Striped Bass (J)	(*)	0.088	1.32	15 x
Spleen				
Striped Bass (A)	(-)	0.320	6.40	20 x
Adrenal Gland				
Striped Bass (A)	(-)	0.320	6.72	21 x
Eye				
Striped Bass (A)	(-)	0.320	7.68	24 x
Blood				
Striped Bass (A)	(-)	0.320	8.64	27 x
Ovaries (Mature)				
Pacific Herring (A)	(*)	0.100	1.40	14 x
Striped Bass (A)	(-)	0.100	2.90	29 x
Muscle				
Striped Bass (J)	(*)	0.088	0.10	1.1 x
Pacific Herring (A)	(*)	0.100	0.63	6.3 x
No. Anchovy (A)	(*)	0.110	1.10	10 x
Striped Bass (A)	(-)	0.320	0.48	1.5 x

Table 10 con't.

Gills				
Striped Bass (J)	(*)	0.088	0.49	5.6 x
Pacific Herring (A)	(*)	0.100	0.73	7.3 x
No. Anchovy (A)	(*)	0.110	4.60	42 x
Brain				
Striped Bass (J)	(*)	0.088	0.63	7.2 x
Pacific Herring (A)	(*)	0.100	0.75	7.5 x
No. Anchovy (A)	(*)	0.110	4.60	42 x
Striped Bass (A)	(-)	0.320	7.36	23 x
Liver				
Striped Bass (J)	(*)	0.088	0.86	9.8 x
Pacific Herring (A)	(*)	0.100	0.53	5.3 x
No. Anchovy (A)	(*)	0.110	6.01	55 x
Striped Bass (A)	(-)	0.320	6.72	21 x
Mesenteric Fat				
Striped Bass (A)	(-)	0.320	35.0	109 x
Intestine				
Striped Bass (J)	(*)	0.088	0.48	5.4 x
Pacific Herring (A)	(*)	0.100	0.83	8.3 x
No. Anchovy (A)	(*)	0.110	22.9	210 x
Gall Bladder (Bile)				
Pacific Herring (A)	(*)	0.100	3.10	31 x
Striped Bass (J)	(*)	0.088	4.70	53 x
No. Anchovy (A)	(*)	0.110	480.	4400 x
Striped Bass (A)	(-)	0.320	3.80	12 x

[a]References: Korn et al., 1976a, 1977; Hirsch et al., ms. in prep.(b); Struhsaker, 1977.

(-) Benzene not labeled; (*) ^{14}C-Benzene and/or metabolites; (A) = Adult, (J) = Juvenile.

prespawning adults and mature ovaries (egg and larval stage studies incomplete). Alevins of coho salmon accumulated more than spawned eggs or emergent fry.

Tissues accumulating highest levels were those involved in metabolism of compounds (^{14}C-benzene and metabolites measured) and/or contained higher levels of lipids. The tissues are arranged in order of minimum to maximum accumulations, the least uptake occurring in testes and the highest in bile (Table 10). In muscle, liver and bile, measurements were made of both 1) ^{14}C-benzene and metabolites and 2) benzene only. In the case of bile, a considerably higher proportion of the measured concentration appears to be metabolites.

The uptake of benzene and toluene as a part of the WSF of Cook Inlet crude oil by starry flounder appeared to be slightly higher than uptake and accumulation of the individual compounds, e.g., benzene in other species (Table 9). This could be a species difference, however.

Figure 5A is a gas chromatogram showing the occurrence

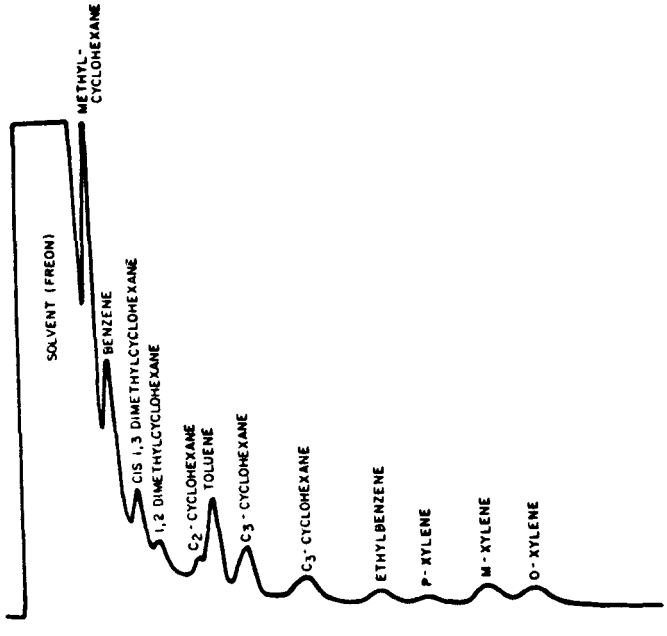


*FIGURE 5A. Chromatogram of low-boiling point hydrocarbons, including monocyclic aromatic hydrocarbons, detected in a maturing ovary of starry flounder (*Platichthys stellatus*). Exposed to 0.115 ppm total MAH in the WSF of Cook Inlet crude oil for seven days (from Whipple* et al., *1978b).*

of the low-boiling point compounds of methylcyclohexanes and monocyclic aromatics in maturing ovarian tissue of starry flounder exposed to the WSF of Cook Inlet crude oil (Whipple *et al.*, 1978b). Highest concentrations were of methylcyclohexane and benzene, but relative accumulations differed because of the much lower concentrations of xylene in the water. Figure 5B shows the relative uptake of different classes of hydrocarbons in the WSF of Cook Inlet crude oil in ovaries of starry flounder. Concentrations of the six commonest monocyclics (M-1) were highest, followed by methylcyclohexanes (CH), other substituted benzenes (M-2), and finally by dicyclic aromatic hydrocarbons (D). Although the MAH accumulate in fishes to relatively high concentrations from the WSF, the relative toxicities of these classes of compounds differ and effects cannot be determined on the basis of concentration alone.

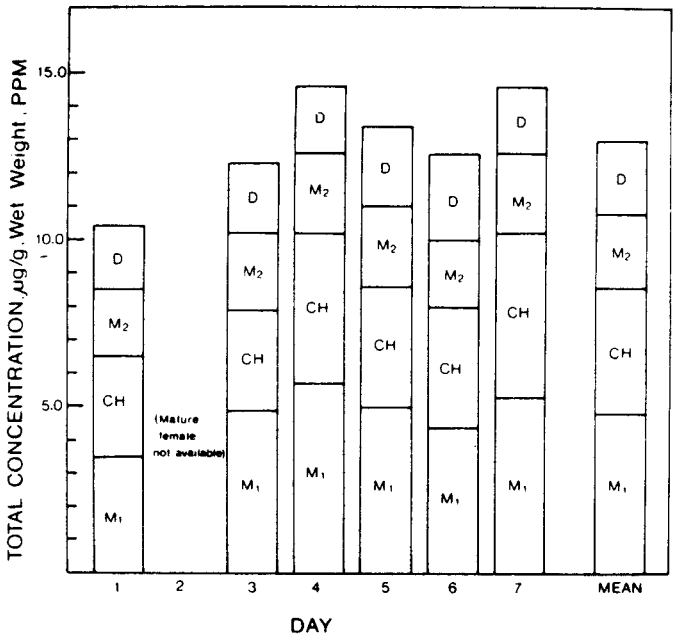


FIGURE 5B. Concentrations of monocyclic aromatics and other compounds in maturing ovaries of starry flounder (Platichthys stellatus). *M1 = six common monocyclics (benzene, toluene, ethylbenzene,* p-, m-, *and* o-*xylenes); CH = total alkyl cyclohexanes; M2 = total higher substituted benzenes; D = total dicyclic aromatics. Exposed to 0.115 ppm total MAH in the WSF of Cook Inlet crude oil for seven days (from Whipple* et al., *1978b).*

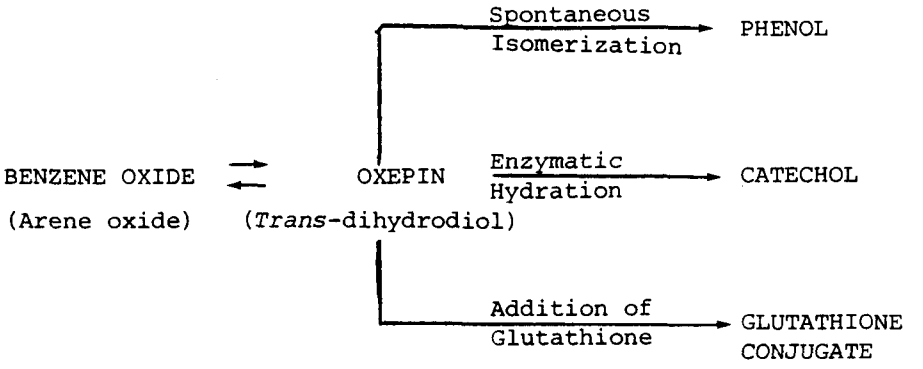
METABOLISM

Although we know that uptake of MAH from water is very rapid, and that bioaccumulation above water concentrations occurs, we still do not know much about what happens to these compounds within the fish. A considerable amount of the MAH taken up is probably returned to the water through the gills, unchanged. Some, however, is probably metabolized (Korn *et al.*, 1976a, 1977).

Fish have oxidative enzyme systems for the metabolic detoxification of xenobiotics, including the aromatic petroluem hydrocarbons (Payne and Penrose, 1975). They are associated with microsomes in the endoplasmic reticulum of cells. These enzymes are NADPH-dependent, and called aryl hydrocarbon hydroxylases (AHH). This area of research is relatively new (1960's to present) and information on the metabolic detoxification of petroleum hydrocarbons in fish is still sparse. Research on mammals is summarized in the papers of LaDu *et al.* (1971), White *et al.* (1973), Jerina and Daly (1974) and Kappas and Alvares (1975). Recently, considerable work has been done on the metabolism of polycyclic aromatics (PAH) in fishes (Lee, 1976, 1977; Stegeman and Sabo, 1976; Malins, 1977a, 1977b; Neff, 1978a, 1978b, 1979; Varanasi and Malins, 1977). There is still little known, however, about the metabolism of MAH in fish. Gibson (1977) summarized the differences in the metabolic processes used by eucaryotic and procaryotic organisms to oxidize aromatic hydrocarbons, including monocyclic aromatics such as benzene and toluene. Bacteria apparently oxidize MAH to dihydrodiol intermediates, followed by formation of catechols. The catechols are then substrates for the enzymatic cleavage of the aromatic ring. Fungi and higher organisms, on the other hand, incorporate oxygen into the aromatic ring to form arene oxides. The oxides undergo enzymatic addition of water to yield *trans*-dihydrodiols. These, in turn, are transformed into phenol, catechols and glutathione conjugates, according to Jerina and Daly (1974).

Most of the studies indicate a basic similarity of aromatic detoxification in mammals and fishes, including evidence that the mixed function oxidases (MFO) are induced in fish exposed to petroleum (Stegeman and Sabo, 1976; Payne and Penrose, 1975).

Jerina and Daly (1974) summarized the hepatic metabolism of benzene in mammals. Studies show that under physiological conditions (*in vitro*), benzene oxide undergoes spontaneous rearrangement to form phenol and react nonenzymatically with the thiol group of glutathione. Benzene oxide also undergoes enzymatic hydration to catechol. The general reactions are as follows:



The rate of metabolism of benzene *in vitro* was relatively low and the arene oxides of benzene and alkylbenzenes very unstable. The hepatic metabolism of toluene and other aromatics is also described in Jerina and Daly (1974). The hepatotoxicity of these compounds in mammals is apparently caused by the arene oxides being covalently bound to hepatic protein, eventually resulting in necrosis. Addition of glutathione inhibits this process.

We do not know if the same process of metabolism of benzene occurs in fishes, but similarities in the MFO system suggest that it does. Roubal *et al.* (1977) compared the accumulation and metabolism of ^{14}C-labeled compounds (benzene, naphthalene and anthracene) in young coho salmon exposed through food and intraperitoneal injection. Although the metabolites of benzene were not identified, the results show that relative to naphthalene and anthracene, a lower proportion of the ^{14}C-activity of labeled benzene appeared in the form of metabolites at 24 hours in the tissues measured (brain, liver, gall bladder, flesh and carcass). Overall, the accumulation of ^{14}C-labeled benzene and metabolites was less than for ^{14}C-labeled naphthalene and anthracene. The paper also indicated that for naphthalene and anthracene, maximum ^{14}C-activity levels were lower and depuration slower when labeled compounds were administered via food as opposed to injection.

Studies of striped bass juveniles indicated that in liver and muscle tissue approximately half of the ^{14}C-labeled compounds were unchanged benzene and half in the form of metabolites when compared to gas chromatographic measurements of unchanged benzene (Korn *et al.*, 1976a, 1977; see also Table 10). In the gall bladder, however, most of the labeled material in bile was probably metabolites, as would be expected.

A recent study (Thomas and Rice, in press) examined the excretion of ^{14}C-labeled toluene and naphthalene from the gut of Dolly Varden char. Results showed that the major avenue for excretion of the parent hydrocarbons and their metabolites was via the gills, and minor portions were excreted via the gut and kidney. Most excretion via the gill was still in the form of the parent hydrocarbons; most excreted via the gut and kidney were in the metabolite phase.

The MAH bear structural similarity to many natural compounds such as steroid hormones, lipids, vitamins and neurotransmitters which also contain aromatic nuclei. This similarity may result in competition with other substrates for metabolism in the MFO system (LaDu *et al.*, 1971). MAH may also mimic effects of natural compounds, stimulating certain neuroendocrine responses and behavior (Doggett *et al.*, 1977; LaDu *et al.*, 1971). More research in this area would be of considerable interest.

TRANSLOCATION

Based on the above studies, we propose that the translocation of MAH through fish occurs as diagrammed in Figure 6. The major route of uptake is from water through the gills. Some uptake may be through the gut from swallowed water, particularly in salt water environments. A minor portion, if any, is taken through the intestinal wall. Most data indicate a minimal uptake of MAH through food (see Tables 7 and 8; Figure 4). The parent compounds readily solubilize in cell membranes (Roubal, 1974; Roubal and Collier, 1975) and are probably carried primarily via the erythrocytes (lipid cell membrane) through the general circulation in the blood (Benville *et al.*, 1980; Figure 3). Some of the monocyclics may also be carried by lipoproteins and leukocytes in the blood. Centrifugation of blood from benzene-exposed striped bass showed that the major portion of the benzene was associated with the erythrocytes, and not the serum fraction (Hirsch *et al.*, ms in prep. (b)). The blood circulates through the fish, and the aromatics apportion into target tissues and organs (Fig. 7) that have high lipid content (e.g., brain) (Korn *et al.*, 1976a, 1977). They are also transported to the liver where they are metabolized (some limited metabolic activity in other tissues is also possible). Benzene and metabolites are then both transported through the general circulation, where they reenter target tissues or are depurated. A considerable portion of the unchanged compounds probably diffuses back through the gills into the water. The

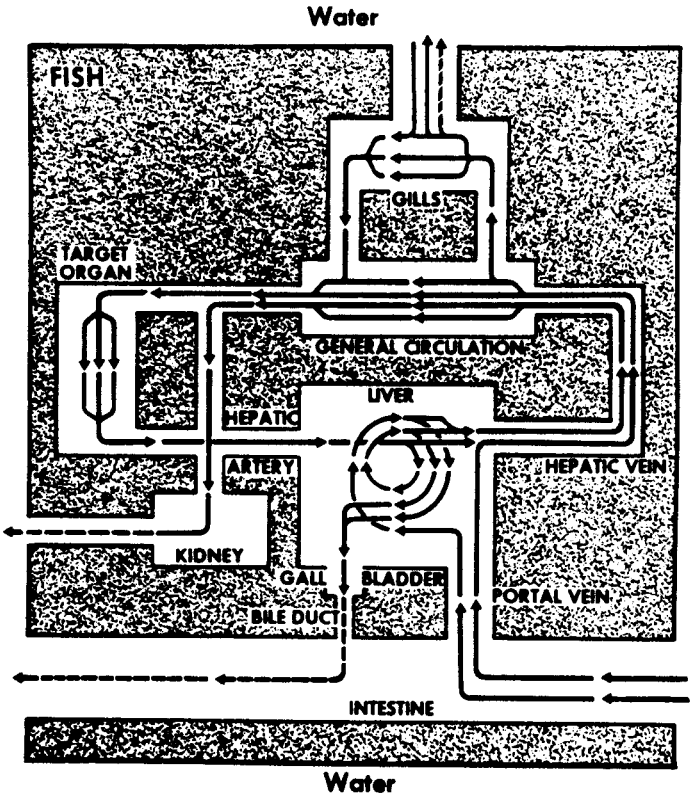


FIGURE 6. Translocation of monocyclic aromatic hydrocarbons and their metabolites in fish exposed through the water column. Dashed lines indicate routes for excretion of metabolites (adapted from Kappas and Alvares, 1975).

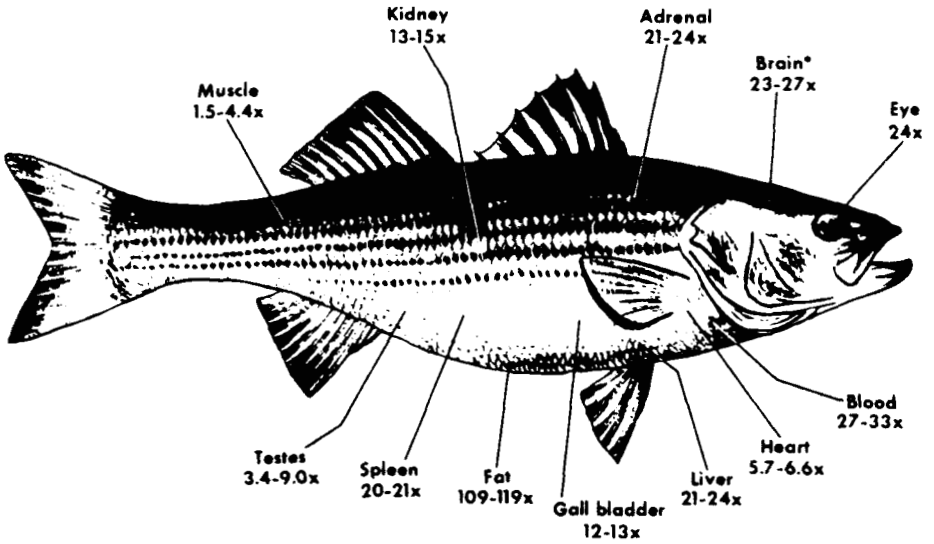


FIGURE 7. Bioaccumulation of benzene in different tissues of the striped bass (Morone saxatilis) *exposed to approximately 1.0 ppm benzene through the water column (from Benville* et al., *1980).*

major route of excretion of metabolites appears to be via the bile into the intestine and out with the feces. Some metabolites are excreted through the gills. A minor portion of metabolites appears to pass through the kidney. At high exposure levels of MAH, the metabolic detoxification capacity of the liver may be exceeded and higher concentrations of unchanged compounds probably accumulate in the target organs.

DEPURATION

MAH are depurated relatively rapidly, parent compounds usually disappearing from most tissues within 48 hrs (e.g., Figs. 3 and 4), although the rate of disappearance is slower than the rate of uptake in most tissues (Fig. 3). The depuration of metabolites takes longer and persistence in tissues involved with metabolism is greater (Korn *et al.*, 1976a, 1977). In striped bass, Pacific herring and northern anchovy, residues of benzene/metabolites were detected in gills, liver and gall bladder seven days after termination of exposure. These organs, of course, are involved with metabolism and excretion of metabolites. In addition, residues were still detectable in fat tissue seven days after termination of exposure.

UPTAKE, ACCUMULATION AND DEPURATION IN LARVAL FISH

Adult and juvenile fishes appear to accumulate MAH very rapidly and to relatively high equilibrium levels within 24 hrs. The unchanged parent compounds are also rapidly depurated, although metabolites may persist for longer periods.

Larval fish, however, appear to have limited capacity to either metabolize or excrete ^{14}C-benzene. Table 9 and Figure 8 show some of the results of an experiment in which striped bass larvae were exposed to ^{14}C-labeled benzene from the water column in a semi-static system. Benzene was added daily to restore a test concentration of approximately 120 ppb benzene (Eldridge and Benville, ms in prep.). Figure 8 shows that the uptake of benzene in feeding larvae fed uncontaminated *Artemia* nauplii was higher than in starved larvae. The major route of uptake is still through water, but when larvae are actively feeding, they appear to continually accumulate benzene and/or metabolites more than when starved. Concentrations were still increasing 16 days after initiation of feeding (Day 24). Possible explanations for this are: 1) larvae have limited enzymatic capacity to metabolize benzene and thus accumulate benzene to high levels (at least 1400 X water concentration) or 2) larvae are unable to excrete accumulated metabolites or 3) both of these. The first explanation seems most plausible. Fetuses and young of mammals also have extremely limited capacity to metabolize aromatic hydrocarbons and other drugs (LaDu *et al.*, 1971).

EFFECTS OF MONOCYCLIC AROMATICS

Acute Toxicity

The bioaccumulation of selected MAH in fishes was summarized previously (Tables 9 and 10). Generally, the effects of MAH will depend upon their concentration in fish and deposition in specific target organs and tissues. For example, the species, stages and tissues which accumulate highest levels, under most conditions, will be most sensitive to MAH; those bioaccumulating less generally will be least sensitive. The effects at a given concentration, however, will also vary according to other inherent and environmental variables as previously discussed and listed in Table 6.

The acute toxicity (96-hr LC_{50}'s) of MAH to fishes ranges from about 2.0 to 300 ppm (Table 11). When the toxicities of

Table 11. Acute bioassays of single monocyclics and for comparison, of water-soluble fraction (WSF) of oil containing monocyclics and other compounds.

Exposed Species/Stage	Salinity Temperature	System[a]	Compound	Concentration (ppm)	Effect[b] (LC_{50}) (TLm)[c]	References
Clupea harengus pallasi						Struhsaker et al., 1974
Pacific herring						
Eggs through early cleavage (survival to hatching)	24 ppt SW 15.2°C	Semi-Static	Benzene	40-45	96h[b]	
Larvae 2 days after hatching	28 ppt SW 12.9°C	Semi-Static	Benzene	20-25	48h[b]	
Engraulis mordax						Struhsaker et al., 1974
Northern anchovy						
Eggs to hatching larvae (survival to hatching)	28 ppt SW 17.5°C	Semi-Static	Benzene	20-25	48h[b]	
Oncorhynchus kisutch						Korn & Rice, (In press)
Coho salmon						
Eggs	0 ppt FW 3.5-6°C	Semi-Static	Toluene	333	96h[b]	
(Hatching)				100	96h[b]	
Alevins						
Early				60	96h[b]	
Middle				20	96h[b]	
Late				9.36	96h[b]	

Eggs hatching	0 ppt FW 3.5-6°C	Semi- Static	Naphthalene	11.8	96h[b]	
Alevins						
Early				9	96h[b]	
Middle				8	96h[b]	
Late				4	96h[b]	
Emergent fry				2-3	96h[b]	
Oncorhynchus tshawytscha						Moles et al., 1979
Chinook salmon Juveniles	0 ppt FW 6°C	Semi- Static	WSF of Prudhoe Bay crude oil	3.59	96h[c]	
	9°C		Benzene	11.73	96h[c]	
Oncorhynchus kisutch						Moles et al., 1979
Coho salmon	0 ppt FW 8°C	Semi- Static	WSF of Prudhoe Bay crude oil	3.67	96h[c]	
	9°C		Benzene	14.09	96h[c]	
Coho Salmon Juveniles	30 ppt SW 8°C	Static				Morrow, 1974
			Benzene	10-50	96h[b]	
			Toluene	10-50	96h[b]	
			Ethyl- benzene	10-50	96h[b]	
			Xylene	10-100	96h[b]	

[a]Systems: Static - Initial dose only, declining over test period; Semi-static - Daily addition to bring test concentration up to dose level over test period; Open flow - Constant exposure to open flow system of test concentrations.
SW = seawater; FW = fresh water

Table 11 con't.

Exposed Species/Stage	Salinity Temperature	System[a]	Compound	Concentration (ppm)	Effect[b] (LC_{50}) (TLm)[c]	References
Oncorhynchus gorbuscha						Rice et al., 1976
Pink salmon Juveniles	26-30 ppt SW 3.7-11°C	Semi-Static	WSF of Cook Inlet crude oil	4.13 2.92	24h[c] 96h[c]	
			WSF of #2 fuel oil	0.89 0.81	24h[c] 96h[c]	
Pink Salmon Smolts	0 ppt FW 4°C	Semi-Static	WSF of Prudhoe Bay crude oil	7.99	96h[c]	Moles et al., 1979
	28-30 ppt SW 4°C		WSF of Prudhoe Bay	3.73	96h[c]	
	0 ppt FW 4°C	Semi-Static	Benzene	17.09	96h[c]	
	28-30 ppt SW 4°C		Benzene	8.47	96h[c]	
Oncorhynchus nerka						
Sockeye salmon Smolts	0 ppt FW 6°C	Semi-Static	WSF of Prudhoe Bay crude oil	2.22	96h[c]	Moles et al., 1979
	28-30 ppt SW 6°C		WSF of Prudhoe Bay crude oil	1.05	96h[c]	
Sockeye Salmon Smolts	0 ppt FW 6°C	Semi-Static	Benzene	10.76	96h[c]	
	28-30 ppt SW 6°C		Benzene	5.55	96h[c]	

Salvelinus malma Dolly Varden Juveniles	0 ppt FW 8°C	Semi-Static	WSF of Prudhoe Bay crude oil	2.75	96h[c]	Moles *et al.*, 1979
			Benzene	11.96	96h[c]	
Smolts	0 ppt FW 8°C	Semi-Static	WSF of Prudhoe Bay crude oil	2.68	96h[c]	
	28-30 ppt SW 8°C	Semi-Static	WSF of Prudhoe Bay crude oil	1.38	96h[c]	
	0 ppt FW 8°C	Semi-Static	Benzene	11.90	96h[c]	
	28-30 ppt SW 8°C		Benzene	6.30	96h[c]	
(Dolly Varden) Smolts	26-30 ppt SW 3.7-11°C	Semi-Static	WSF of Cook Inlet crude oil	3.25 2.94	24h[c] 96h[c]	Rice *et al.*, 1976
			WSF of #2 Fuel Oil	2.29	96h[c]	

Table 11 con't.

Exposed Species/Stage	Salinity Temperature	System[a]	Compound	Concentration (ppm)	Effect[b] (LC_{50}) (TLm)[c]	References
Morone saxatilis						
Striped bass Juveniles	25 ppt SW 16.0°C	Semi-Static	Benzene	6.9 5.8	24h[c] 96h[c]	Benville and Korn, 1977
			Toluene	7.3 7.3	24h[c] 96h[c]	
			Ethyl-benzene	4.3 4.3	24h[c] 96h[c]	
			p-Xylene	2.0 2.0	24h[c] 96h[c]	
			m-Xylene	9.2 9.2	24h[c] 96h[c]	
			o-Xylene	11.0 11.0	24h[c] 96h[c]	
Striped Bass Juveniles	29 ppt SW 17.4°C	Open Flow	Benzene	10.9	96h	Meyerhoff, 1975

Eleginus gracilis Saffron cod	26-30 ppt SW 3.7-11°C	Semi- Static	WSF of Cook Inlet crude oil	2.48 2.28	24h[c] 96h[c]	Rice *et al.*, 1976
			WSF of #2 fuel oil	>4.56 2.93	24h[c] 96h[c]	
Aulorhynchus flavidus Tube-snout	26-30 ppt SW 3.7-11°C	Semi- Static	WSF of Cook Inlet crude oil	--- 1.34	24h[c] 96h[c]	Rice *et al.*, 1976
			WSF of #2 fuel oil	--- ---	24h[c] 96h[c]	
Thymallus arcticus Arctic grayling	0 ppt FW 9°C	Semi- Static	WSF of Prudhoe Bay crude oil	4.40	96h[c]	Moles *et al.*, 1979
	9°C		Benzene	14.71	96h[c]	
Cottus cognatus Slimy sculpin Juveniles	0 ppt FW 9°C	Semi- Static	WSF of Prudhoe Bay crude oil	6.44	96h[c]	Moles *et al.*, 1979
	9°C		Benzene	15.41	96h[c]	
Gasterosteus aculeatus Three-spined stickleback Adults	0 ppt FW 5°C	Semi- Static	WSF of Prudhoe Bay crude oil	>10.45	96h[c]	Moles *et al.*, 1979
	8°C		Benzene	24.83	96h[c]	

Table 11 con't.

Exposed Species/Stage	Salinity Temperature	System[a]	Compound	Concentration (ppm)	Effect[b] (LC_{50}) (TLm)[c]	References
Lepomis macrochirus						Pickering and Henderson, 1966
Bluegill	0 ppt FW	Static	Cyclohexane	42.33	24h[c]	
Adults	25°C			40.00	48h[c]	
				34.72	96h[c]	
			Benzene	22.49	24h[c]	
				22.49	48h[c]	
				22.49	96h[c]	
			Toluene	24.00	24h[c]	
				24.00	48h[c]	
				24.00	96h[c]	
			Ethylbenzene	35.08	24h[c]	
				32.00	48h[c]	
				32.00	96h[c]	
			Xylene	24.00	24h[c]	
				24.00	48h[c]	
				20.87	96h[c]	
			Phenol	25.85	24h[c]	
				23.88	48h[c]	
				23.88	96h[c]	
Carassius auratus						Pickering and Henderson, 1966
Goldfish	0 ppt FW	Static	Cyclohexane	42.33	24h[c]	
Adults	25°C			42.33	48h[c]	
				42.33	96h[c]	
			Benzene	34.42	24h[c]	
				34.42	48h[c]	
				34.42	96h[c]	
			Toluene	57.68	24h[c]	
				57.68	48h[c]	
				57.68	96h[c]	

			Ethylbenzene	94.44	24h[c]	
				94.44	48h[c]	
				94.44	96h[c]	
			Xylene	36.81	24h[c]	
				36.81	48h[c]	
				36.81	96h[c]	
			Phenol	49.86	24h[c]	
				49.13	48h[c]	
				44.49	96h[c]	
Poecilia reticulata Guppy 6 months old	0 ppt FW 25°C	Static	Cyclohexane	57.68	24h[c]	Pickering and Henderson, 1966
				57.68	48h[c]	
				57.68	96h[c]	
			Benzene	36.00	24h[c]	
				36.00	48h[c]	
				36.00	96h[c]	
			Toluene	62.81	24h[c]	
				60.95	48h[c]	
				59.30	96h[c]	
			Ethylbenzene	97.10	24h[c]	
				97.10	48h[c]	
				97.10	96h[c]	
			Xylene	34.73	24h[c]	
				34.73	48h[c]	
				34.73	96h[c]	
			Phenol	49.86	24h[c]	
				49.86	48h[c]	
				39.19	96h[c]	

Table 11 con't.

Exposed Species/Stage	Salinity Temperature	System[a]	Compound	Concentration (ppm)	Effect[b] (LC_{50}) (TLm)[c]	References
Pimephales promelas						
Fathead minnow	0 ppt FW	Static	Cyclohexane	35.08	24h[c]	Pickering and Henderson, 1966
Adults	Soft water			35.08	48h[c]	
	25°C			32.71	96h[c]	
			Benzene	35.56	24h[c]	
				35.95	48h[c]	
				33.47	96h[c]	
			Toluene	46.31	24h[c]	
				46.31	48h[c]	
				34.27	96h[c]	
			Ethylbenzene	48.51	24h[c]	
				48.51	48h[c]	
				48.51	96h[c]	
			Xylene	28.77	24h[c]	
				27.71	48h[c]	
				26.70	96h[c]	
			Phenol	40.60	24h[c]	
				40.60	48h[c]	
				34.27	96h[c]	

Pimephales promelas Fathead minnow Adults	0 ppt FW Hard water 25°C	Static	Cyclohexane	42.33 42.33 42.33	24h[c] 48h[c] 96h[c]	Pickering and Henderson, 1966
			Benzene	34.42 32.00 32.00	24h[c] 48h[c] 96h[c]	
			Toluene	56.00 56.00 42.33	24h[c] 48h[c] 96h[c]	
			Ethylbenzene	42.33 42.33 42.33	24h[c] 48h[c] 96h[c]	
			Xylene	28.77 28.77 28.77	24h[c] 48h[c] 96h[c]	
			Phenol	38.62 38.62 32.00	24h[c] 48h[c] 96h[c]	

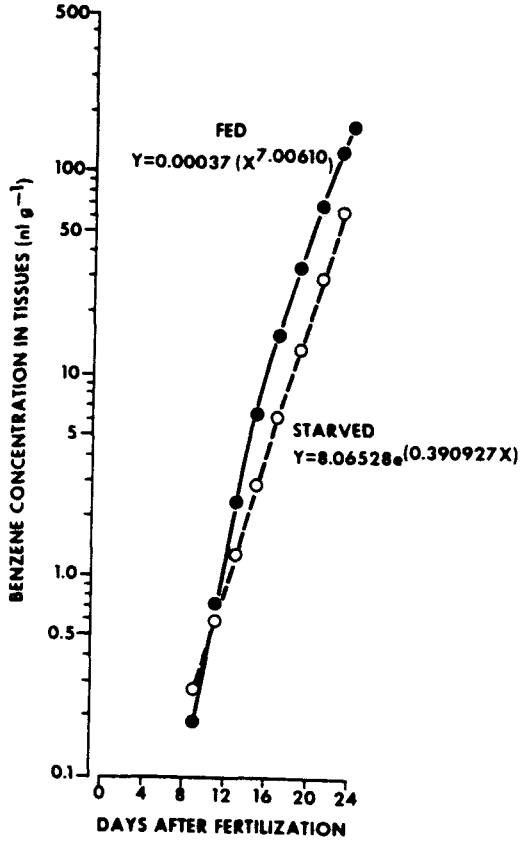


*FIGURE 8. Uptake and accumulation of ^{14}C-labeled benzene in feeding and starved larvae of the striped bass (*Morone saxatilis*), constantly exposed to a concentration of approximately 0.100 ppm ^{14}C-benzene through the water column (from Eldridge and Benville, ms in prep.).*

benzene to juveniles of different species are compared, salmonids appear slightly more sensitive than striped bass. Freshwater species such as the grayling, sculpin, stickleback, etc., appear less sensitive. The most sensitive to benzene was the sockeye salmon and the least sensitive, the guppy.

Among life history stages the most sensitive, at both acute and chronic levels, appears to be the feeding larvae, followed by gametes, prespawning adults, eggs, embryos and nonspawning adults. The least sensitive stage is the juvenile. This is in accord with the relative bioaccumulations at these stages (Table 9). Most acute bioassays are done with juvenile fish, although they appear to be the least

sensitive to MAH. More susceptible stages should be selected for bioassays.

Recent work on striped bass (*Morone saxatilis*) and starry flounder (*Platichthys stellatus*) indicates that there may be intraspecific genotypic differences in the accumulation of MAH. This may mean that there is also intraspecific variability in susceptibility to the effects. In the striped bass, for example, intraspecific differences in color pattern and some meristic characteristics appear to correlate positively with the MAH concentration in liver and gonads (Whipple, 1979; Whipple *et al.*, ms in prep.). Factor analytic results are being examined for correlations between genotypic characters and concentrations of MAH (and other pollutants) in striped bass. Right- and left-eyed starry flounder also appear to vary in their relative uptake of MAH and other components in the WSF of Cook Inlet crude oil (Yocom *et al.*, ms in prep.). If laterality in starry flounder is genetically based, the variants may also differ physiologically and metabolically in their susceptibility to MAH.

Fish may also vary in sensitivity to MAH if they are already stressed by other environmental factors, or are low in energy reserves due to spawning or other stress (see Table 6). In striped bass, for example, there appears to be a strong relationship between poor condition (estimated by condition factors) and a higher concentration of MAH (Whipple, 1979; Whipple *et al.*, ms in prep.). The degree of parasitism (number of types, abundance, severity of host reactions) also appears to correlate with the tissue concentration of MAH (Whipple, 1979; Whipple *et al.*, ms in prep.). Cause and effect relationships, however, are as yet unclear. For example, does a heavily parasitized fish take up more MAH, or does a fish stressed by MAH also become more heavily parasitized, or do both events occur? Laboratory tests are planned to clarify this issue. Moles (1980) found that coho salmon fry (*Oncorhynchus kisutch*), infested with clam glochidia of *Anodonta oregonensis* were significantly more sensitive to toluene, naphthalene and the WSF of Prudhoe Bay crude oil than uninfested fish.

Work is also being done to measure the pollutant load in striped bass from San Francisco Bay, including not only MAH, but heavy metals, PCB's, pesticides, etc. (Whipple, 1979, 1980; Whipple *et al.*, 1979; Whipple *et al.*, ms in prep.). This work is still underway; however, results so far show high loads of zinc, copper, mercury, PCB's and others (Jung and Bowes, 1980). The interaction of the existing pollutant load with MAH uptake in striped bass is being studied.

The relative toxicity of individual monocyclics (Table 11) varies with their solubility and bioaccumulation. The

position of the alkyl substitution on the aromatic ring also appears to affect toxicity (Benville and Korn, 1977). In general, *p*-xylene and benzene appear most toxic to fish, while toluene, *m*- and *o*-xylene are least toxic. Ethylbenzene is usually intermediate in toxicity. In comparison, cyclohexane and phenol (phenol is a major metabolite of monocyclics) are about as toxic as toluene (Pickering and Henderson, 1966; Table 11).

Exposure periods in most acute bioassays (Table 11) are 24, 48 and 96 hrs. For most MAH the toxicity at 24 hrs does not vary significantly from that at 96 hrs. We have found that, although death may not occur at selected exposure levels, delayed mortality often occurs after 96 hrs (Struhsaker *et al.*, 1974). We feel that it is important to examine surviving fish for some time after termination of exposure. The length of exposure relative to MAH concentration is very important and should be considered in tests of the effects of MAH on fishes, particularly when interested in chronic low level effects, such as may occur in a polluted estuary.

The source of exposure in most acute bioassays is through the water column only. As discussed above, there appears to be little accumulation through food and thus toxic effects would not be expected through this route.

Studies done at Auke Bay, Alaska (Rice *et al.*, 1976; Moles *et al.*, 1979) showed that temperature and salinity affect the toxicity of MAH and the WSF of crude oil (Table 11). MAH are usually more toxic to fish in seawater than in freshwater, and more toxic at lower temperatures. Freshwater fish also appear to be slightly more sensitive to some MAH in hard water (higher alkalinity) than in soft water (Pickering and Henderson, 1966; Table 11).

The interaction of the MAH with one another, with other hydrocarbons in the WSF of crude oil, and with other classes of pollutants (such as chlorinated hydrocarbons and heavy metals) is obviously complex. Little is known about whether effects are additive, synergistic or antagonistic (Caldwell *et al.*, 1977). An acute bioassay of benzene and the WSF of Prudhoe Bay crude oil on salmonids (Table 11) shows that the toxicity of the WSF is greater than that of benzene alone. Generally in acute bioassays, the polycyclic aromatics appear to be more toxic and more persistent in tissues than the MAH (Rice *et al.*, 1977; Neff, 1979). However, in the environment their solubility in water is much less and the uptake probably lower. Field data from San Francisco Bay also indicate that dicyclics are not being bioaccumulated in striped bass (Tables 4 and 5). Alkyl cyclohexanes may be an important chronic pollutant interacting with MAH, since they are bioaccumulated

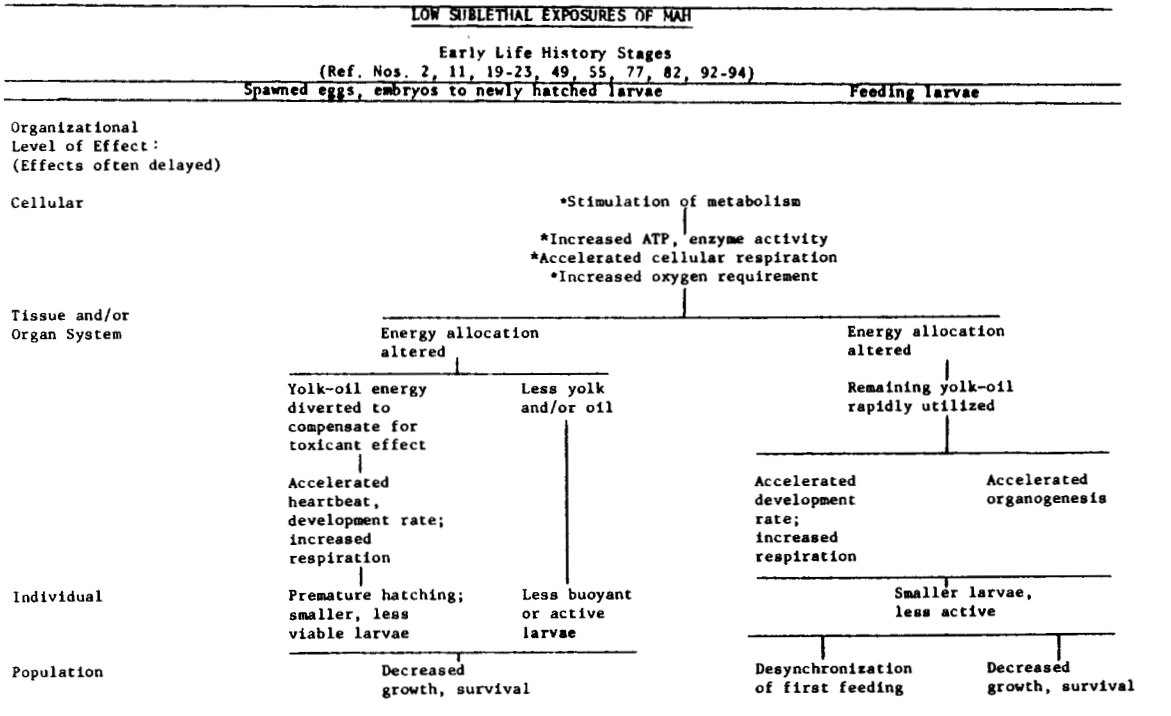
to a higher degree and are more persistent (Tables 4, 5 and 8) and methylcyclohexane is about as toxic as toluene (Table 11). Competitive inhibition or stimulation between chlorinated hydrocarbons and petroleum hydrocarbons is probable, depending upon relative levels and their demand on the MFO system for detoxification (LaDu *et al.*, 1971). Heavy metals often act as inhibitors of the enzymes in the MFO system and could potentiate the effects of hydrocarbons, including monocyclics, by decreasing the rate of metabolic detoxification and increasing accumulated levels in target organs (Maines and Kappas, 1977). Further studies on the interactions of components within these major classes of pollutants are badly needed before we can determine their combined effects on fishes in the environment.

Hypothesized Modes of Action

We still know very little about the modes of action of MAH or other petrochemicals. We know that effects of MAH will differ according to several factors discussed previously, among them: 1) the life history, 2) the exposure level (lethal, high or low sublethal) and 3) the length of the exposure (short-term acute vs. long-term chronic). Similar effects probably occur whether fish are exposed to MAH, other aromatics (e.g., PAH), the total WSF of crude oils, or even other classes of hydrocarbons. Effects specifically attributable to single compounds are rare, or at least difficult to ascertain. From the literature it appears that effects of exposure to chlorinated hydrocarbons (pesticides) are similar to those observed in fishes exposed to petroleum hydrocarbons. A possible explanation is that the modes of action of most toxic hydrocarbons are similar, and that many effects are a result of a generalized enzymatic and hormone-mediated syndrome of responses to stress, similar no matter what the stressor. We hypothesize that four effect syndromes are generally seen: 1) effects due to inhibition or stimulation of enzymatic systems in metabolism, 2) hormone-mediated adaptive responses, 3) damage to organs due to hormone-mediated responses and 4) damage due to specific effects of an individual compound. Which responses occur probably depends primarily on the concentration and length of the exposure.

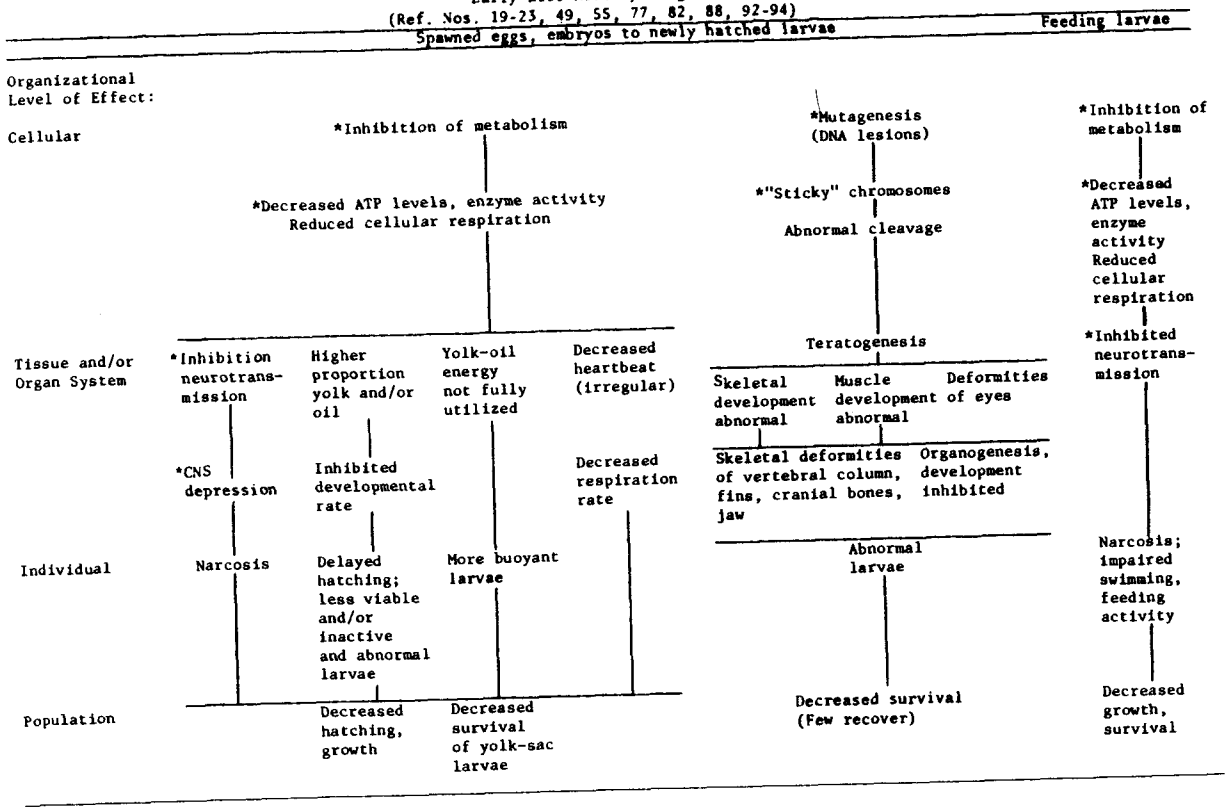
The hypothesized modes of action and effects are summarized in Table 12. Several of these modes of action are still hypothetical, and are meant to act only as temporary guidelines for study of the effects of MAH on fishes. Where observations or research tend to substantiate these hypotheses, references are noted.

Table 12. Some hypothesized modes of action and effects (functional and structural) of monocyclic aromatic hydrocarbons (MAH) at different life history stages and at different concentration levels of exposure. Hypothetical relationships are indicated by an asterisk.



HIGH SUBLETHAL EXPOSURES OF MAH

Early Life History Stages
(Ref. Nos. 19-23, 49, 55, 77, 82, 88, 92-94)



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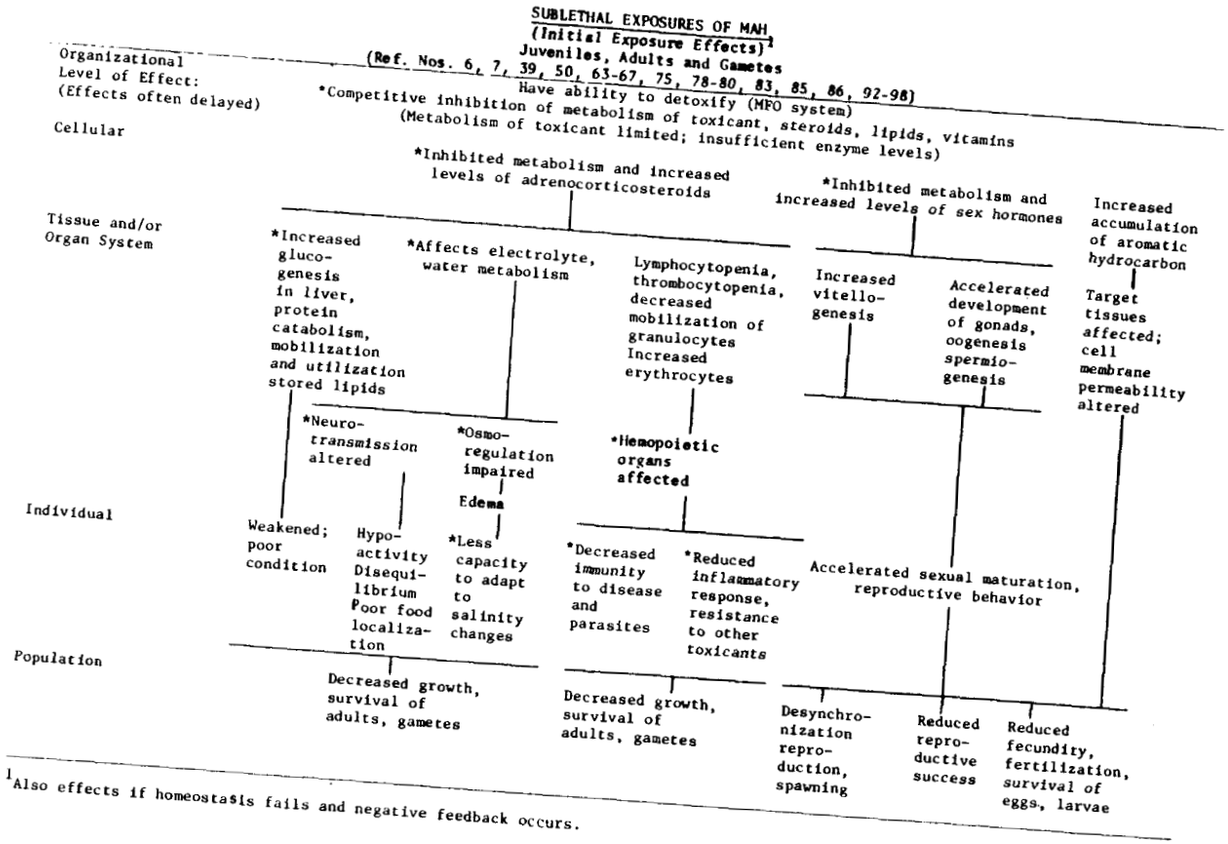
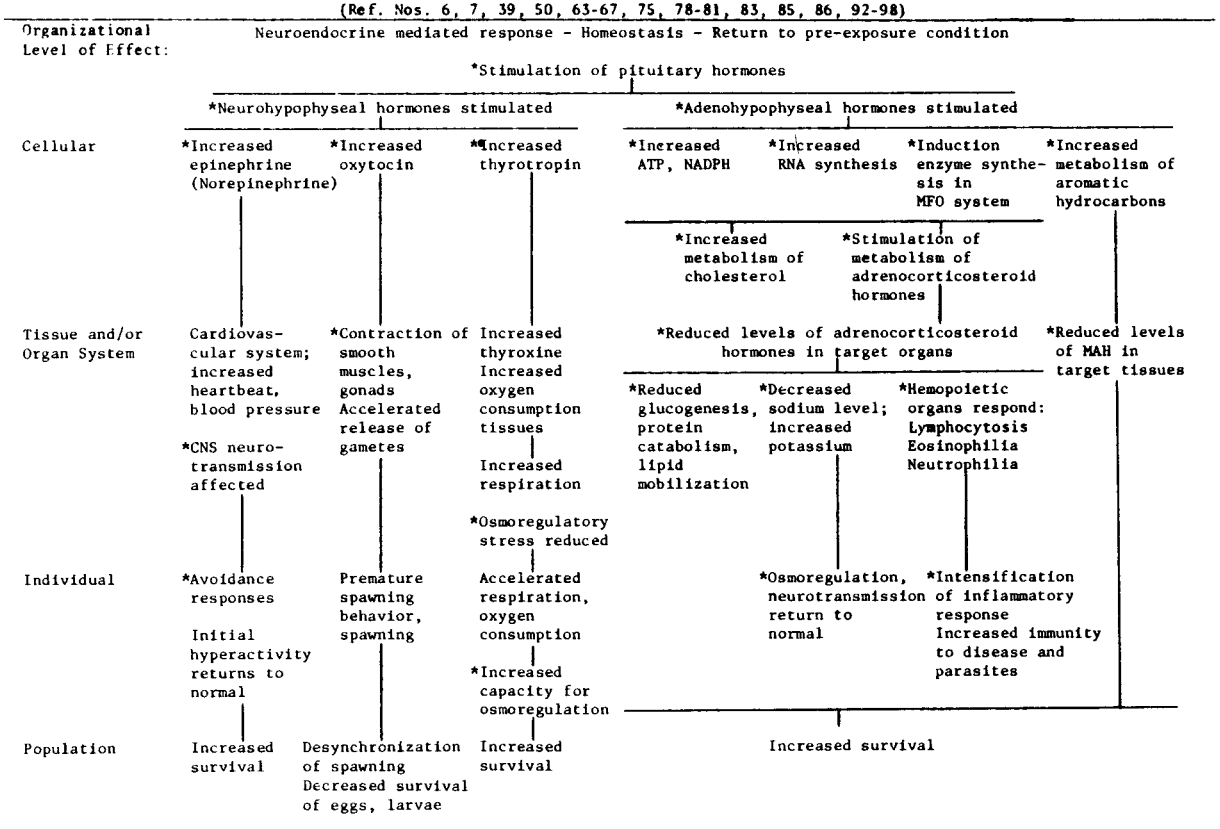


[1]Also effects if homeostasis fails and negative feedback occurs.

SUBLETHAL EXPOSURES OF MAH
(Adaptation to Exposure)
Juveniles, Adults and Gametes
(Ref. Nos. 6, 7, 39, 50, 63-67, 75, 78-81, 83, 85, 86, 92-98)



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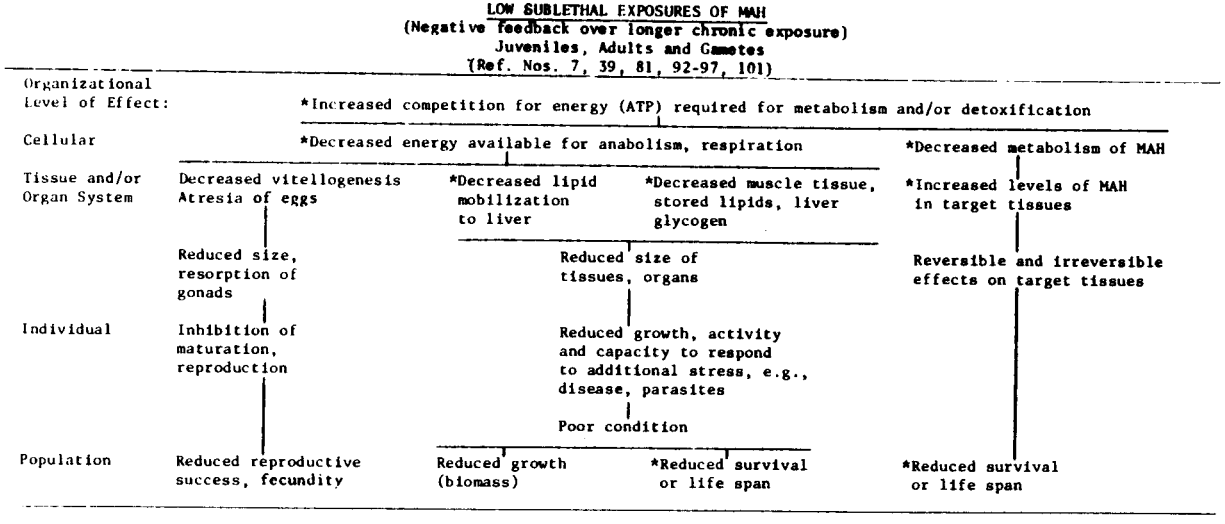


SUBLETHAL EXPOSURES OF MAN

Toxic effects on target tissues possibly resulting from hormone-mediated, adaptive responses; chronic exposure (Ref. Nos. 6, 7, 39, 50, 63-67, 75, 78-81, 86, 92-98)

Organizational Level of Effect				
Cellular	Induction of MFO			
	•Increased epoxidation of aromatics with accumulation of toxic metabolites (increased endoplasmic reticulum - ribosomes)			
	•Mutagenesis (DNA) and •Carcinogenesis (DNA)			
	•Induction of prophage	•Abnormal cell division	•Increased production leukocytes	•Abnormal leukocytes •Abnormal erythrocytes
Tissue and/or Organ System		•Tumors: liver, kidney, epidermis, etc.		•Splenomegaly
			•Leukemia	•Acute anemia
Individual		Weakened, poor condition		Weakened, poor condition
Population		Decreased survival		Decreased survival

Table 12 con't.

SUBLETHAL AND LETHAL EXPOSURES OF MAH

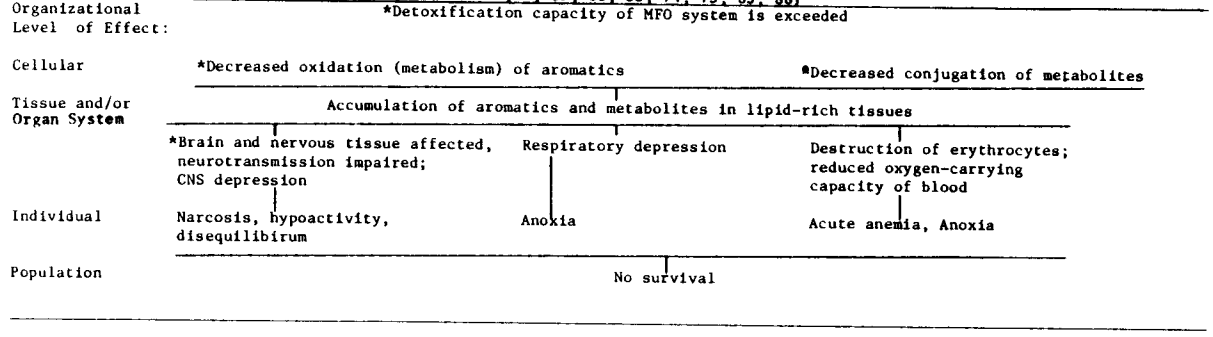
(Some toxic effects on target tissues resulting from increased levels of MAH and/or metabolites)

Most life history stages

(Ref. Nos. 4-7, 19-24, 39, 49, 50, 63-67, 74, 75, 77-81, 86-88, 92-98, 101)

Organizational Level of Effect: (Effects often delayed)	Detoxification capacity of MFO exceeded and/or toxic metabolites not depurated				
	Accumulation of aromatics and/or metabolites in target tissues and organs				
Tissue and/or Organ System	Liver: Lipid deposition *Change in type of lipid *Glycogen depletion Disruption of hepatic muralia Fibrotic infiltration Hypertrophy Hemorrhage; sinusoidal congestion with erythrocytes Necrosis	Ovaries: Increased vitellin in eggs, or atresia-resorption Egg membranes disrupted Egg vacuolization Reduced blood supply to eggs Erythrocyte destruction Hemorrhage Necrotic foci Testes: Resorption Hemorrhage Necrotic foci *Reduced sperm motility	Spleen: *Hypoplasia or *Hyperplasia Fibrotic infiltration Granulomas Blood: Abnormal erythrocytes, leukocytes Destruction erythrocytes *Increased erythrocyte fragility *Cell membrane disruption *Increased lipoprotein	Heart: *Fatty infiltration Blood Vessels: Sloughing of endothelial lining *Vasodilation *Increased capillary permeability Gills: Increased mucus secretion Increased number mucus cells Lesions, vacuoles Epithelial sloughing	Brain & Nerve Tissue: *Lesions in hypothalamus, pituitary, optic nerve tract, olfactory bulbs Adrenals: Enlarged size Muscle: *Decrease in muscle tissue, less lipid; edema
Individual	Decreased metabolic efficiency Poor condition	Decreased reproduction	Decreased respiration, immunity, resistance to environmental extremes	Decreased cardio-vascular efficiency Decreased respiratory efficiency and osmoregulation	Impaired neuro-transmission and behavioral responses Neuroendocrine stress responses Reduced activity
Population	Decreased growth, survival	Decreased fecundity Reduced fertilization and reproductive success	Decreased growth, survival	Decreased growth, survival	Decreased growth, survival

LETHAL EXPOSURES OF MAH
All life history stages
(Ref. Nos. 5, 6, 19, 63, 65, 74, 75, 85, 88)



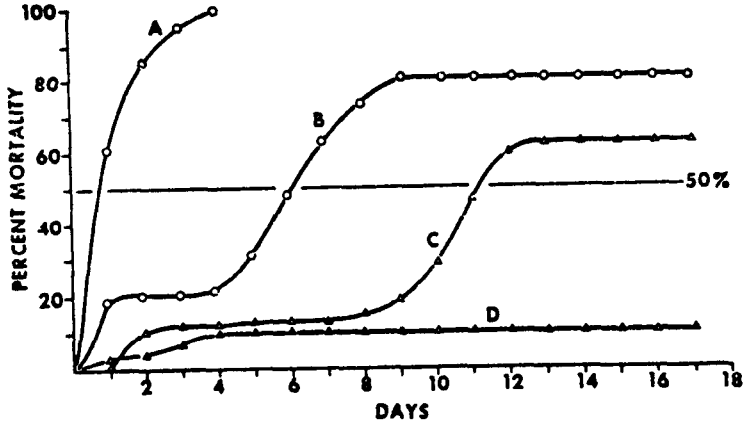


*FIGURE 9. Mortality curves of anchovies (*Stolephorus purpureus*) exposed to different levels of osmoregulatory stress in the laboratory. A = lethal (or acute) stress; B and C = High sublethal (eventually lethal) stress; D = Low sublethal (chronic) stress (Adapted from Struhsaker* et al., *1974).*

To illustrate the responses of fish to stress, Figure 9 shows a family of mortality curves generated by exposing anchovies to osmoregulatory stress (Struhsaker *et al.*, 1975). The percent mortality over a 17-day period is shown at different levels of stress (from lethal to low sublethal). The configuration of two of these curves (B and C) is similar to the general adaptation syndrome (GAS) described by Selye (1950) (see also Rosenthal and Alderdice, 1976). A lethal level of stress is represented by curve A, where continuous mortality occurs until 100% of the fish are dead. At this level, no homeostatic or adaptive response was adequate to overcome the effects of the stress. In curves B and C, there was an initial mortality, followed by an adaptive response with zero mortality, followed by a negative feedback where capacity to adapt was exceeded and mortality again increased. Finally, mortality leveled off with some of the fish surviving. The survivors probably represent a genetically resistant portion of the population with greater adaptive capacity. Curve D is equivalent to what may occur with low sublethal exposures, where there may be a small initial mortality (or none) followed by an adaptive response of long duration. Ultimately, however, negative feedback may occur. There is disagreement among researchers as to whether there is a threshold level of "no effect" for many pollutants.

Some believe that there is no "safe level" of exposure if organisms are exposed to pollutant stress for a sufficiently long period of time. For example, the negative feedback may shorten the life span, decrease growth or inhibit reproduction. This issue has not been resolved at the present time.

Chronic Effects

Many of the observed effects of MAH are probably due to a generalized stress response. A possible specific toxic effect may occur with the component benzene. In mammals, benzene affects the blood-forming organs, resulting in leukemia. This effect was observed to be specific to benzene and was not induced by other aromatic compounds (Gerarde, 1960). Preliminary studies of field-captured striped bass indicate that there are effects on red and white blood cells which are correlated with high levels of benzene in the liver (Whipple, 1979). Further studies on hemopoietic tissues are being done. The degree of a toxic effect will depend largely on the levels of the monocyclics and/or metabolites, such as phenol, in the target tissues. Our research has also shown that prespawning females, their ovaries, eggs and larvae experience irreversible damage to tissues at very low levels of MAH in the water column (50-100 ppb) and thus are potentially the most sensitive in the actual environment (Struhsaker, 1977; Whipple *et al.*, 1978b).

Several other effects are shown in Table 12. Many of the hypothesized modes of action and effects have been derived from work on mammals (summarized by LaDu *et al.*, 1971). In the table, hypothetical concepts are indicated by asterisks, while those with studies substantiating the hypotheses are referenced by numbers.

Many researchers have noted an apparent beneficial effect of exposure of fishes to low levels of toxicants, including the MAH (Eldridge *et al.*, 1977). This probably results from the hormone-mediated adaptive responses of the organisms to stress, at least temporarily stimulating metabolic functions. This response is referred to by Smyth (1967) as a "sufficient challenge". Under chronic conditions, however, we feel that some negative feedback eventually occurs (Table 12).

Hypothesized Effects at the Population Level

The effects of monocyclics summarized in Table 12 include potential effects at a population level. In essence, these effects may result in the reduction in production of a

fishery as determined by fecundity, reproduction and larval recruitment, growth and survival. For example, our work has shown that such effects of MAH are possibly contributing to the decline of the striped bass population and fishery in the San Francisco Bay area (Whipple, 1979, 1980; Whipple *et al.*, ms in prep.). Chronic levels of MAH occur in tissues of striped bass which either alone, or in concert with other pollutants identified (e.g., zinc), are sufficient to cause negative effects. Although striped bass are relatively hardy, many appear to be stressed beyond their capacity to adapt. We hypothesize monocyclic aromatic petrochemicals reduce the ability of fishes to adapt to natural stresses, particularly during spawning migration and early life history stages. Whether or not a population can genetically adapt to this stress over longer periods depends upon the differential selection of a hardier genotype. Although such intraspecific diversity appears to exist in striped bass, present observations indicate that it is not sufficient to ensure population persistence.

Estimates of reduction in fecundity and larval survival due to the uptake of monocyclic aromatics (both laboratory and field data) indicate that a reduction of larval recruitment as high as 40-50% may occur in some of the species studied, for example, Pacific herring and striped bass (Struhsaker, 1977; Whipple *et al.*, ms in prep.) at the chronic levels now occurring in San Francisco Bay.

SUMMARY

Recent investigations of the sources of monocyclic aromatic hydrocarbons (MAH) and their potential effects on aquatic resources indicate that this hydrocarbon class is prevalent in estuarine ecosystems such as the San Francisco Bay-Delta, and may constitute a chronic pollution threat. These toxic petrochemicals, interacting with other pollutants, could cause quantitative reductions in the production of fish populations by decreasing growth, reproduction, egg viability, larval recruitment and survival. There are also qualitative effects on fisheries, such as condition of fish flesh and increased parasitism and disease. This paper briefly discusses the sources and fates of MAH and summarizes their effects on fishes.

The chronic effects of monoaromatics are examined in the context of a qualitative conceptual model, suggesting interactive effects of inherent environmental factors (abiotic and biotic). Variability in uptake, bioaccumulation and

effects of monocyclics is considered in relation to inherent differences in fishes (e.g., interspecies variation, intraspecific variation in genotype, sex, age or life history stage and condition). The interaction of these inherent differences with certain environmental variables (e.g., temperature, salinity, pollutants and parasitism) is also discussed. The definition of variability is important in determining potential chronic effects on the biota, and in making selections of critical stages and effects to study and monitor.

Monocyclic aromatics discussed include: benzene, toluene, xylenes, ethylbenzene and substituted benzenes, in general. Examples of fish species are drawn from our research in the San Francisco Bay-Delta area. However, most of these species are widely distributed into other areas. Species discussed include: striped bass (*Morone saxatilis*), starry flounder (*Platichthys stellatus*), Pacific herring (*Clupea harengus pallasii*), northern anchovy (*Engraulis mordax*) and Chinook salmon (*Oncorhynchus tshawytscha*). The possible relationship of selected genotypic differences to variability in uptake, bioaccumulation and effects is discussed for striped bass and starry flounder. Differences among the following life history stages also occur: spawning adults, gametic eggs, spawned eggs, embryos, larvae, juveniles and nonspawning adults. Larvae, for example, appear to bioaccumulate very high levels of monocyclic aromatics.

Uptake, bioaccumulation, translocation and depuration of these components are discussed in relation to effects in terms of hypothesized modes of action and alterations of structure and function (morphology, physiology and behavior). Many responses appear to be nonspecific responses to pollutant stress, but some specific effects (e.g., blood parameters) may also occur.

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