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THE PHOTO-INDUCED TOXICITY OF
POLYCYCLIC AROMATIC HYDROCARBONS TO FISH
(LEPOMIS SPP., LEPOMIS MACROCHIRUS, AND PIMEPHALES PROMELAS)

presented by

James Thomas Oris

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John P. Giesy
Major professor

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THE PHOTO-INDUCED TOXICITY OF POLYCYCLIC AROMATIC HYDROCARBONS TO FISH (*LEPOMIS* SPP., *LEPOMIS MACROCHIRUS*, AND *PIMEPHALES PROMELAS*)

By

James Thomas Oris

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ABSTRACT

THE PHOTO-INDUCED TOXICITY OF POLYCYCLIC AROMATIC HYDROCARBONS TO FISH (LEPOMIS SPP., LEPOMIS MACROCHIRUS, AND PIMEPHALES PROMELAS)

By

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The acute toxicity of polycyclic aromatic hydrocarbons (PAH) to fish in the presence of solar radiation has been assessed. These studies were conducted in a laboratory system under simulated sunlight. Anthracene, a linear 3-ring PAH was used as a model compound in the examination of light intensity and photoperiod effects, the elucidation of possible sites and modes of toxic action, and the development of an environmental hazard assessment. The primary test species in these studies was juvenile sunfish (Lepomis spp. and Lepomis macrochirus). Eleven other PAH were examined for potential photo-activity with larvae of the fathead minnow (Pimephales promelas). A structure-activity relationship has been developed based on molecular structure and photochemical properities which can predictively classify a compound as being phototoxic or non-phototoxic.

The results of these experiments are environmentally significant since when compared to current natural PAH concentrations in water and in fish tissue, there are waters in which photo-induced PAH toxicity may presently occur. It is concluded that solar radiation is an important accessory parameter that deserves consideration in the toxicity assessment of PAH in the aquatic environment.

ACKNOWLEDGEMENTS

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INTRODUCTION

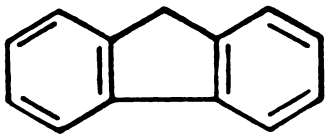
Assessing the potential hazards of chemicals in the aquatic enviroment is a monumental task. Until recently, much of the information used for predictive hazard assessment has been obtained from acute and chronic laboratory bioassay information interfaced to simulation models. These techniques have been well developed, but are predicated on the input of accurate information to effectively predict the potential impacts a compound will have on target organisms or the ecosystem in general. While toxicity tests may be very precise they may not give an accurate estimation of the toxicity of compounds under field conditions. By design, standard laboratory toxicity tests are insensitive to the complex interactions between an organism and its environment. Cairns (1981) has stressed the need for the implementation of environmental realism in predictive toxicity testing. Environmental pollutant realism is attained when the characteristics of a compound in the natural environment are incorporated into the laboratory test system. The concepts of

ecological relevance and pollutant realism have been discussed by Blanck and Gustafsson (1978). It is important to assess the effects of a chemical on an ecological level, including population, community and ecosystem effects. For example, community composition and diversity of an aquatic community can be extremely sensitive indicies of environmental change (Giesy and Odum 1980), and Gaufin (1973) has shown that on a long term basis, even slight changes in environmental conditions can lead to changes in community structure and diversity.

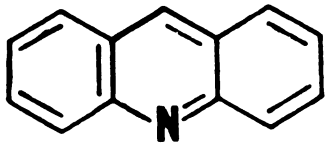
PAH consist of a large class of organic compounds comprised of two or more fused benzene rings with occassional heteroatom or cyclopentane inclusions in the ring structure, or variously substituted alkyl side chains. Compounds in this class, of environmental importance, range from two ring napthalene (M.W. 128.16) to seven ring coronene (M.W. 300.36). Some representative examples of other environmentally important PAH are shown in Figure 1. PAH occur as natural products in plants and microbes (Gerarde and Gerarde 1962). PAH can be formed under anaerobic conditions from quinones and related precursors produced by fungi, plants and animals (Neff 1979), but evidence for direct synthesis of PAH by plants and animals is inconclusive. Major sources of PAH in the environment are oil spills, industrial processes, fossil fuel combustion and other pyrolytic processes attributed to human activity (Suess 1976; Braunstein et al. 1977;

Figure 1. The structure of some common polycyclic aromatic hydrocarbons.

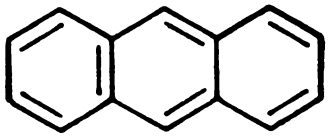
Figure 1.



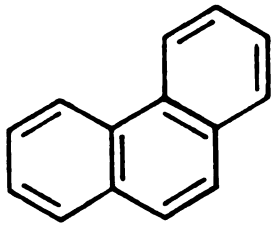
FLUORENE



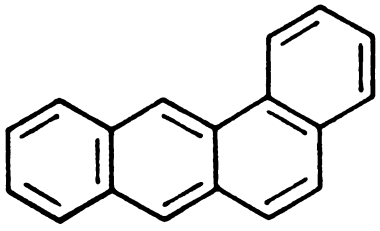
ACRIDINE



ANTHRACENE

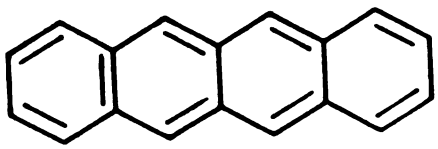


PHENANTHRENE

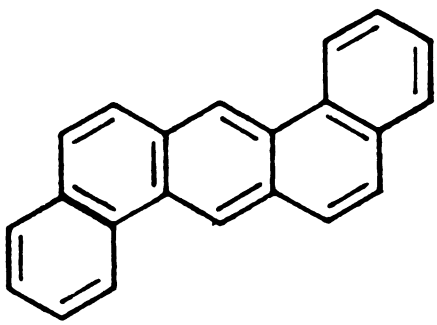


BENZO(a)ANTHRACENE

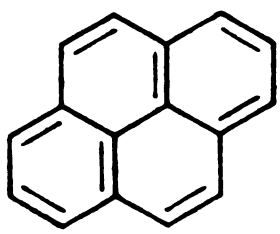
Figure 1. (continued)



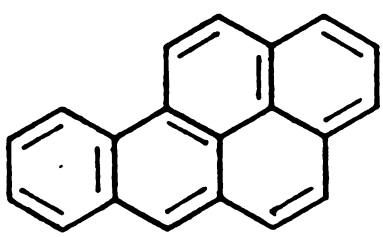
BENZO(b)ANTHRACENE



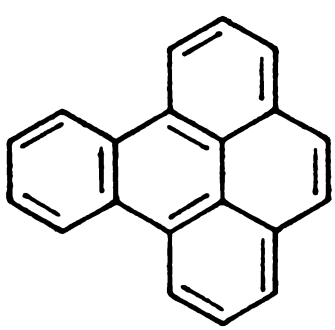
DIBENZ(a,h)ANTHRACENE



PYRENE

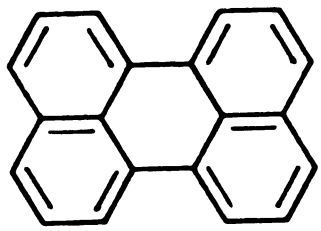


BENZO(a)PYRENE

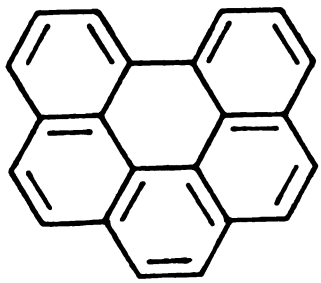


BENZO(e)PYRENE

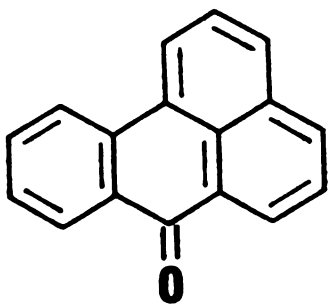
Figure 1. (continued)



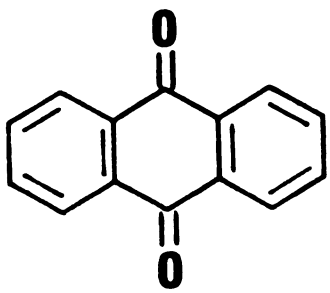
PERYLENE



BENZO(g,h,i)PERYLENE



BENZANTHRONE



ANTHRAQUINONE

Schemltz and Hoffman 1976; Laflamme and Hites 1978). Of the estimated 900 to 1300 tons of annual benzo(a)pyrene (BAP) emmissions in the United States (Baum 1978; NAS 1972), open refuse burning contributes 42 to 46% of the total, heat and power generation contributes 37 to 38%, 15 to 19% is generated in coke production, and 1 to 1.5% arises from the use of motor vehicles (Dipple 1983).

Increasing inputs of polycyclic aromatic hydrocarbons (PAH) into aquatic systems from greater utilization of coal combustion and petroleum products (Gehrs 1976) has created the need to develop an environmentally realistic assessment of the impact these compounds may have on fish and other aquatic organisms. Nearly 230,000 metric tons of PAH are estimated to enter the world's oceans and surface waters every year (Neff 1979). PAH in freshwaters near industrialized regions can be elevated as a result of aerial inputs (Andelman and Snodgrass 1972). Concentrations of PAH in water resulting from aerial inputs have been reported to range from 0.025 to 3 ug/L (Neff 1979; USEPA 1980). The major input of organic contaminants in the Great Lakes region has been determined to be non point-source atmospheric deposition (Eisenreich et al. 1981). The flux of total PAH and the concentration of 12 individual PAH into Lake Michigan from aerosols was measured, and in southern Lake Michigan the flux of total PAH was on the order of 10^5 kg/yr in dry flux and 10^6

kg/yr in wet flux (Strand and Andren 1980). These fluxes resulted in increases of PAH in the surface waters of the lake.

PAH may also enter freshwater systems from groundwater sources. For example, ash from incinerated sewage sludge, which is often stored in landfills and is susceptable to groundwater leaching, has been measured to contain between 65 and 240 ng/g of anthracene (Wszolek and Wachs 1972). Total PAH concentrations in sewege sludge ash from Saginaw, MI, were measured to be greater than 865 ng/g. The runoff from coal piles, which are frequently located near major waterways, may also contribute to the contamination of freshwaters. PAH have been identified in extracts of runoff water from Illinois #6 coal at a total concentration of approximately 100 ug/L (Stahl et al. 1984). Additionally, a wide range of PAH have been identified at enriched concentrations in river sediments adjacent to coal piles (Herbes et al. 1979). It is apparent that PAH are entering aquatic ecosystems at an ever increasing rate and that a pro-active, environmentally realistic approach toward assessing their potential effects in aquatic systems is needed.

There has been a considerable effort exerted in the study of PAH in the aquatic environment. However, most studies of PAH in aquatic organisms have been concerned with fate and transport mechanisms, long term chronic effects, and determining vectors of carcinogen transfer to

humans. Standard laboratory toxicity tests generally have indicated that PAH are not acutely toxic to aquatic organisms within the limits of aqueous solubility (Herbes et al. 1976), and reported median-lethal concentration (LC50) values often exceed maximum solubilities by a factor of 100 to 1000 (Neff 1979). Such values suggest that carrier solvents were used in the tests or that the PAH of interest may have precipitated or formed micels, which possibly altered the solution behavior or bioavailability of the compound. Additionally, PAH studies in the laboratory may be conducted under specialized lighting to prevent photodegradation of the parent compound. This use of lights with spectral emissions greater than 400 to 500 nm may have been responsible for a misinterpretation of the actual degree of acute toxicity of PAH to aquatic organisms.

Medical and biochemical researchers have long recognized that PAH are involved in photosensitization and phototoxic reactions in the skin of mammals (Tannenbaum et al. 1975; Forbes et al. 1976). The first references to the photosensitizing activity of organic compounds can be found in Egyptian medical writings dating back to 1550 B.C. (Benedetto 1977). It was written that inflammation of the skin would occur after ingestion of a particular plant material, now known to contain psoralens, and subsequent exposure to bright sunlight. In more recent

times, the relationship between ultraviolet (UV) light, PAH and skin cancer was first examined (Findlay 1928, Lewis 1935, Doniach and Mottram 1937, Mottram and Doniach 1938). Burkhardt (1939) reported hypersensitivity and pronounced edema in patients treated with coal tar and exposed to sunlight. High boiling point fractions, which included fluoranthene, methylanthracene and anthracene, were identified as the photosensitizing agents. Complex mixtures such as coal tar as well as individual PAH have been observed to cause erythema in the presence of UV-B (285 to 345 nm) and UV-A (345 to 400 nm) radiation (Kochevar et al. 1982). Due to the hyperconjugation across the rings of aromatic compounds there is a pronounced red-shift in the UV absorption spectra of many PAH such that the major absorption and excitation bands occur in the UV-B and UV-A regions of the electromagnetic spectrum (Cheng and Prather 1978). An extremely large number of PAH strongly absorb radiation in the solar UV range, and can be considered potential phototoxins (Table 1). Almost every organic molecule which absorbs radiation in the region of the electromagnetic spectrum from 320 to 900 nm has been proposed as a potential photosensitizing compound (Krinsky 1976). There are several possible reasons for the photosensitizing potential of PAH. These include the great absorbance of PAH in the portion of the solar spectrum that penetrates the atmosphere, high quantum yields of singlet and triplet

Table 1. Solar radiation absorption characteristics of selected polycyclic organic compounds (Weast 1972; Jacob et al. 1984). "*" indicates a log molar extinction coefficient greater than 3.0.

Compound	Absorption in Atmospheric Solar Range		
	UV-B (285-345 nm)	UV-A (345-400 nm)	Visible (400-700 nm)
Acridine	*	*	-
Anthracene	*	*	-
Anthanthrene	*	*	*
Benz(a)acridine	*	*	-
Benzanthrone	*	*	*
Benzo(a)anthracene	*	*	-
Benzo(b)anthracene	-	*	*
Benzo(b)chrysene	*	*	-
Benzo(a)fluoranthene	*	*	*
Benzo(c)fluorene	*	-	-
Benzo(g,h,i)perylene	*	*	*
Benzo(a)pyrene	*	*	*
Benzo(e)pyrene	*	*	-
Chrysene	*	*	-
Coronene	*	-	-
Dibenz(a,j)acridine	*	*	-
Dibenz(a,c)anthracene	*	*	-
Dibenz(a,h)anthracene	*	*	-
Fluoranthene	*	*	-
Fluorene	*	-	-
Napthalene	*	-	-
Perylene	*	*	*
Phenanthrene	-	-	-
Pyrene	*	*	-

excited states, and long lifetimes of these excited states (Prusik et al. 1979). These factors have not previously been addressed in the study of the impact of PAH in the aquatic environment. There is a need to identify compounds which, due to their environmental mobility and bioavailability, have a great potential to act as phototoxins in the aquatic environment, and to identify environmental parameters which may serve to attenuate or magnify the actinic effects of these compounds.

Recently, there has been some recognition that PAH and related compounds can be extremely toxic when aquatic organisms are concurrently exposed to PAH and to solar ultraviolet radiation (SUVR). For example, algae appear to be sensitive to SUVR-PAH exposure. BAP and several metabolites of BAP inhibited the growth of the green alga Selenastrum capricornutum in the presence of fluorescent blacklight (Cody et al. 1984). Several polyacetylene compounds are also known to be phototoxic to marine and freshwater algae (Arnason et al. 1981). The phototoxic effects of PAH to algae are inconclusive, however, since no adverse effects to the green alga Chlorella pyrenoidosa exposed to anthracene were found in the presence of natural or simulated sunlight (Oris et al. 1984). Allred and Giesy (1984) have studied the photo-induced toxicity of anthracene to Daphnia pulex, and have found this organism to be extremely sensitive to anthracene in the

presence of UV-A radiation. The toxicity of a wide range of PAH in the presence of UV radiation to brine shrimp (Artemia salina) has also been examined (Morgan and Warshawsky 1977). Furthermore, the photo-induced toxicity of anthracene has been demonstrated with larvae of the mosquito Aedes aegypti (Oris et al. 1984). In addition to anthracene, larval A. aegypti are known to be photosensitized by polyacetylenes and other thiophene derivatives at small ug/L concentrations (Wat et al. 1981; Kagan et al. 1983). The nontoxic xanthene dye fluorescein, was found to synergise the toxicity of rose bengal to larval A. triseriatus in sunlight, under fluorescent light, and upon illumination of laser light in the visible wavelengths (400 to 700 nm) (Carpenter et al. 1981). Exposure to solutions containing parts per million concentrations of the dye erythrosin-B in the presence of visible light caused mortality in larvae of the mosquito Culex pipiens quinquesfasciatus (Carpenter and Heitz 1981).

Light-mediated PAH toxicity is not limited to aquatic invertebrates. Dunbar (1951) may have unknowingly reported one of the first observed cases of light-mediated toxicity in fish. The report describes mortality of rainbow trout fingerlings held in "black asphaltum painted" troughs after two days of exposure to bright sunlight. Direct effects of UV radiation were considered, though no association was made between possible photosensitization

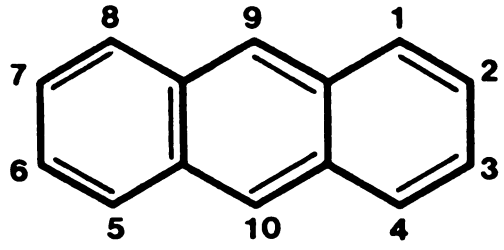
by PAH which leached into the water and the observed mortality. Investigators involved in hatchery reared fishes have also recognized the photosensitizing potential of organic compounds. SUVR-induced skin lesions have been reported in rainbow trout fingerlings (Salmo gairdneri) and in plaice (Pleuronectes platessa) fed the antihelmenthic drug phenothiazine (Bullock and Roberts 1979; Bullock 1981). Bowling et al. (1983) unexpectedly observed 100 percent mortality of juvenile bluegill sunfish (Lepomis macrochirus) exposed to 12.7 ug anthracene/L within 48 h in outdoor artificial stream channels. This concentration caused no mortality of fish of comparable origin in laboratory experiments of similar duration. It was determined that the solar UV portion of the electromagnetic spectrum significantly enhanced the toxicity of anthracene. Kagan et al. (1984) has shown that late embryonic stages of the frog Rana pipiens, that share similar habitats with juvenile sunfish are extremely sensitive to anthracene in the presence of natural sunlight. LC50 values of 65 and 25 ug/L for 30 and 60 min sunlight exposures, respectively, were determined. While these studies have indicated the potential for light-induced PAH toxicity to fish, there have been no detailed examinations of this phenomenon under controlled conditions.

The purpose of the present study was to examine the

photo-induced toxicity of PAH to fish in the laboratory under well-defined conditions of lighting and water quality. Three different species of fish were used in these experiments. A natural population of juvenile sunfish (Lepomis spp.) and a hatchery population of juvenile bluegill sunfish (Lepomis macrochirus Rafinesque) were used as the primary test species. Larvae and sexually immature fathead minnows (Pimephales promelas R.) were also used. These fish are easily maintained in laboratory cultures and are commonly used in standard toxicity tests as representative warm water fishes. All studies, with the exception of the experiments concerning structure-activity relationships, were conducted with anthracene. Anthracene (Figure 2) is a commonly occurring PAH of both natural origin and human activities. It has been identified as a constituent in coal tar and airborne coal tar emmissions, in coal and oil, and as a pyrolysis product from various combustion sources (Neff 1979). Anthracene has been used in many studies as a model PAH because it is inexpensive to obtain in pure form, it is relatively easy to extract and analyze in water and organisms, it possesses a median level of water solubility compared to other PAH, its symetrical structure reduces the number of potential transformation and metabolic products, and it is classified as being non-carcinogenic.

Figure 2. Structure and properties of anthracene.

Figure 2.



SYNONYMS -- ANTHRACIN, PARANAPTHALENE

COLORLESS SOLID CRYSTALS

M.W. = 178.23

DENSITY = 1.283

MELTING POINT = 216° C

BOILING POINT = 342° C

VAPOR PRESSURE = 3.7×10^{-6} torr

$K_{ow} = 2.8 \times 10^{4}$

SOLUBILITY IN WATER AT 22° C = 35 ug/L

Objectives

The objectives of the present study were as follows:

I. Overall objective -- To study in detail the toxicity of anthracene and other PAH to fish under simulated sunlight in the laboratory in an effort to ascertain the potential hazard that these compounds may pose in the aquatic environment.

II. Specific objectives --

1. To examine the dose-response relationships among anthracene concentration, solar ultraviolet radiation intensity, and time to death of fish under conditions of continuous laboratory simulated sunlight.

2. To use the information gained in 1) to derive a basis for a mathematical relationship that would predict the level of anthracene toxicity to fish given a particular anthracene concentration in water and solar ultraviolet radiation intensity under natural conditions.

3. To determine the site(s) and mode(s) of toxic action using physiological and histological techniques.

4. To study inter- and intra-species specific differences in sensitivities of fish to the photo-induced toxicity of anthracene.

5. To examine the dose/dose-rate relationships of anthracene phototoxicity to fish under various lengths of photoperiod to further the development of an environmental hazard estimation, as well as to provide first of its kind information on rates of photosensitized damage versus physiological repair in the fish.

6. To examine the phototoxic potential to fish of a wide range of PAH in an attempt to develop structure-activity relationships based on the chemical and physical characteristics of the compounds.

Organization of dissertation

This dissertation is organized into two chapters. The first chapter is concerned with studies designed to examine in detail the photo-induced toxicity of one compound, anthracene, to juvenile sunfish and minnows. The first chapter comprises the major portion of this dissertation, and addresses Objectives 1 to 5 as outlined above. Portions of Chapter 1 can be found in published form in Oris and Giesy (1985). The second chapter specifically addresses Objective 6 above. Finally, a general discussion is presented that considers, based on a review of the literature, the potential ecological consequences of increased loading of PAH into the aquatic environment.

CHAPTER 1

THE PHOTO-INDUCED TOXICITY OF ANTHRACENE TO JUVENILE SUNFISH (*Lepomis* spp.), JUVENILE BLUEGILL SUNFISH (*L. macrochirus*), AND FATHEAD MINNOWS (*Pimephales promelas*)

1.1 INTRODUCTION

Solar ultraviolet radiation (SUVR) induced effects of polycyclic aromatic hydrocarbons (PAH) on mammals (Burkhardt 1939; Morimura et al. 1964; Forbes et al. 1976; Kochevar et al. 1982) and on microbes (Dworkin 1958; Harrison and Raabe 1967; Shimizu-Takahama 1981) have been known for many years. Only recently aquatic ecologists and toxicologists have recognized the importance of SUVR in aquatic ecosystems. Concern over the potential degradation of the atmospheric ozone layer and the subsequent increase in global intensities of injurious SUVR has been the impetus for studies of the damaging effects of direct UV irradiation to fish (Hunter et al. 1981) and other aquatic organisms (Damkaer et al. 1980;

Barcelo and Calkins 1979; Damkaer et al. 1981). Concern has also been expressed over potential increases of PAH inputs to aquatic systems from non-point source combustion of fossil fuels (Gehrs 1976).

PAH are extremely toxic when aquatic organisms are exposed simultaneously to natural or artificial SUVR. This acute, photo-induced toxicity has been observed in a variety of aquatic organisms including fish (Bowling et al. 1983), tadpoles (Kagan et al. 1984), cladocerans (Allred and Giesy 1985), mosquito larvae (Oris et al. 1984), and algae (Cody et al. 1984). Acute toxicity of some PAH due to this photosensitization is as much as 3 to 4 orders of magnitude greater than that determined in tests conducted in the absence of SUVR. These studies have demonstrated the need for the incorporation of environmental realism in the assessment of PAH toxicity in the aquatic environment. The studies presented in this chapter were designed to provide a detailed examination of the photo-induced toxicity of anthracene (Figure 2) to fish under controlled, laboratory conditions.

The first set of experiments examines the effect of SUVR intensity and anthracene concentration under conditions of continuous lighting on the photo-induced toxicity of anthracene to a natural population of juvenile sunfish (Lepomis spp.). These studies provide information on the dose-response relationship among time to mortality, SUVR intensity, and anthracene dose. Information is also

presented, based on physiological and histological evidence, concerning the sites of acute toxic action. Possible mechanisms of toxic action are discussed in relationship to observed sites of damage. An additional objective of these experiments was to determine whether the rate of mortality of fish exhibits reciprocity with SUVR intensity. This information was used in a statistical relationship to predict the rate of mortality of fish given the SUVR intensity and concentration of anthracene in water. The predictive relationship is based on the Bunsen-Roscoe Law of Reciprocity (Dworkin 1958). Finally, in this set of experiments, species sensitivities of different fish populations are examined. Differences in sensitivity among a natural population of juvenile sunfish (L. spp.), two hatchery populations of juvenile bluegill sunfish (L. macrochirus) and sexually immature fathead minnows (Pimephales promelas) are presented and discussed.

The second set of experiments presented are concerned with the effect that different daily photoperiod regimes have on the photo-induced toxicity of anthracene to a hatchery population of juvenile bluegill sunfish (L. macrochirus). To be able to predict the response of an organism to a photodynamic compound one must know the dose of both compound and UV radiation. In addition, the responses of organisms to toxicants is determined, in

part, by the rate of damage caused by the toxicant relative to the rate at which an organism can repair the damage. In this study, photoperiod dependent effects on rates of damage versus rates of repair are examined and discussed. Also, a relationship to predict no-effect anthracene concentrations (LC1) from knowledge of daily light-cycle duration at one SUVR intensity has been developed. This assessment is presented as a preliminary estimate of photo-induced PAH toxicity to fish under natural conditions, and chronic hazard is discussed in relation to current environmental SUVR intensities and PAH concentrations in aquatic systems.

1.2 GENERAL MATERIALS AND METHODS

Laboratory system

Sunlight was approximated in the laboratory with a combination of Chroma F40C50 white (General Electric, Cleveland, OH) and FS40 ultraviolet (Westinghouse, Bloomfield, NJ) fluorescent bulbs. The lights were mounted on a 1.22 X 2.74 m frame on 15.24 cm centers, alternating every other bulb. A 5 mil thickness of cellulose triacetate (CTA) filter was used to eliminate wavelengths shorter than 285 nm. This combination of light provided a functional approximation of natural sunlight (Figures 3 and 4). The ratio of UV-A:UV-B under the laboratory lighting system was 1.42, whereas the UV-

Figure 3. Spectral characteristics of Westinghouse FS40 ultraviolet fluorescent lamps (Modified from Westinghouse 1980).

Figure 3.

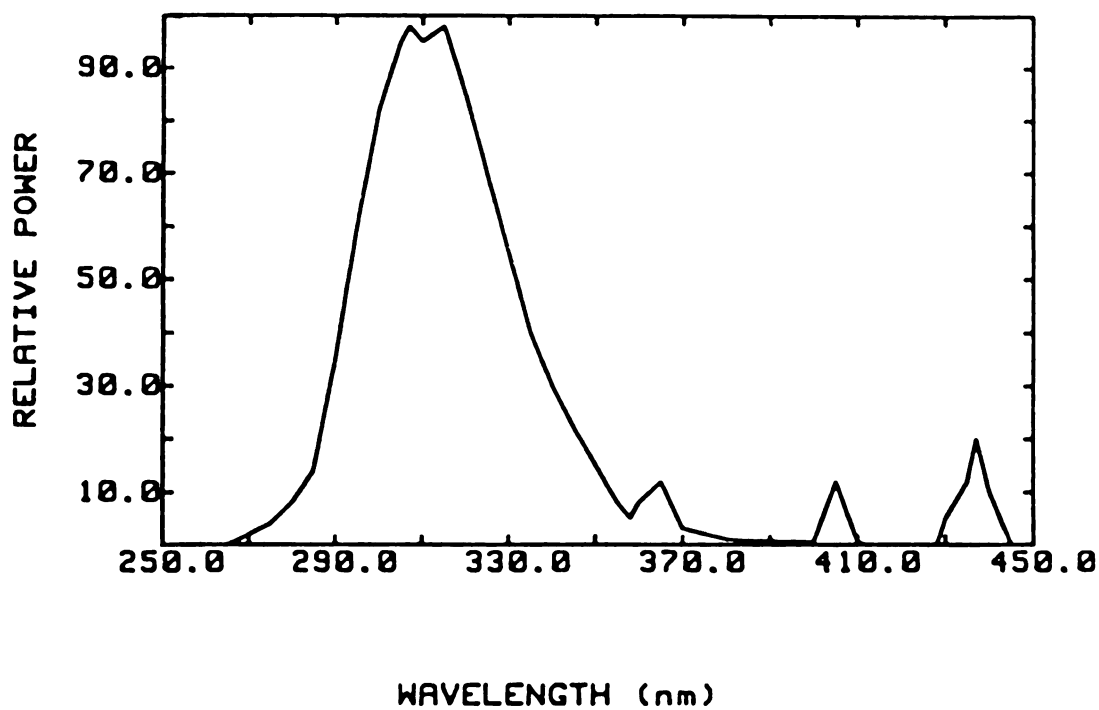
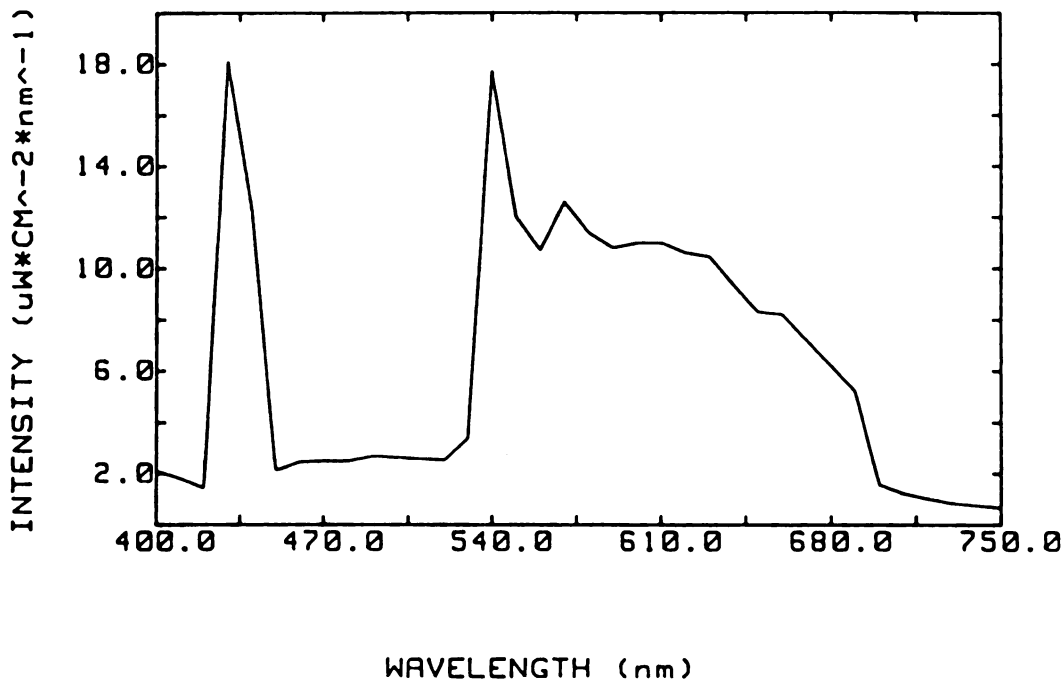

RELATIVE POWER
90.0
70.0
50.0
30.0
10.0
250.0
290.0
330.0
370.0
410.0
450.0
WAVELENGTH (nm)

Figure 4. Spectral characteristics and radiation intensity from 400 to 750 nm of the combination of FS40 ultraviolet and F40C50 white fluorescent lamps, 60 cm below the light bank.

Figure 4.


INTENSITY (uW*CM^-2*nm^-1)
18.0
14.0
10.0
6.0
2.0
400.0
470.0
540.0
610.0
680.0
750.0
WAVELENGTH (nm)

A:UV-B ratio under natural sunlight was measured to be 8.21. Thus, the laboratory lighting system was weighted in the UV-B region. Light intensity was varied by changing the height of the light bank over the bioassay table or by changing the thickness, by adding or removing layers, of the CTA filter.

Aqueous solutions of anthracene (M.W. 178.23, Sigma grade III, no. A-3885) were obtained from a once-through aqueous elution column, which obviated the use of carrier solvents in the bioassays. Columns were made by pouring anthracene, dissolved in acetone, onto a thin layer of silica sand at 0.2% wt/wt, and allowing the acetone to evaporate in the dark. When dry (24 h) the sand was packed into a 7.5 X 45 cm glass column and flushed with water for 48 h to remove loose anthracene crystals. Anthracene eluted from the column, as part of the laboratory water delivery system, at aqueous solubility (ca. 35 ug/L at 22^{o} C) and was diluted in the flow through system to a desired concentration before entering the aquariums.

Light measurement

UV-B (310 +/- 34 nm) and UV-A (365 +/- 36 nm) were quantified using a Macam Photometrics (Livingstone, Scotland) Model UV-103 radiometer equipped with Model SD104 cosine-corrected photodiodes fitted with water-tight, wavelength selective filters. Visible light (400 -

700 nm) was measured using a Techtum Instruments QSM-2500 (Sweden) scanning quantum spectrometer coupled to a LI-COR model LI188-B integrating quantum meter. SUVR intensities for all bioassays are shown in Table 2.

Analytical

Anthracene concentrations in water were determined directly by reverse-phase HPLC. Twenty microliters of sample or standard were injected onto a Partisil ODS-3, 10 um C18 column, at 30° C. An isocratic elution was performed with 9:1 acetonitrile:water at 1.0 ml/min. A Kratos FS-970 fluorescence detector was used at an excitation of 252 nm with a 370 nm emmission filter. Peaks were recorded and quantified on a Hewlett-Packard 3390-A integrator. The limit of detection for anthracene, using this technique, was 2.0 pg total mass, or 0.1 ug/L.

Anthracene concentrations in fish tissue were determined as follows. Live fish were removed from test chambers, blotted, weighed, wrapped in aluminum foil, placed in labelled plastic bags filled with N_2, and stored in a freezer until analysis. For extraction and analysis, thawed fish and anhydrous Na_2SO_4 was added at a ratio of 1 fish:200 Na_2SO_4 wt/wt to a glass mortar. This mixture was ground to a fine powder with a glass pestal. The ground mixture was put into a 250 ml erlenmeyer flask and 5 ml acidified benzene was added. Flasks were vigorously

shaken at 5 min intervals for 30 min. This extraction was followed by another 5 ml of acidified benzene, 2X 5 ml ethyl acetate, 2X 5 ml 15% ethyl ether in petroleum ether. Each volume of solvent was successively poured through a small column of activated Florisil^{R}. The Florisil^{R} was eluted with a final 15 ml volume of the ether mixture. The volume of the extracts was reduced by rotary evaporation and nitrogen evaporation to 0.2 ml. The extracts were then made up to 1.0 ml in acetonitrile. Fifty microliters of the final extract were injected onto a 10 um Partisil ODS-3 reverse phase column at 30^{o} C. Samples were eluted at 1.0 ml/min with a 10 min gradient from 50% acetonitrile in water to 100% acetonitrile, and were detected and quantified using the same equipment as for the water samples.

Organisms

For the SUVR intensity studies, a natural assemblage of juvenile sunfish (Lepomis spp.) was used. These fish were collected by seine from Park Lake, Clinton Co., Michigan. Other continuous light studies were conducted using juvenile bluegill sunfish (L. macrochirus R.) obtained from Osage Catfisheries (Osage Beach, MO) or from ByBrook Bass Hatcheries (Ashford, CT). All fish were 2 to 3 cm in length and were 0.5 to 1 g in weight. Sexually immature fathead minnows (Pimephales promelas; 3.6 to 4.3 cm, 0.8 to 1.4 g) were collected by trap from a pond at

the Limnological Research facility at Michigan State University (Kalamazoo Street). The photoperiod studies were conducted with hatchery bluegill sunfish (L. macrochirus R.) obtained from Osage Catfisheries. These fish were slightly larger than the bluegill sunfish used in continuous light studies. Fish were 3 to 3.5 cm in length and were 1 to 1.5 g in weight.

Different populations of fish were kept segregated and were held in large flow-through fiberglass tanks with charcoal filtered, aerated tap-water (Temp = 22 +/- 1 ^{o} C, pH = 8.20 +/- 0.27, D.O. = 7.15 +/- 0.24 mg/L, Hardness = 328 mg/L $CaCO_3$, Alkalinity = 346 mg/L $CaCO_3$). Juvenile and sexually immature fish were held for at least two weeks prior to bioassays on an 18:6 (hours light:hours dark) photoperiod under a low pressure sodium lamp (UV fluence negligible) and were fed twice a day with Biodiet-Starter (BioProducts Inc., Worrenton, OR).

Bioassays

Juvenile and sexually immature fish were exposed to anthracene in 20 L glass aquariums in a flow-through system under the laboratory lighting system (Table 2). Water quality was the same as that for holding fish. Sufficient flow was maintained in all aquariums to provide at least six turnovers per day. Fish were transferred to dosing aquariums 48 h prior to bioassays in the absence of UV radiation for acclimation to the test system and to

Table 2. Simulated solar ultraviolet radiation (SUVR) intensities used in bioassays with anthracene. UV-A = 310 +/- 34 nm, UV-B = 365 +/- 36 nm, PL = Park Lake juvenile sunfish, OS = Osage Catfisheries juvenile bluegill sunfish, BB = ByBrook Hatcheries juvenile bluegill sunfish, FH = fathead minnow eggs, larvae and adults.

		Experiment				
UV-A	UV-B	Continuous Light				Photoperiod
(uW/cm^2)		PL	OS	BB	FH	OS
22	15	*	*	-	-	-
100	70	*	-	*	*	*
240	170	*	-	-	-	-

establish a nominal body burden of anthracene approximately 80% of the theoretical steady state (Spacie et al. 1983). Actual body concentrations of anthracene in juvenile sunfish were 137.2 +/- 47.34 (+/- SE) percent of the estimated bioconcentration factor of 998 calculated from Spacie et al. (1983). Ten or eleven fish per aquarium and two aquariums per anthracene concentration were used in all bioassays. During the acclimation period in the SUVR intensity experiments the photoperiod was changed from 18:6 to continuous light in 2 h per day increments. During the acclimation period in the photoperiod experiments the photoperiod was changed (50% per day) from 18:6 to one of four different photoperiods: 24:0, 18:6, 12:12, or 6:18. The lights were wired into four independent circuits on separate 24 h time clocks. In the photoperiod experiments a 30 min transition period between light and dark was achieved by turning on (or off) each circuit at 10 mmin intervals at the initiation of each light or dark cycle. As part of a standard protocol (ASTM, 1984) fish were not fed for 48 h prior to, or for the first 96 h of a bioassay. After 96 h, fish were fed sparingly every other day for the duration of the test. Fecal and other particulate material was siphoned from the aquariums as needed. Mortality, gross physical damage, and behavioral changes were noted and recorded at least twice daily. A fish was considered dead when no opercular

movements could be detected. Water flows were checked and calibrated twice daily, and anthracene concentrations in water were monitored at the beginning of and at least once per additional 96 h of a bioassay.

Control experiments were performed during preliminary testing and during the bioassays. Preliminary tests were conducted where fish were exposed to anthracene as long as 96 h under darkness, under cool-white fluorescent lamps (General Electric, F40CW-RS-WM; > 400 nm), and under gold fluorescent lamps (General Electric, F40GO; > 500 nm) to determine any effects due to anthracene in the absence of SUVR. Also, in preliminary tests, fish were exposed to anthracene for 48 h in the dark and then transferred to clean water under SUVR for 96 h to ascertain any effects due to presence of anthracene in the water during SUVR exposure. Similarly, fish were exposed to anthracene for 48 h in the dark and transferred to clean water in the dark for 144 h to allow for depuration of the compound. These fish were then transferred to clean water and exposed to SUVR as long as 96 h. Concurrent with all anthracene-SUVR bioassays, a no-anthracene treatment was performed as an SUVR-only control. This treatment is reported as an anthracene concentration in water less than the analytical detection level of 0.1 ug anthracene/L.

Median lethal times (LT50) and/or median lethal concentrations (LC50) were calculated from the recorded

time-mortality and anthracene concentration data (Finney 1971). In the photoperiod experiments two different types of LT50 estimates were derived for each anthracene concentration at each photoperiod. One LT50 value was calculated on the basis of the real-exposure-time (R-LT50), including the time of anthracene exposure in both periods of SUVR-exposure and periods of darkness. For each R-LT50, another LT50 value was calculated solely on the basis of accumulated-SUVR-exposure-time (UV-LT50). Thus fish exposed during the 12:12 photoperiod, for example, only accumulated 50% of the SUVR-exposure-time compared to fish exposed during the same period of real-time in the 24:0 photoperiod.

Histology

At various times during continuous light bioassays, randomly selected fish were removed from each aquarium, swim bladders punctured, and were fixed in buffered formalin for 24 h. Whole fish were dehydrated in an ethyl alcohol series, cleared in xylene and embedded in Paraplast-Plus. Ten micron thick parasagittal sections were cut and then were stained with hemotoxylin and eosin. Sections were examined by light microscopy for abnormalities or lesions and compared to fish from the SUVR-only treatment.

1.3 RESULTS AND DISCUSSION OF CONTINUOUS LIGHT EXPERIMENTS

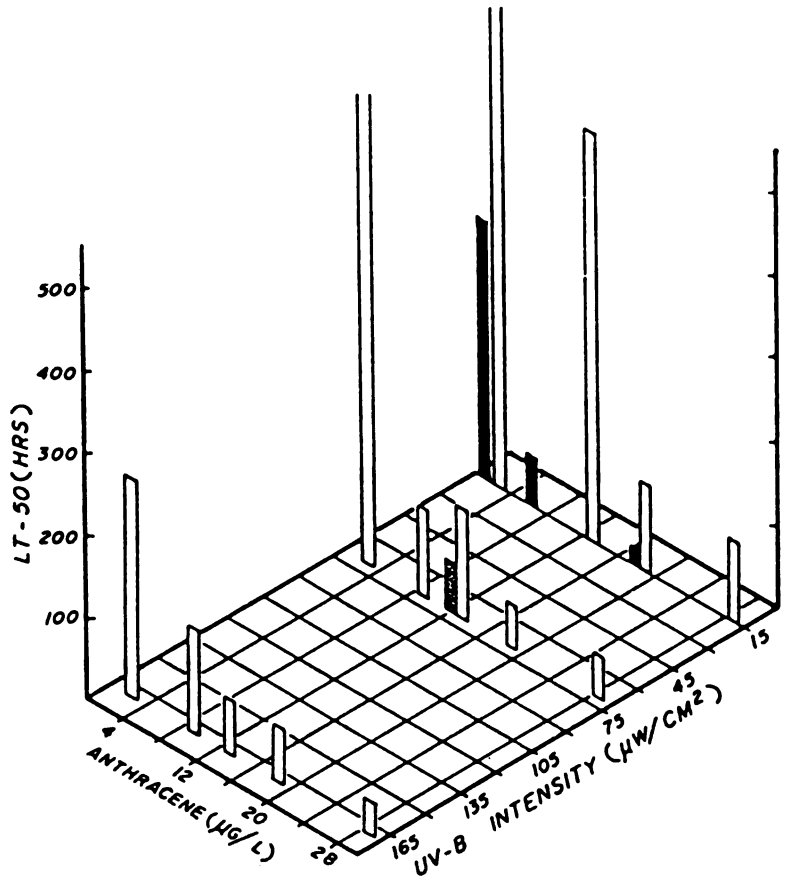
RESULTS

The toxic effects of anthracene were observed only in the presence of SUVR. Fish exposed to anthracene under darkness, under cool-white fluorescent bulbs, or under gold fluorescent bulbs were not adversely affected. Fish that were exposed to anthracene for 48 h in the dark and then transferred to clean water under SUVR exhibited signs of toxicity, but recovered slowly as anthracene was eliminated from the body. Sunfish that had attained steady-state body burdens of anthracene and were allowed sufficient depuration to elimate anthracene in the dark were not adversely affected when subsequently exposed to SUVR. These results are in agreement with the findings of Bowling et al. (1983). Mortality of fish exposed to simulated sunlight without anthracene as SUVR-only controls was less than 10 percent in all bioassays.

Under continuous laboratory illumination, the time to reach 50% mortality (LT50) for sunfish was dependent on both SUVR intensity and anthracene concentration (Figure 5). LT50 values for Park Lake fish ranged from 36.5 h at 26.8 ug anthracene/L and 170 uWatts/cm^2 UV-B to intermediate values (40 - 500 h) at intermediate and low light intensities and anthracene concentrations, with no mortality in 144 h at the lowest measured anthracene

Figure 5. Median lethal times (LT50) of juvenile sunfish exposed to continuous simulated sunlight as a function of UV-B intensity (310 +/- 34 nm) and anthracene concentration. Bars open at top indicate no mortality in 144 h. White bars = natural assemblage of juvenile bluegill sunfish collected from Park Lake, MI. Black bars and stipled bar = juvenile bluegill sunfish obtained from Osage Catfisheries, MO, and ByBrook Hatchery, CT, respectively. SUVR-only controls are not shown.

Figure 5.


LT-50(HRS)
500
400
300
200
100
4
12
20
28
ANTHRACENE (µG/L)
165
135
105
75
45
15
UV-B INTENSITY (µW/CM²)

concentration and UV-B intensity.

Affected fish showed signs of irritation and hypoxia. An increase in opercular ventilation rate was observed in an anthracene dose-related fashion (Figure 6), suggesting a respiratory involvement in the toxic response. Dead fish exhibited signs of asphyxia: open mouth, splayed opercula, and pale gill filaments (Reichenbach-Klinke and Landolt 1973). Compared to SUVR-only controls, gills of SUVR-anthracene treated fish lacked a defined epithelial cell layer, and in most cases, only the structural supporting pillar cells appeared to remain intact (Figure 7). Dorsal surfaces became thickened and aquired a creamy white appearance similar to the description of sunburn in fish presented by Bullock (1982). Sections of dorsal epidermis, anterior to the dorsal fin, from SUVR-anthracene treated fish were structurally disorganized and extensively eroded compared to SUVR-only controls (Figure 8). A severe necrotic condition and rapid loss of the upper epidermal cell layers was evident in these fish.

Based on the comparison of 96 h LC-50 values, the fathead minnows were the least sensitive among all fish to the light-anthracene combination (Table 3). In fact, the 96 h LC50 for the fathead minnows exceeded the aqueous solubility of anthracene. The natural assemblage of juvenile sunfish was not as sensitive as were the hatchery bluegill sunfish (Table 3). Bluegill sunfish from Osage Catfisheries were 10 times more sensitive than sunfish

Figure 6. Opercular ventilation rate as a function of anthracene concentration in juvenile bluegill sunfish exposed concurrently to simulated sunlight. Error bars represent 2 SE, and a > b > c (Student's T, alpha = 0.05).

Figure 6.

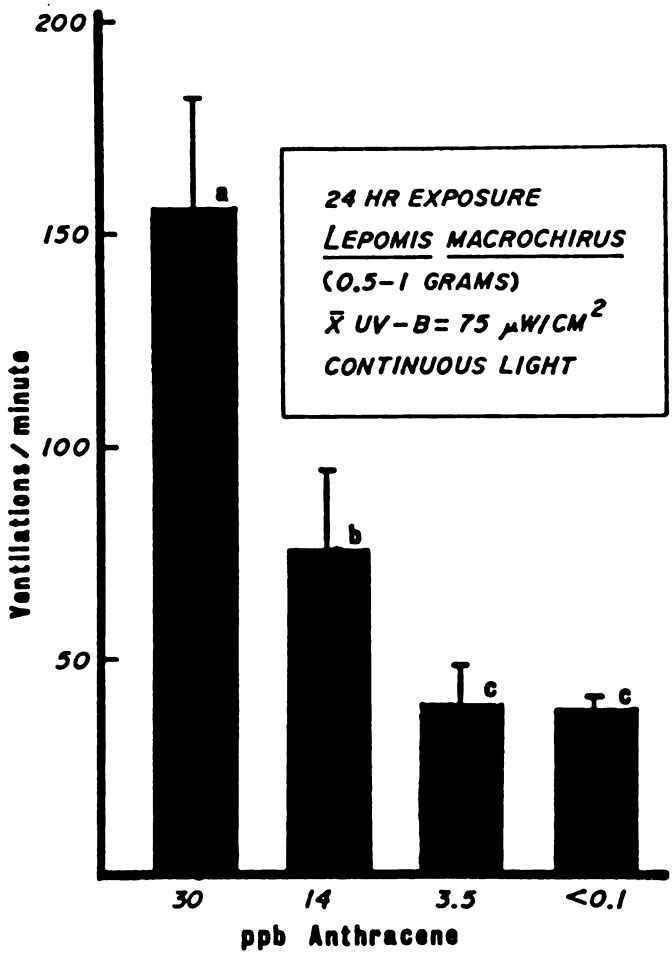

200
150
100
50
Ventilations / minute
24 HR EXPOSURE
LEPOMIS MACROCHIRUS
(0.5-1 GRAMS)
X̄ UV-B= 75 μW/CM²
CONTINUOUS LIGHT
a
b
c
c
30
14
3.5
<0.1
ppb Anthracene

Figure 7. Photomicrographs of gill filaments from juvenile bluegill sunfish exposed to <0.1 ug anthracene/L (SUVR-only control) for 144 h (A) and to 9.98 ug anthracene/L for 24 h (B), at 14.8 uW/cm^2 UV-B (310 +/- 34 nm) continuous simulated sunlight. Scale bar represents 50 um.

Figure 7.

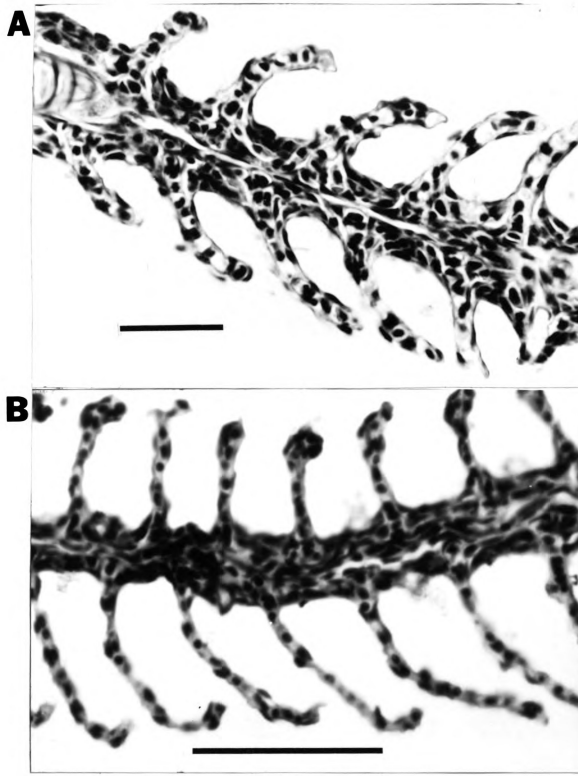


Figure 8. Photomicrographs of dorsal epidermis, anterior to the dorsal fin, from juvenile bluegill sunfish exposed to <0.1 ug anthracene/L (SUVR-only control) for 144 h (A) and to 9.98 ug anthracene/L for 24 h (B), at 14.8 uW/cm^2 UV-B (310 +/- 34 nm) continuous simulated sunlight. Scale bar represents 20 um.

Figure 8.

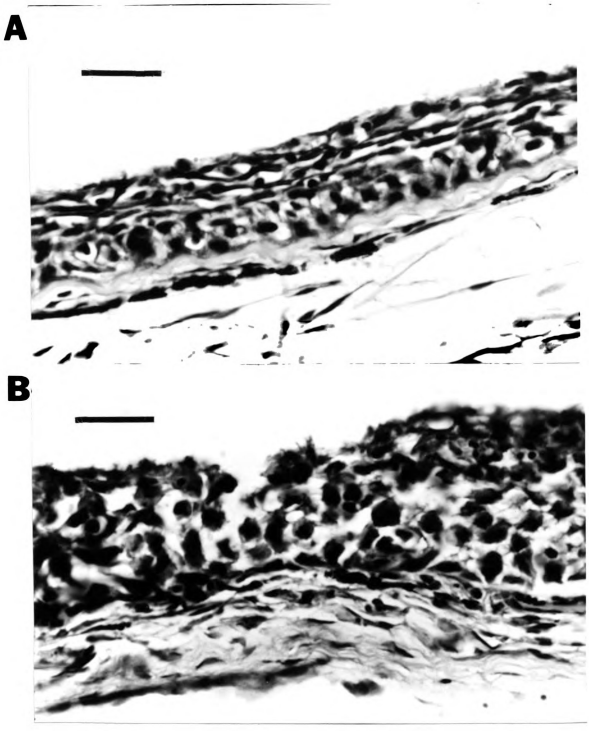


Table 3. 96 h LC50 values for different populations of fish exposed to anthracene at different SUVR intensities in the laboratory under conditions of continuous light. PL = Park Lake juvenile sunfish, OS = Osage Hatcheries juvenile bluegill sunfish, FH = sexually immature fathead minnows.

FISH	UV-B Intensity (uW/cm^2)	LC50 (ug/L)	95% Fiducial limits Lower	Upper
PL	15	26.47	22.62	34.48
	70	18.23	16.14	21.11
	170	11.92	10.15	13.40
OS	15	2.78	1.94	3.92
FH	70	41.38	35.71	53.08

from Park Lake. Although no LC50 value could be calculated for ByBrook bluegill sunfish, for at least one anthracene concentration and UV-B intensity these fish were approximately twice as sensitive as fish from Park Lake. LT50 values were 63 h and 135 h for fish from ByBrook and from Park Lake, respectively, at comparable anthracene concentrations and at the same UV-B intensity (Figure 5). No direct comparisons between bluegill sunfish from Osage and ByBrook could be made.

DISCUSSION

The incorporation of an important environmental parameter in aquatic ecosystems (ie. SUVR) into laboratory toxicity tests has demonstrated that at least one common PAH is extremely toxic to fish. Anthracene has not been reproted to be acutely toxic to fish in laboratory experiments when SUVR was not a consideration. Spacie et al. (1983) studied the pharmacokinetics of anthracene in L. macrochirus under gold fluorescent lighting and observed no mortality after 48 h exposures as great as 32 ug/L. Exposure of rainbow trout for 72 h to anthracene at a concentration of 50 ug/L in the absence of SUVR did not cause any signs of toxicity (Linder et al. 1985). In similar studies, no adverse effects were reported after dosing young coho salmon with 14 or 28 ug anthracene by injection or feeding, respectively (Roubal et al. 1977).

Applegate et al. (1957) reported a 24 h no-effect anthracene concentration of 5 mg/L for juvenile bluegill sunfish. This no-effect value is 190, 274, and 420 times greater than the calculated 96 hr LC50 values in these experiments at UV-B intensities of 15, 70, and 170 uW/cm^2, respectively, for the natural assemblage of juvenile sunfish (Table 3). On the basis of LC50 values, bluegill sunfish from the Osage hatchery were 1800 times more sensitive than the no-effect concentration at a UV-B intensity of 15 uW/cm^2 (Table 3).

While studying the fate of anthracene in outdoor artificial stream microcosms at the Savannah River facility in South Carolina, Bowling et al. (1983) observed unexpected acute mortality among juvenile bluegill sunfish held in cages exposed to full sunlight in the channels. It was concluded that SUVR significantly enhanced the toxicity of anthracene to fish. Calculated LT50 values from the data presented by Bowling et al. (1983) for 12 ug anthracene/L are 38 h and 23.6 h for real exposure time (R-LT50) and for cumulative light exposure (UV-LT50), respectively. Cumulative light exposure LT50's were calculated based on the total number of hours of light during the anthracene-light dosing period. This manipulation was necessary in order to compare results from studies conducted outdoors, under fluctuating conditions of SUVR, to the results under conditions of

continuous light. In downstream reaches of the artificial channels, where anthracene concentrations fluctuated from 12 ug/L to 5 ug/L on a daily basis due to photolytic degradation, calculated toxicity values are 45.5 h and 28 h for R-LT50 and for UV-LT50, respectively. These values are comparable to the results obtained in this study (cf. Figure 5), making the necessary assumption that SUVR intensities were greater in the outdoor experiments, where no radiometry was reported, than in the present laboratory system. This assumption has validity since the maximum light intensity achieved in the present laboratory system was approximately 5 to 10 times less than what has been measured at the water surface during the summer months on Lake Michigan (unpublished data).

Site of toxic action

Signs of hypoxia and death from apparent asphyxiation suggested that SUVR-anthracene treatment adversely affected the gills. Ventilations per minute were increased from 2 to greater than 3 times the rate observed for the SUVR-only treatment in one experiment (Figure 6), except in the case of the 3.5 ug anthracene/L treatment where no fish died during the experimental period of 144 h. In all bioassays, mortality reached 100 percent within 144 h in treatments with fish exhibiting a statistically significant (Student's T, alpha = 0.05) increase in ventilation rate after 24 h SUVR-anthracene exposure.

Ventilation rate of SUVR-only controls, and SUVR-anthracene treated fish that were not adversely affected, were not significantly different from the rates reported for free swimming normal controls of juvenile L. macrochirus (Carlson 1982). Ventilation rates of fish significantly increased by SUVR-anthracene treatment ranged from the same to 2 times greater than the rates reported for free swimming juvenile bluegill sunfish exposed to an acutely toxic concentration of $ZnSO_4$, a known gill toxicant, for 24 h (Carlson 1982). This evidence, along with the observed damage to the gill epithelium (Figure 7), implicates the gills as being a major site of SUVR-anthracene toxic action. However, destruction of dorsal epidermal cells (Figure 8) indicates that the actions of anthracene phototoxicity are not specifically located at the gills. Instead, a more general mode of action is presumed.

The mechanism of toxic action is not known but evidence from a broad base of phototoxicity literature indicates that damage to cell membranes might be involved. Anthracene and other lipophilic molecules are known to bind to membrane constituents (Sinha and Chignell 1983) and, in the presence of SUVR, can induce cellular damage. Membrane function can be altered through the inactivation of enzymes (Gietzen et al. 1980; Wat et al. 1980; Sandburg et al. 1981), the cross linking of proteins and peroxidation of lipids (Sinha and Chignell 1983), the

alteration of membrane fluidity and permeability (Kahn and Fleischaker 1971; Copeland et al. 1976; Utsumi and Elkind 1979; Keefe et al. 1980), and the photolysis of amino acids, unsaturated fatty acids, cholesterol, or carboxylic acids (Gennari et al. 1974; Sysak et al. 1977; Logani et al. 1981). Photo-induced lysosomal membrane alteration and subsequent leakage of hydrolytic enzymes (Allison et al. 1966; Honigsmann et al. 1974; Santus et al. 1983) could cause the destruction of cells of the gill epithelium and of the dorsal epidermis. Coincident with gill membrane damage, increased respiratory activity could be induced via deleterious changes in respiratory enzymes. Additionally, respiratory stress could be brought about as a result of photosensitized hemolysis of red blood cells (Wat et al. 1980) or of decreased blood microcirculation (Castellani et al. 1963). Respiratory activity may also increase during a general stress syndrome in response to greater metabolic demand for the repair of damaged physiological systems or for the compensation of osmoregulatory stress induced by the loss of cell membrane integrity. It is postulated that the photo-induced toxicity of anthracene is manifested in a general disruption of cellular membranes leading to an overwhelming metabolic demand on the organisms for repair processes and for the compensation of osmotic imbalance.

Population sensitivity differences

At present, it is not known why the different populations of fish varied so widely in sensitivity to the light-anthracene combination. One possibility for the observed differences in sensitivities between the sunfish populations is the fact that the natural population of sunfish was a mixed and unavoidably hybridized population. Hybrid sunfish are known to be less sensitive to low oxygen conditions compared to L. macrochirus due to differences in the oxygen affinities of the respective hemoglobins (Hochachka and Somero, 1973). Since the physiological and histological findings reported here indicate that adversely affected fish are under respiratory stress, undergo damage to gill surfaces, and die of asphyxiation, it would be expected that the natural population of sunfish would be less sensitive to anthracene photo-induced toxicity than the hatchery bluegill sunfish. The difference of sensitivity between sunfish populations and the minnows can also be partially explained by oxygen stress susceptability. Cyprinid fish are well known to be less sensitive to oxygen stress compared to centrarchids. However, the minnows were larger and more pigmented than the sunfish which may have also contributed to the lesser sensitivity. In addition, other biochemical and genetic differences or subtle differences in the health status of the different populations are possible explanations for the observed

ranges of sensitivities. It seems likely, however, that qualitative predictions of species sensitivity to acute photo-induced anthracene toxicity could be made on the basis of sensitivity to respiratory stress. More detailed study is needed in this area before any definitive conclusions can be made with regard to population differences in sensitivity to anthracene phototoxicity.

Predictive model

While the importance of investigating the site and mode of toxic action of photo-induced anthracene toxicity is recognized, it is also important to consider the potential environmental consequences involved with this phenomenon at the organism and population levels of organization. In order to establish guidelines and to protect fish and other aquatic organisms it is necessary to develop the ability to predict the toxicity of anthracene in the environment given a particular intensity of SUVR and aqueous concentration of anthracene. A preliminary predictive relationship based on the Bunsen-Roscoe photochemical law of reciprocity has been developed. This law states that for any photochemical reaction, the product of the light intensity and the reaction time are constant for a fixed concentration of sensitizer if there are no complicating side reactions (Dworkin 1958). In other words, given a fixed anthracene

concentration in water, if an LT50 of 50 h is calculated at a UV intensity of 100 uW/cm^2, then at 200 uW/cm^2 the law of reciprocity predicts an LT50 of 25 h. The relationship between UV-B intensity multiplied by the time to 50% mortality, and anthracene concentration from laboratory experiments conducted under conditions of continuous light was investigated (Figure 9). The regression is linear (r^2 = 0.78) and significant (P = 0.004). However, when making predictions in the environment it is desirable to obtain a fairly high degree of certainty in the accuracy of the prediction. The necessary level of accuracy is dependent on the margin of safety which is utilized when standards or guidelines are developed from laboratory toxicity tests. It is evident from the continuous confidence bands for the 90th and 95th percentile drawn around the regression (Figure 9) and noting the log-scale on the Y-axis, that the degree of confidence in the predictions is not very great. Additionally, the model consistently overpredicts toxicity at the greater light intensities and underpredicts toxicity at lesser light intensities. These discrepencies indicate that the toxic phenomenon does not entirely follow the reciprocity law, and that complicating processes such as biological repair may be occurring.

To test the applicability of the predictive model, an acute static-renewal anthracene phototoxicity bioassay was conducted under natural sunlight, and the results compared

Figure 9. Regression, based on the Bunsen-Roscoe law of reciprocity, of the product of light intensity and median lethal time versus anthracene concentration in water for continuous simulated sunlight exposures. ▲= experiments conducted at UV-B intensity of 170 uW/cm^2, ■ = experiments conducted at 70 uW/cm^2, and ● = experiments conducted at 15 uW/cm^2 UV-B. Lines A and B are continuous confidence bands around the regression for the 95th and 90th percentile, respectively.

Figure 9.

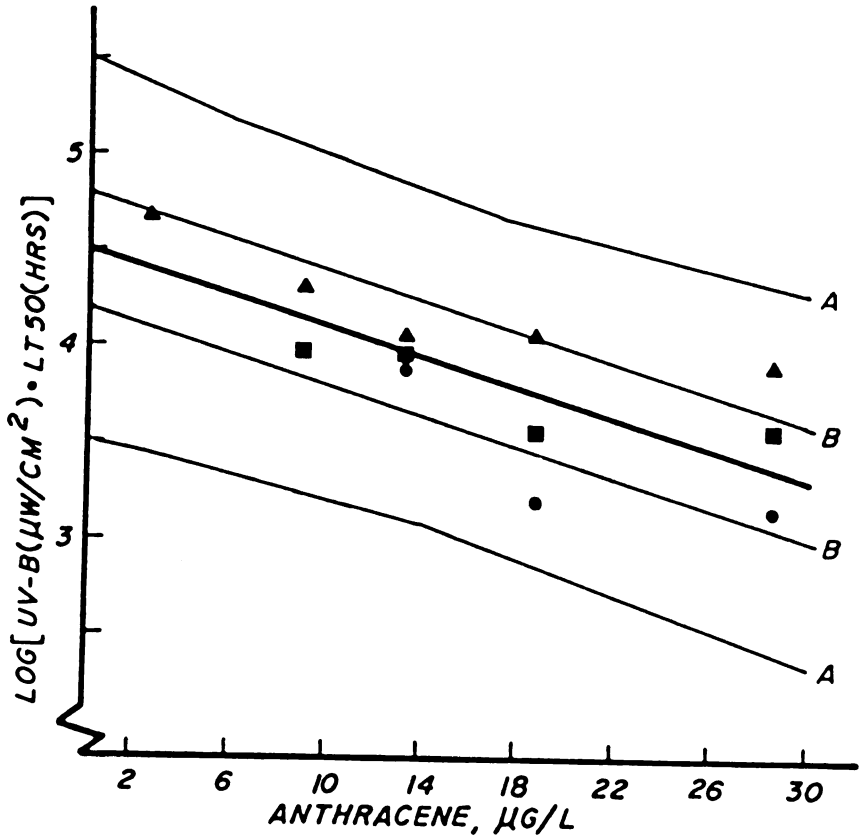

LOG[UV-B(μW/CM²)·LT50(HRS)]
5
4
3
A
B
B
A
2
6
10
14
18
22
26
30
ANTHRACENE, μG/L

Table 4. Comparison of lab predictions to field results. Laboratory predictions are for 24 hr continuous light at 400 uW/cm^2 UV-B. The field experiment had a photoperiod of 11.75:12.25 hr L:D and a measured mean light intensity of 400 uW/cm^2 UV-B. Field results are expressed in total hours of light exposure, making the assumption that no biological repair processes took place during the dark hours.

Anthracene (ug/l)	Lab LT-50 Prediction (h)	90% Confidence Range (h)	Field LT-50 Result (Light-h)
1.5	70.72	39.17 - 123.86	66.57
7.0	43.05	23.88 - 77.26	37.83
22.0	11.12	6.14 - 19.86	10.45

to laboratory bioassays (Table 4). The LT50 values for the field experiment fall well within the range of accepted statistical confidence from the laboratory predictions. LT50 values calculated from Bowling et al. (1983) also correspond to laboratory predictions. The closeness of agreement among the laboratory predictions and the field results indicates that although there are many potential complicating factors, accurate predictions concerning the toxicity of anthracene under natural sunlight can still be made. These factors include the constantly changing intensity of SUVR under natural conditions, and biological repair processes. The predictions in Table 4 are based on the measured mean UV-B intensity over a 48 h period while the predictive relationship was developed under continuous light and intensity conditions. That relatively accurate predictions from controlled laboratory conditions to fluctuating environmental conditions can still be made is interesting given all of the possible confounding interactions of PAH and biological systems.

1.4 RESULTS AND DISCUSSION OF PHOTOPERIOD EXPERIMENTS

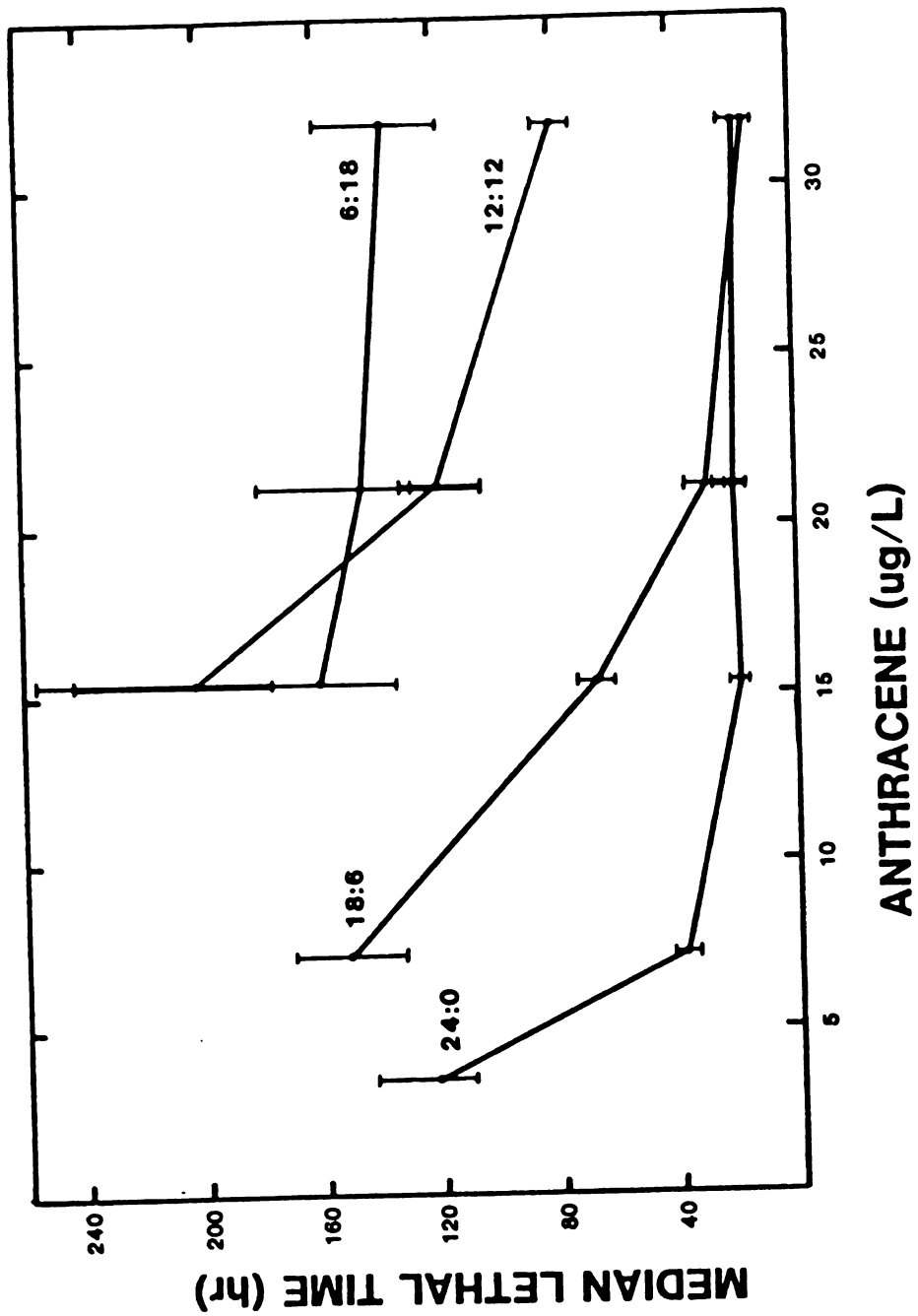
RESULTS

SUVR-anthracene treated fish exhibited identical signs of adverse effect as in the SUVR intensity study. Affected fish exhibited tremors and signs of irritation and hypoxia. Low levels of external stimuli would induce bouts of uncontrolled coughing and uncoordinated swimming in affected fish. Severely affected fish were observed at the bottom of the aquariums along the sides or in the corners, probably to avoid SUVR. Fish that had been observed to be adversely affected by SUVR-anthracene treatment during a light cycle exhibited signs of partial recovery after a period of darkness. Signs of hypoxia were less evident and these fish were less prone to bouts of coughing and uncoordinated swimming. However, within 1 h after the initiation of another light cycle, signs of intoxication would return. Over several photoperiod cycles, the condition of these fish progressively deteriorated until mortality occurred.

Time to mortality was dependent both on anthracene concentration and on daily light-cycle length (Figure 10). Real-time median lethal times (R-LT50) ranged from 20 h, at an anthracene concentration of 15 ug/L or greater and photoperiod of 24:0, to 202 h at 15 ug/L and photoperiod

Figure 10. Plot of Real-time median-lethal-time (R-LT50) versus anthracene concentration in water for photoperiods of 24:0 (hours light:hours dark), 18:6, 12:12, and 6:18. Error bars = 95 % C.I.

Figure 10.


MEDIAN LETHAL TIME (hr)
240
200
160
120
80
40
ANTHRACENE (ug/L)
5
10
15
20
25
30
6:18
12:12
18:6
24:0

of 12:12 or 6:18. Above 15 ug/L R-LT50 was independent of anthracene concentration during the continuous photoperiod. Anthracene dose-independence of R-LT50 was observed above 20 ug/L during the 18:6 photoperiod. During the 18:6 photoperiod dose-independence of mortality rate occurred at greater anthracene concentrations than during the 24:0 photoperiod, however the maximum rate of mortality between the two photoperiods was not significantly different (Figure 10).

DISCUSSION

An important objective of studying photoperiod effects was to examine rates of phototoxic damage versus rates of physiologic repair. The assumption that damage is non-repairable during periods of darkness has allowed for an initial estimate of LT50 values for any given anthracene concentration and SUVR intensity (Section 1.3). The present study was conducted, in part, to test the validity of that assumption.

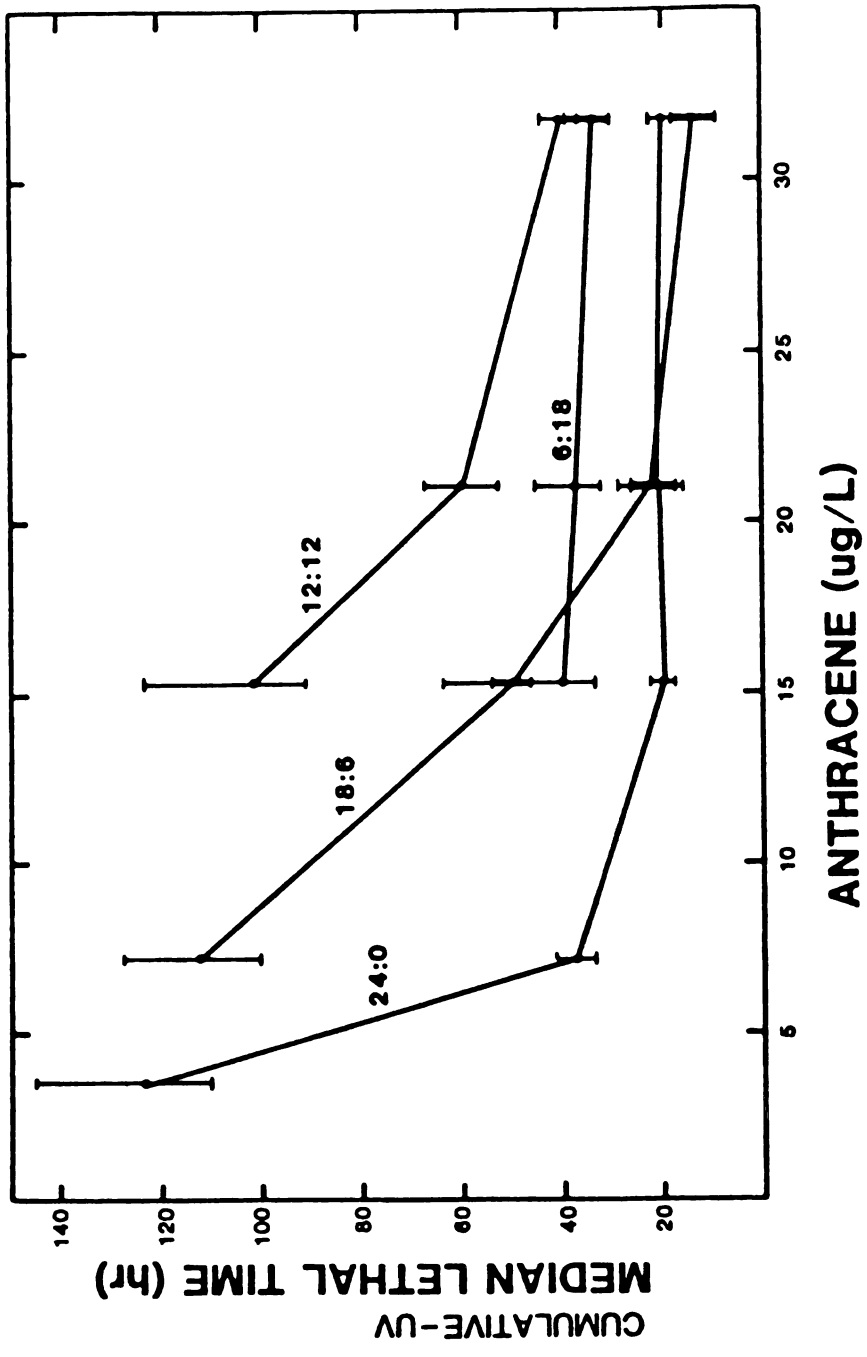
Assessment of the repairability of damage can be accomplished by the examination of the different LT50 estimations. R-LT50 is considered to be an integrated measure of the difference between rate of damage and rate of repair. UV-LT50 is considered to measure the rate of damage for cumulative-SUVR-exposure, assuming that little or no repair occurs during SUVR-exposure periods. If

damage only occurs during the light cycle and there is little or no repair during the dark cycle, then the damage would be expected to accumulate as a direct function of the total amount of SUVR exposure, regardless of the length of cycling periods of darkness. Therefore, if damage is cumulative then UV-LT50 values for a given anthracene concentration should all be the same magnitude, independent of daily light cycle length. Where damage is shown to be cumulative, then knowledge only of total SUVR dose (intensity X duration) and of PAH dose are needed to predict the photo-induced toxic response. That is, reciprocity would exist between SUVR dose, dose-rate and duration, and toxicity could be expressed on an SUVR-exposure-time basis.

The results obtained in this study suggest that photo-induced anthracene toxicity to bluegill sunfish involves a combination of cumulative and repairable damage. LT50 values calculated on the basis of SUVR-exposure-time were more similar among photoperiods than the R-LT50 values but were still dependent on daily light cycle duration (Figure 11). These results indicate that damage is not entirely cumulative and that damage incurred in the light cycle is partially repaired as a direct function of the dark cycle length. The observation of partial recovery after a dark cycle corraborates this evidence. The achievement of a maximum rate of mortality across anthracene concentration at the two longest

Figure 11. Plot of median-lethal-time, calculated based on cumulative-light-hours exposure (UV-LT50), versus anthracene concentration in water for photoperiods of 24:0 (hours light:hours dark), 18:6, 12:12, and 6:18. Error bars = 95 % C.I.

Figure 11.


CUMULATIVE-UV
MEDIAN LETHAL TIME (hr)
140
120
100
80
60
40
20
ANTHRACENE (ug/L)
5
10
15
20
25
30
12:12
6:18
18:6
24:0

photoperiods also implies that damage involves an equilibrium (reversible) process, as well as that a constant threshold amount of damage must occur before mortality is observed. In addition, the UV-LT50 anthracene dose-response curve for the 6:18 photoperiod is inverted in position relative to the curve for the 12:12 photoperiod (Figure 11) when compared to the same dose-response curves for R-LT50 values (Figure 10). This inversion suggests that at some point between 12 h and 6 h of SUVR per day there is a shift away from reversible to cumulative damage and that below a threshold length of SUVR exposure, anthracene becomes more toxic per SUVR-hour than at longer photoperiods. Due to this discontinuous response across daily light-cycle duration of UV-LT50 values and because there is evidence that damage is only partially cumulative, knowledge of daily SUVR exposure period is desirable in addition to total SUVR dose to accurately describe anthracene phototoxicity to juvenile bluegill sunfish.

Predictive Hazard Assessment

In the continuous SUVR studies it was demonstrated that median lethal time (LT50) values for photo-induced anthracene toxicity to juvenile sunfish could be predicted from knowledge of total SUVR dose and concentration of anthracene in water. This prediction was made on the assumption that no repair of photo-induced toxic damage

occurred in the absence of SUVR. That is, that damage accumulated as a direct function of total SUVR dose. While the previous predictive relationship still represents a sound preliminary estimate of photo-induced anthracene toxicity, this study has demonstrated that the assumption of cumulative damage is not totally valid. A predictive relationship that would account for variations due to both SUVR intensity and daily SUVR-exposure-time would be the most accurate mechanistic description of photo-induced anthracene toxicity. Also, it is often more desirable to obtain estimates of lethal concentration (LC) values, rather than lethal time (LT) values that require a priori knowledge of toxicant concentrations, especially for the determination of guidelines and interim water quality criteria. Therefore, in developing a predictive hazard assessment for photo-induced anthracene toxicity to bluegill sunfish the two independent variables considered were SUVR intensity and photoperiod duration. The dependent variable chosen was the anthracene concentration that would cause lethality in 1% of the population after exposure for an infinitely long period of time (∞ LC1). This value was estimated from laboratory derived acute dose-response (log anthracene concentration - probit) data using the methods outlined in Mayer et al. (1984).

The derivation of this chronic toxicity estimate is slightly different than that proposed in the original

method (Mayer et al. 1984) which uses the chronic LC0. I have chosen to use the chronic LC1 as opposed to the chronic LC0 since it was felt that my estimate was a conservative and acceptable level of risk for fish populations and because of the inherent large amount of uncertainty involved with extrapolating to LC0 values in a probability relationship. It is important to note that any level of risk, such as LC0.1 or LC10, could have been assigned for this value. The greater proportion of effect assigned possesses a higher degree of statistical certainty in the prediction, and vice versa. While admittedly arbitrary, my level of ∞LC1 was chosen as the most statistically economic value weighed against what was decided to be an acceptable level of long-term effect on natural fish populations. It is also important to note that these estimates are based solely on acute lethality test data (24 - 144 h). Presently, there are no chronic lethality tests available concerning photo-induced PAH toxicity to fish and I wanted to examine the probability of chronic effects at environmentally relevant SUVR intensities and anthracene concentrations. Since chronic testing was beyond the scope of the present study, chronic estimates from acute data were necessary. The method used to derive these estimates has been shown to be relatively accurate, and it was felt that the estimates were of sufficient certainty to be used in an attempt to assess the environmental hazard of the photo-induced toxicity of

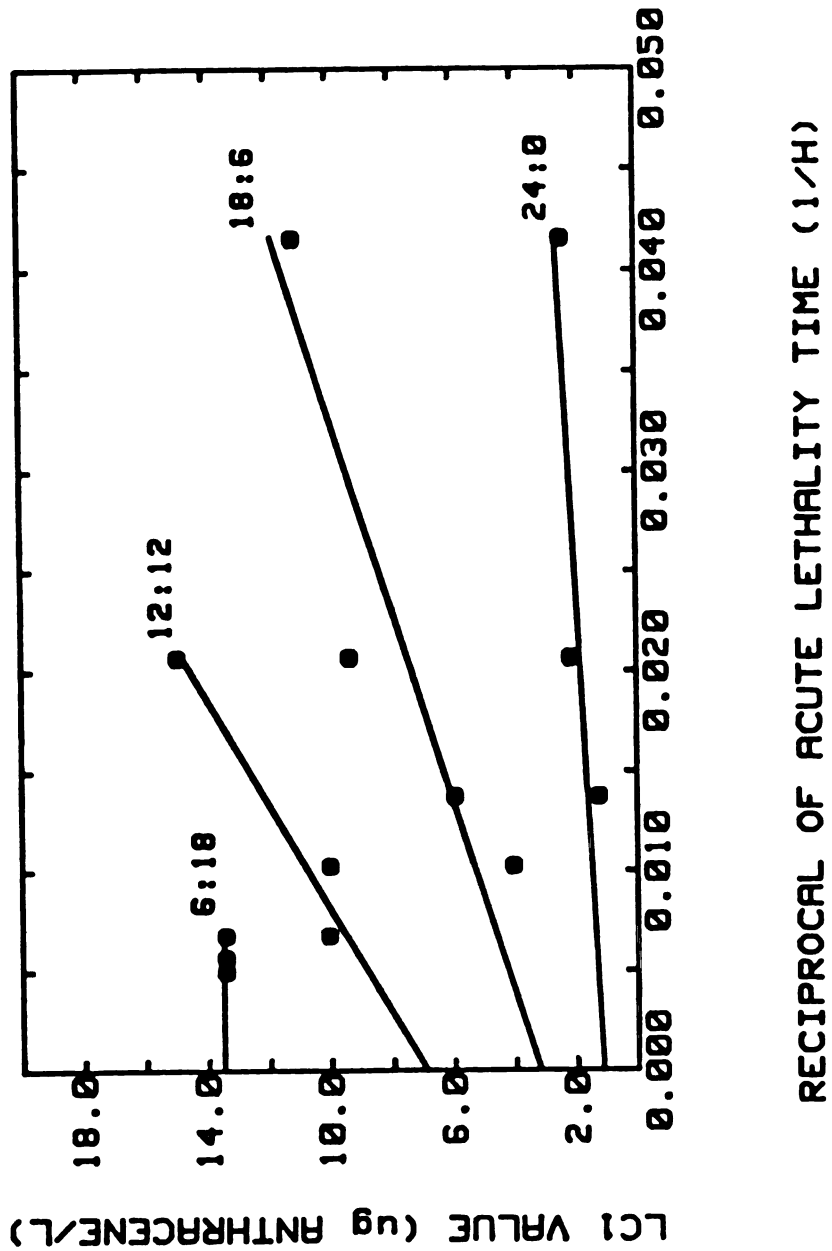
anthracene.

The predictive relationship for chronic hazard assessment for the photoperiod experiments was developed as follows. Acute LC1 values were estimated (Finney 1971) from the tests conducted for each photoperiod for real-time periods of 24, 48, 72, 96, and 144 h. These values were regressed against the reciprocal of the respective time periods (Figure 12). The estimated ∞LC1 value for each photoperiod was then derived as the least squares linear regression estimate of the intercept from each of these plots. This procedure resulted in estimates of LC1 for each photoperiod for a UVA (365 +/- 36 nm) intensity of 100 uW/cm^2.

The incorporation of predictions of chronic LC1 values for all SUVR intensities from the continous light study with the predictions for the different photoperiods from this study was not possible. The fish used in the SUVR intensity experiments were collected from a natural population of hybridized sunfish, and these fish were found to be more resistant to anthracene-SUVR toxicity than the bluegill sunfish used in this study. Even though the slopes of the anthracene dose-response curves at the common SUVR intensity between the two studies were very similar, I did not feel justified in making any adjustments for differential species sensitivity for that or other SUVR intensities. Even if species sensitivities

Figure 12. The relationship between anthracene concentration and acute LC1 values as a function of the reciprocal of acute lethality time period. Plotted points represent estimated LC1 values calculated from log-anthracene, probit analysis. Plotted lines are linear least squares estimates for each photoperiod. Chronic LC1 values are predicted as the Y-intercepts from these regressions: 6:18 -- Y = 13.5 + 0.00(X), 12:12 -- Y = 6.901 + 380.53(X), 18:6 -- Y = 3.196 + 207.38(X), 24:0 -- Y = 1.151 + 34.23(X).

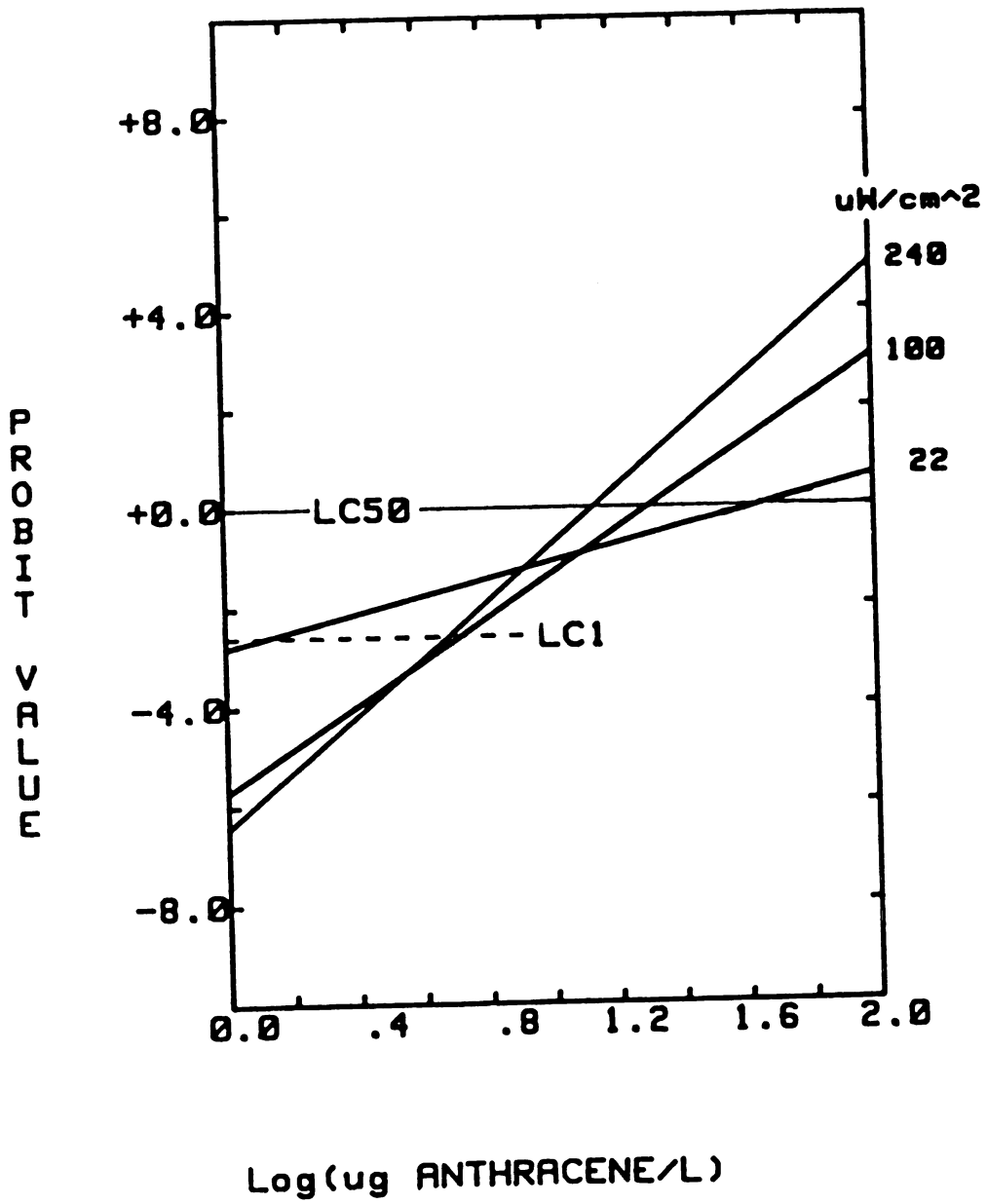
Figure 12.


LC1 VALUE (ug ANTHRACENE/L)
18.0
14.0
10.0
6.0
2.0
0.000
0.010
0.020
0.030
0.040
0.050
RECIPROCAL OF ACUTE LETHALITY TIME (1/H)
6:18
12:12
18:6
24:0

were similar, the data from the SUVR intensity experiments could not be incorporated in the chronic toxicity estimates using the proposed method. One of the requirements of using this method for estimating chronic toxicity values is that the dose-response curves for each of the SUVR intensities must not intersect. The anthracene dose-response curves for the different SUVR intensities do, in fact, intersect (Figure 13) implying an interaction between anthracene dose and SUVR intensity as they relate to photo-induced toxicity. Only the 96 h dose-response curves are shown as a representative example of what occurs at several acute lethality time periods. The interaction between anthracene concentration and SUVR intensity causes the acute LC1 value estimates to become reversed in order of magnitude compared to the acute LC50 values for each respective SUVR intensity. The end result of this interaction is that chronic toxicity predictions are also reversed in order of magnitude compared to acute toxicity estimates for each respective SUVR intensity. The simultaneous exposure of fish to both anthracene and SUVR can be considered as a multiple toxicant exposure. Apparently the method used to derive chronic toxicity predictions cannot account for multiple toxicant exposure where interaction among toxicants occurs. Until a method is developed that will account for these interactions, it is suggested that chronic photo-induced anthracene LC values be predicted on the basis of the intermediate SUVR

Figure 13. Plot of calculated probit values versus log10(anthracene concentration) showing the interaction between UV-A (365 +/- 36 nm) intensity and anthracene concentration.

Figure 13.


+8.0
+4.0
+0.0
-4.0
-8.0
PROBIT VALUE
uW/cm^2
240
100
22
LC50
LC1
0.0
.4
.8
1.2
1.6
2.0
Log(ug ANTHRACENE/L)

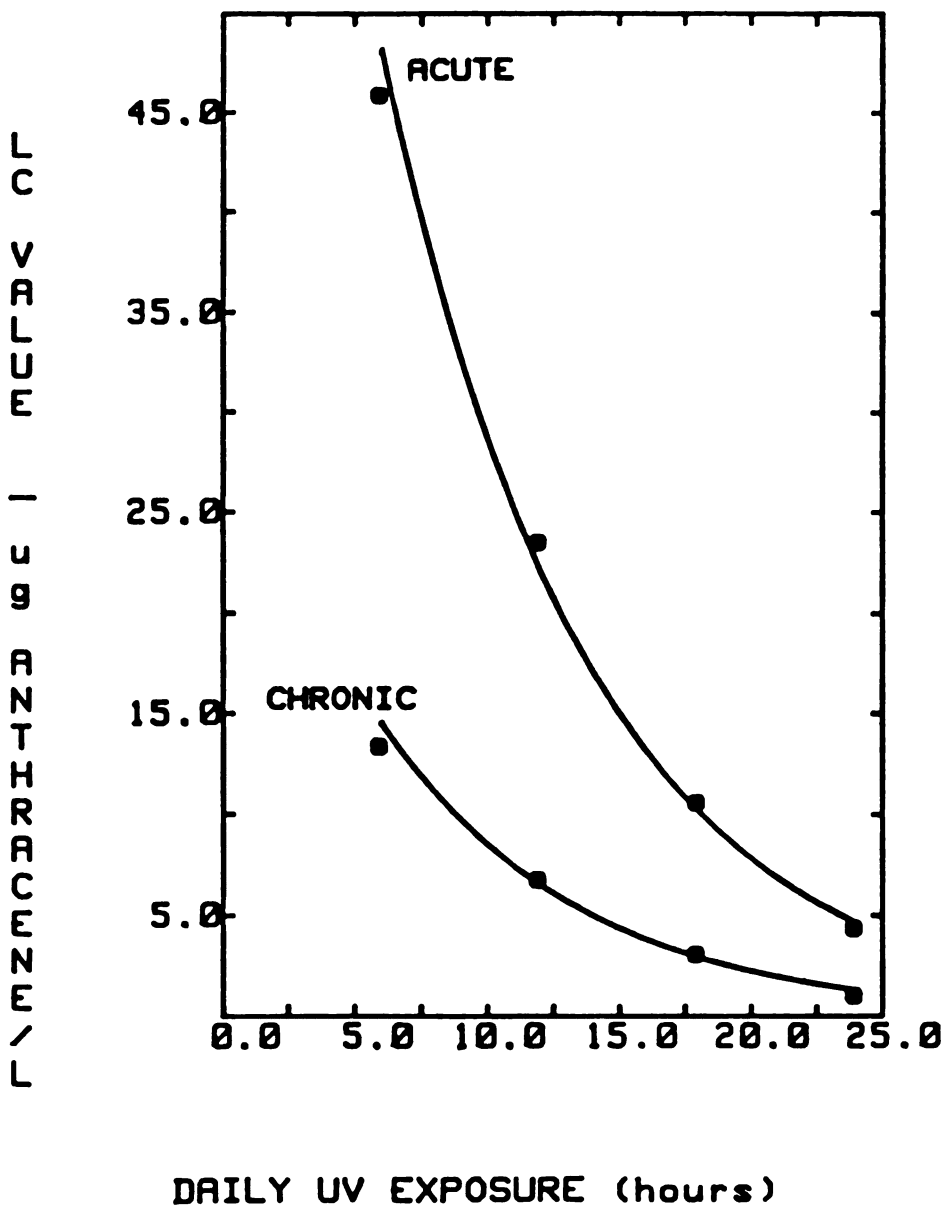
intensity as a median value for all SUVR intensities or that LT50 values be predicted utilizing the assumption of cumulative-damage (Section 1.3). These predictions should be used only on a tentative basis until actual chronic toxicity experiments can be performed.

The relationship between the number of daily SUVR-exposure-hours and LC values for both acute (96 h LC50) and chronic (∞LC1) estimates was examined (Figure 14). 96 h LC50 estimates ranged from 4.5 ug anthracene/L for the 24:0 photoperiod to 46 ug/L for the 6:18 photoperiod. Predicted ∞LC1 values ranged from 1.2 ug anthracene/L for the 24:0 photoperiod to 13.5 ug/L for the 6:18 photoperiod. Both sets of estimates were negative-exponentially related to the number of daily SUVR-exposure-hours (Figure 14). The difference in magnitude of LC values between acute estimates and chronic predictions becomes smaller as the number of daily SUVR-exposure-hours increase. However, the ratio between acute estimates and chronic predictions is constant (Mean = 3.46, SE = 0.202). This ratio is small and is indicative of a threshold toxicity response. Therefore care must be taken in evaluating and establishing guidelines for PAH concentrations in water since the difference between estimated acute and predicted chronic toxicity is so small.

These estimates were derived for an SUVR intensity

Figure 14. The relationship between estimated lethal concentration values and the number of daily hours of SUVR exposure(HL/D) for both acute (96 h LC50) and chronic (∞LC1) toxicity estimates. Ln(96 h LC50) = 4.656 - 0.130(HL/D), r^2 = 0.997. Ln(∞LC1) = 3.48 - 0.134(HL/D), r^2 = 0.992.

Figure 14.


ACUTE
CHRONIC
45.0
35.0
25.0
15.0
5.0
0.0
5.0
10.0
15.0
20.0
25.0
LC VALUE - ug ANTHRACENE/L
DAILY UV EXPOSURE (hours)

that is ecologically relevant and can be assigned to a depth in any body of water using a measured UVA extinction coefficient (K_{UVA}) and water surface UVA intensity (I_o). The penetration of solar radiation in a column of water can be described using the negative-exponential relationship :

$$Ln(I_z) = Ln(I_o) - K*(z)$$

Where z = depth, I_z = intensity at depth z, I_o = intensity at z=0, and K = extinction coefficient for a specified wavelength or waveband (fitted parameter). The equivalent depth for these estimates in offshore Lake Michigan (K_{UVA} = 0.45 m^{-1}; I_o = 5000 uW/cm^2) is 8.7 m, and the equivalent depth in a small local eutrophic impoundment (Fink's Pond, Ingham Co., MI; K_{UVA} = 1.32 m^{-1}; I_o = 5000 uW/cm^2) is 3.0 m. The water surface UVA intensity used in these equivalent depths is approximately equal to our measured summer maximum in mid-Michigan (43° latitude).

Concentrations of anthracene in most natural waters are presently less than the estimated acute, as well as the predicted chronic no-effect concentrations. However, as has been mentioned the input of anthracene from non-point sources into the Great Lakes region is considerable (Strand and Andren 1980), and this input is expected to increase with the increased utilization of fossil-fuel combustion (Gehrs 1976). In addition, anthracene is only one of many PAH that can cause photo-induced toxicity

(Kochevar et al. 1982). Results presented in Chapter 2 (this volume) and other recent findings (Newsted and Giesy 1985) suggest that anthracene exhibits a median level of phototoxicity compared to other PAH commonly identified in aquatic systems. Therefore, the results presented here can be considered to represent an average assessment of total PAH phototoxicity to fish, if one assumes simple additivity of toxicity and similar modes of toxic action. In terms of the summed totals of molar PAH concentrations, there are natural waters in which the present concentration of PAH approach or exceed our estimated chronic toxicity threshold (Neff 1979; USEPA 1980).

The hazard assessment presented here is based only on water borne PAH. Because of the complicating factor that fish can derive a significant PAH body-burden from food sources and because body-burden has been shown to be a controlling factor in photo-induced anthracene toxicity (Bowling et al. 1983), it is suggested that in the future the hazard of phototoxic PAH to fish be evaluated on a body-burden basis. Using a 48 h bioconcentration factor for anthracene in bluegill sunfish of 1367 (Section 1.2), a UVA intensity equivalent to 3.0 m deep in a typical eutrophic impoundment (100 uw/cm^2), a photoperiod of 16:8 h and a safety factor of 50, an estimated no-effect anthracene body-burden of 131 ug anthracene/kg (0.736 uM/kg) has been calculated. This no-effect body-burden

would be considerably less at shallower depths.

It is difficult to assess the significance of this no-effect value with regard to current natural body-burdens in fish since information on anthracene concentrations in fish is scarce. There is, however, a considerable body of literature concerning the concentrations of benzo(a)pyrene (BAP) in fish, and BAP has been shown to exhibit a less degree of photo-induced toxicity compared to anthracene on a molar body-burden basis to larvae of the fathead minnow (Pimephales promelas) (Chapter 2). Worldwide, BAP body-burdens have been reported to range from 0.004 to 5 uM/kg for 12 different species of marine fish (Neff 1979). Concentrations of BAP in codfish from the west coast of Greenland and sardine from the Bay of Naples, Italy, have been measured to be 0.06 and 65 uM/kg, respectively (Braunstein et al. 1977). Carp and pike from Hamilton Harbor, Lake Ontario, have been reported to contain 0.62 and 0.31 uM/kg BAP, respectively, while in the Detroit River the same two species were found to contain 0.16 and 0.20 uM/kg, respectively (Hallett and Brecher 1984). BAP concentrations measured in 4 species of fish from three contaminated rivers were found to range from 0.0003 to 0.07 uM/kg (Baumann et al. 1982). The concentrations of BAP and two other PAH with demonstrated photo-induced toxicity to fish (Chapter 2), benz(a)anthracene and pyrene, have been measured in brown bullhead taken from

the Black River, OH (Baumann et al. 1982). Mean values were 0.05 uM BAP/kg, 0.08 uM benz(a)anthracene/kg and 3.8 uM pyrene/kg. It is apparent that there are some areas where photo-induced PAH toxicity to fish may be of concern.

To my knowledge, there have not been any direct examinations of photo-induced toxicity to fish from natural exposures to PAH, however a recent example of potential PAH photo-induced toxicity was observed with sediments from the Elizabeth River (Chesapeake Bay system) (Hargis et al. 1984). Acute toxicity was observed to spot (Leiostomus xanthurus), a bottom feeding sciaenid, when exposed outdoors under ambient sunlight to water containing Elizabeth River sediments that were heavily contaminated with PAH. Overflow water from sediment tanks was also acutely toxic. These effects were not observed when fish were exposed to the same sediments and water under laboratory lighting. Outdoors, fish developed dermal necroses similar to those observed in fish collected from the Elizabeth River (Huggett 1984). PAH-induced dermal necroses in fish exposed to ecologically relevant UV-A intensities have also been reported in this study (Section 1.3). While SUVR was not considered in these experiments (Hargis et al. 1984; Huggett 1984), PAH photo-induced toxicity probably played an important role in the differential level of toxicity between outdoor and

indoor experiments.

Some of the environmental parameters not considered in the hazard assessment presented here may need to be added to experimental designs in future investigations. The effect of lower water temperature, which is integrally linked to photoperiod duration especially in north temperate regions, significantly reduces the water solubility of PAH (May et al. 1982), thus reducing bioavailability of the compounds. In addition, lower water temperature would reduce metabolic, and thus respiratory, demand in poikilothermic organisms and would serve to decrease acute anthracene phototoxicity, which has been shown to be partly due to respiratory stress (Section 1.3). The presence of suspended and dissolved organic matter in the water column could also ameliorate anthracene phototoxicity by decreasing light penetration (Baker and Smith 1982), by decreasing bioavailability (Leversee et al. 1983), or by increasing rates of photodecomposition (Miller and Zepp 1979).

1.5 CONCLUSIONS

In this study the importance of an environmentally realistic assessment of the hazards posed by PAH in the aquatic environment has been stressed. An important accessory parameter that in laboratory experiments

significantly enhances the toxicity of at least one PAH to fish compared to previously published reports has been identified. Currently, there are only a few areas where photo-induced PAH toxicity may be of concern but there is a need to prevent further increases of PAH loading to aquatic systems. There are many other environmental parameters which may also affect PAH toxicity to aquatic organisms which have not been accounted for in the present laboratory system, but SUVR is one of the most important of these considerations. While the present experimental design is far from being completely realistic, the approach of identifying the key environmental parameters involved in this phenomenon can be considered as being a conservative representation of occurrences in natural aquatic systems.

CHAPTER 2

THE PHOTO-INDUCED TOXICITY OF SELECTED POLYCYCLIC AROMATIC HYDROCARBONS TO LARVAE OF THE FATHEAD MINNOW (Pimephales promelas): COMPARATIVE TOXICITIES AND STRUCTURE-ACTIVITY RELATIONSHIP

2.1 INTRODUCTION

In the previous studies, it was demonstrated that anthracene is acutely toxic to juvenile sunfish and fathead minnows under laboratory and field conditions of solar ultraviolet radiation (SUVR), and that this toxicity could be predicted from knowledge of UV intensity and anthracene concentration. Because polycyclic aromatic hydrocarbons (PAH) belong to a very large class of compounds, it is desirable to determine the extent and likelihood that other PAH are capable of eliciting photo-induced toxicity to fish. However, conducting toxicity tests in the laboratory with all PAH found in the aquatic environment would be extremely time consuming and costly. To be able to predict whether a PAH has the potential of

being phototoxic to fish based on a structure-activity measurement would therefore be beneficial. It has already been suggested that many PAH can be considered as potential phototoxic compounds, and there have been reports describing the photo-activity of a large number of PAH to mammals (Kochevar et al. 1982) and to aquatic organisms (Morgan and Warshawsky 1977). There are no known reports, however, concerning the range and extent of PAH photo-induced toxicity to fish. The present study was conducted in an effort to 1) determine the relative photo-induced toxicity of a group of PAH possessing a wide range of chemical characteristics, and 2) develop a structure-activity relationship that, based on known chemical characteristics, can predict the photo-induced toxicity of a compound.

Because flow-through toxicant exposures consume a large amount of compound and because many PAH are carcinogenic, as well as very expensive to obtain in pure form, these experiments were conducted in a static-renewal system with larvae of the fathead minnow (Pimephales promelas). The experiments were designed to examine the toxicity of 12 different PAH at a concentration in water that would, based on bioconcentration values, result in equimolar body burdens of PAH in fish. Toxicity is reported in terms of a calculated efficacy for each compound based on molar PAH body-burdens and absorption spectra characteristics, as well as the more traditional

median lethal time (LT50). Finally, a structure-activity relationship is developed from multivariate discriminate analysis that classifies PAH as phototoxic or non-phototoxic using phosphorescence lifetime and first order molecular connectivity measurements.

2.2 MATERIALS AND METHODS

Compounds and Test Solutions

Anthracene (ANT), benzo(a)anthracene (BAA), benzo(b)anthracene (BBA), acridine (ACR), dibenz(a,h)anthracene (DBA), perylene (PER), benzo(g,h,i)perylene (BGP), pyrene (PYR), benzo(a)pyrene (BAP), benzo(e)pyrene (BEP), benzanthrone (BAN), and phenanthrene (PHE) (Figure 1) were obtained at the highest purification available commercially. All compounds except BAN were used without further purification. Saturated aqueous solutions of each PAH were obtained using a shell coating technique to avoid the use of carrier solvents in the tests. A concentrated stock solution of each PAH was made in acetone and was added to oven-dried acid, base, and solvent washed 1000 ml erlenmyer flasks at a volume of 150% in excess of a particular compound's aqueous solubility. The stock solutions were swirled in the

flasks and the acetone was allowed to evaporate to dryness to leave a thin film of PAH on the walls of the flask. One liter of charcoal filtered, aerated tap water (Section 1.2) was then added to each flask. The flasks were covered with aluminum foil to eliminate light and were gently agitated for 24 h on a shaker table. Concentrations of PAH in the aqueous solutions were determined by HPLC as in Section 1.2 changing only the excitation and emmission wavelengths of the fluoresence detector. Excitation and emmission wavelengths were 250 and 370 nm, respectively, for ACR, ANT, PHE, and PYR. Excitation and emmission wavelengths were 280 and 389 nm, respectively, for all other compounds. Once the concentration of PAH in these aqueous stock solutions was determined, appropriate dilutions were made to achieve the desired concentration in a test. The design of this study required that final PAH concentrations in the organisms be equimolar. Therefore, PAH concentrations in water were selected on the basis of published bioconcentration values and molecular weights, normalized to the least water soluble compound (BGP). On this basis, a nominal PAH body burden of 100 nM/g was selected. Nominal and actual PAH concentrations in water and in fish are presented in Table 5.

Table 5. Nominal and actual concentrations of PAH in water and in organisms. Nominal PAH body-burdens were 100 nM/g for all compounds based on water solubility of BGP, bioconcentration factor, and molecular weights. N.D. indicates that no compound(s) was detected.

Compound	Water nominal (ug/L)	Water actual (ug/L)	Organism actual (nM/g)
ACR	526	525	120.6
ANT	14.7	5.4	111.8
PHE	14.5	10.0	293.2
BAA	1.9	1.8	6.7
BBA	1.1	1.9	N.D.
DBA	0.25	0.15	0.94
PYR	7.21	25.6	87.1
BAP	0.82	5.6	486.7
BEP	0.43	2.9	6.0
PER	0.79	1.7	7.5
BGP	0.20	0.15	3.7
BAN	31.6	49.5	52.9
SUVR-only control	0.0	N.D.	N.D.

Organisms and Bioassay Procedure

Larvae of the fathead minnow (Pimephales promelas) were obtained 4 d post-hatching from the Michigan Department of Natural Resources Surface Water Quality Division. Larvae were transferred by pipette to a small flow-through aquarium with charcoal filtered, aerated tap water at 24°C. The larvae were maintained in the flow-through aquarium for two days after transfer and were fed newly hatched brine shrimp nauplii (Artemia salina; Metaframe Corp.) ad libitum twice a day. On the seventh day post-hatching, larvae were transferred by pipette to 300 ml Pyrex^{R} crystallizing dishes (5 cm ht. X 10 cm diam.) containing 150 ml of PAH solution or dilution water. Treatments consisted of 20 to 25 larvae per dish and two dishes per PAH examined. Dishes were covered with aluminum foil to allow the larvae a 24 h pre-incubation period in the absence of SUVR. After the pre-incubation period, larvae were fed brine shrimp ad libitum for 0.5 h, all solutions were then replaced and the dishes with larvae were placed in random positions under the laboratory system light bank (Section 1.2). In these experiments, the lights were filtered with a 5 mil thickness of Mylar^{R} to eliminate radiation of wavelengths shorter than 315 nm, giving a UV-A:UV-B ratio 4.75. SUVR intensities for all exposures were UV-B = 20 uW/cm^{2} and UV-A = 95 uW/cm^{2}. After the initial pre-incubation,

solutions were changed twice a day at 12 h intervals. Larvae were fed brine shrimp ad libitum once a day for 0.5 h prior to changing solutions. Solutions were changed by slowly siphoning all but approximately 20 ml of old solution, taking care to remove any remaining brine shrimp or any other debris without disturbing the fish larvae. A 150 ml volume of new solution was then carefully poured into the dish. Bioassay dishes were examined for larval mortality at least 4 times daily. Tests were conducted until 100% mortality was achieved or for a maximum of 96 h, whichever came first. PAH concentrations in water were determined at the beginning of a bioassay at zero and 12 h and at least once more for initial and 12 h solutions during the test period. PAH concentrations in water are reported as the geometric mean between the measured concentrations in the initial and 12 h old solutions. In tests where mortality occurred, a 96 h dark exposure to the PAH was performed to assess any effect due to PAH in the absence of SUVR. No PAH tested exhibited toxicity in the dark. PAH body burdens were determined on all larvae at the end of a test (Section 1.2). Percent recoveries of PAH from spiked fish larvae samples were 72 +/- 12% (mean +/- SE).

Efficacy and Relative Potency Factor

The efficacy of each phototoxic compound was determined in a manner similar to Morgan and Warshawsky (1977). Efficacy ( ) is defined analagously to quantum yield in photochemistry and is a descriptor of the rate of larval mortality versus the rate of quanta absorbed by a compound in the larvae. The rate of mortality versus time can be described by equation 1.

$$\frac{d(\%\text{Mortality})}{dt} = \frac{\sum [(I_{o\lambda} T_{\lambda}) \cdot (\epsilon\ b\ C_a)]}{n} \cdot \Phi = A \cdot \Phi \quad (1)$$

where A represents the average number of quanta absorbed per time and

λ = waveband (UV-B=315-336 nm, UV-A=336-400 nm, VIS1=400-420 nm, and VIS2=420-450 nm)

$I_{o\lambda}$ = waveband radiation intensity (uW/cm^2; Table 6)

T_{λ} = optical transmittance of epidermis for waveband (Table 6)

ϵ = mean molar extinction coefficient of compound in octanol for waveband (L/mole/cm)

b = depth of radiation penetration in organism (b = 0.2 cm)

C_a = molar concentration of compound in organism (moles/1000 g)

n = number of wavebands (n = 4)

t = time (s)

Φ = efficacy of compound

Table 6. Waveband specific radiation intensities ($I_{o\lambda}$) and epidermal optic transmittance (T_λ; Wan et al. 1981).

Waveband	$I_{o\lambda}$ (uW/cm^2)	T_λ
UV-B (315-336 nm)	20	.55
UV-A (336-400 nm)	95	.62
VIS1 (400-420 nm)	35	.69
VIS2 (420-450 nm)	270	.72

Integration of equation 1 yields:

$$\%Mortality = A \cdot \Phi \cdot t + B \qquad (2)$$

which is in the form of a linear equation where, in plots of %Mortality versus time, B is the intercept and ΦA is the slope of the line (Figure 15). Efficacy, therefore, can be determined algebraically from knowledge of the calculated A and the slope of the time mortality curve for each individual compound. The Relative Potency Factor (RPF) is an index of the relative efficacy of a compound compared to the least efficacious of the compounds tested. Therefore, efficacy is a unique descriptor of the phototoxic activity of a compound and RPF gives a relative index of activity for the group of compounds used in this study.

2.3 RESULTS

Six of the 12 compounds tested exhibited acute photo-induced toxicity (Table 7). LT50 values ranged from 0.83 h for BAN to 65.1 h for BAA. Of the remaining six compounds, four compounds exhibited no effect compared to SUVR-only controls (BEP, DBA, PER, PHE) and the other two compounds exhibited a marginal level (<20% mortality in 96 h) of photo-induced toxicity (BBA, BGP). Mortality in

Figure 15. Percent mortality of fathead minnow larvae Pimephales promelas exposed to benzo(a)anthracene under continuous laboratory simulated sunlight as a function of time to illustrate the derivation of compound specific absorption (A) and efficacy (Φ). Slope of line is equal to $A\Phi$.

Figure 15.

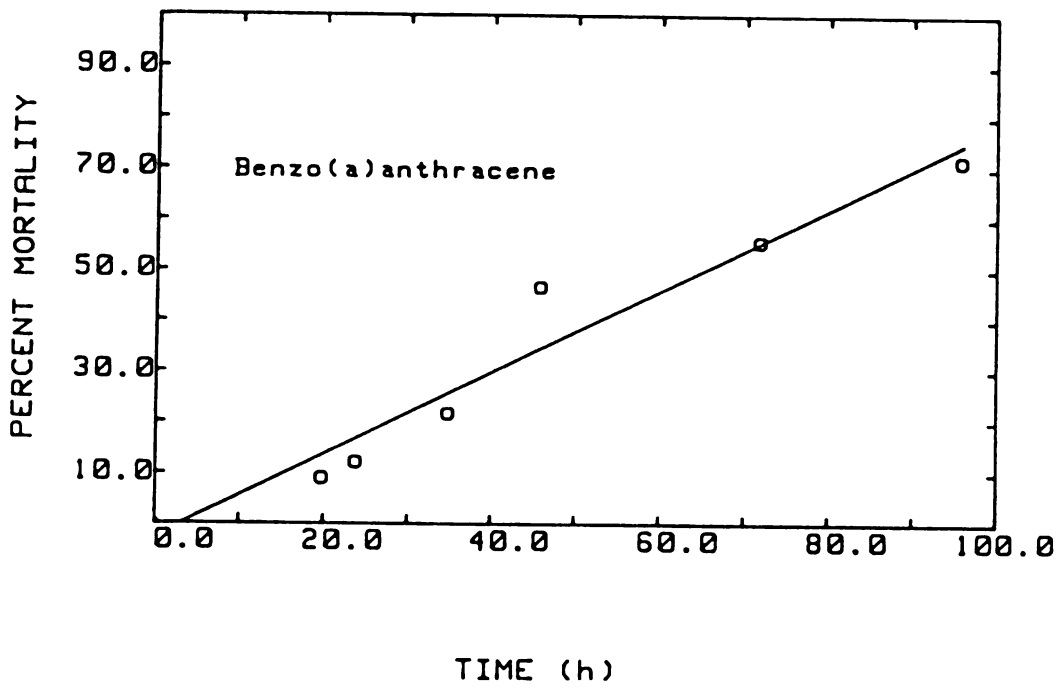

PERCENT MORTALITY
90.0
70.0
50.0
30.0
10.0
Benzo(a)anthracene
0.0
20.0
40.0
60.0
80.0
100.0
TIME (h)

Table 7. Tabulated values of median lethal times (LT50), average quanta absorbed (A), efficacy (Φ), and Relative Potency Factor (RPF) for all phototoxic compounds. Compounds are listed in decreasing order of relative potency.

Compound	LT50 (h)	A	Φ	RPF
BAN	0.83	0.183	5.46 E-2	337.1
PYR	3.20	0.372	1.45 E-2	100.1
ACR	4.30	0.397	7.00 E-3	48.3
ANT	15.75	0.218	3.12 E-3	21.5
BAA	65.09	0.100	2.38 E-3	16.4
BAP	40.05	2.913	1.45 E-4	1.0

SUVR-only controls was less than 5% in all tests. Contrary to the original design of this experiment, equimolar body-burdens of PAH were not obtained (Table 5), even though PAH concentrations in water were relatively close to the selected nominal concentrations. The equation used to calculate A and Φ takes into account the concentrations of compound in the animal, so even though the achievement of equimolar body-burdens was desirable, it was not entirely necessary. Since BBA was not detected in fish tissue (Table 5) this compound was not used for further analysis.

On the basis of RPF, BAN exhibited the greatest and BAP exhibited the least level of concentration and absorption-specific photo-induced toxicity among the compounds that were phototoxic (Table 7). Values of LT50 were strongly correlated to A, Φ and RPF with the exception of BAA and BAP. Since BAA was measured at a much less concentration than BAP and since BAP has a much greater absortion of radiation in the wavebands examined, the concentration and absorption-specific activity of BAA is much greater than BAP.

Various attempts were made to correlate the measures of mortality to the chemical characteristics of the compounds to determine a structure-activity relationship. The factors considered included octanol-water partition coefficients, first and second order molecular connectivity indicies, energies of lowest singlet excited

state splitting, energies of lowest triplet excited state splitting, the difference between singlet and triplet splitting energies, phosphorescence lifetimes, average molar extinction coefficients in octanol for each of the four wavebands examined, and the summed total of all molar extinction coefficients across all wavebands (315 to 450 nm). No significant univariate correlations were observed between any of the measures of mortality (LT50, RPF, A Φ) and any of the above chemical characteristics.

Since no simple correlations were observed, an attempt was made using discriminate analysis (SAS 1982) to classify the compounds as being either phototoxic or non-phototoxic. All compounds were classified as being toxic or non-toxic on the basis of bioassay results, and a stepwise discriminant analysis was performed to determine which variables could be used to best classify the compounds into the two groups. Stepwise discriminant analysis determined that the best canonical discriminant model for classification of the compounds consisted of phosphorescence lifetime and first order molecular connectivity index (MC1 ; Table 8). Phosphorescence lifetime exhibited the main effect in the classification with a partial r^2 in the model of 0.69 ($P > F = 0.003$) compared to MC1 with a parital r^2 in the model of 0.39 ($P > F = 0.056$). Discriminant analyses were then run in an attempt to calibrate a classification model to predict

Table 8. Phosphorescence lifetime (Morgan et al. 1977) and first order molecular connectivity (Koch 1983) values for all compounds tested. T_p = phosphorescence lifetime, MC1 = first order molecular connectivity.

Compound	T_p (s)	MC1
BAN	0.020	6.2635
PYR	0.630	5.5594
ACR	0.150	4.5856
ANT	0.090	4.8094
BAA	0.359	6.2201
BAP	0.105	6.9701
BGP	0.438	7.7201
DBA	1.600	7.6308
BEP	2.120	6.9761
PHE	2.940	4.8154
PER	3.500	6.9761

photo-induced PAH toxicity. One analysis was conducted with only phosphorescence time in the model and another was conducted with both phosphorescence time and MCl in the model. In the classification results for the calibration analysis with only phosphorescence time in the model, all compounds except BGP were correctly classified (Table 9). In the classification results for the calibration analysis with both phosphorescence time and MCl in the model, all compounds were correctly classified (Table 10). BGP, which exhibited a marginal level of photo-induced toxicity to fish larvae, had a posterior probability of 55% for classification as non-toxic (Table 10).

To test the predictive capability of the classification criterion, a test classification was performed using the eleven compounds tested in the present study plus 17 other PAH for which MCl was calculated and for which information on phosphorescence lifetimes was available (Table 11). Of the compounds examined in the test classification, 12 (43%) were designated toxic and 16 (57%) were designated non-toxic (Table 12). Ten of the 12 compounds designated as toxic had posterior probabilities of greater than 90% for membership in the toxic classification and 13 of the 16 compounds designated as non-toxic had posterior probablities of greater than 90% for membership in the non-toxic classification (Table 12).

Table 9. Classification results for discriminant analysis calibration data, in model which considers only phosphorescence lifetime. TOXIC = demonstrated or predicted to be phototoxic, NOTOX = demonstrated or predicted to be non-phototoxic.

Compound	From:	Classified into:	Posterior probability of membership in: NOTOX	TOXIC
BAN	TOXIC	TOXIC	0.057	0.943
PYR	TOXIC	TOXIC	0.295	0.705
ACR	TOXIC	TOXIC	0.049	0.951
ANT	TOXIC	TOXIC	0.051	0.949
BAA	TOXIC	TOXIC	0.071	0.929
BAP	TOXIC	TOXIC	0.050	0.950
BGP	NOTOX	*TOXIC*	0.098	0.902
DBA	NOTOX	NOTOX	1.000	0.000
BEP	NOTOX	NOTOX	1.000	0.000
PHE	NOTOX	NOTOX	1.000	0.000
PER	NOTOX	NOTOX	1.000	0.000

* = misclassified observation

Table 10. Classification results for discriminant analysis calibration data, in model which considers both phosphorescence lifetime and first order molecular connectivity. TOXIC = demonstrated or predicted to be phototoxic, NOTOX = demonstrated or predicted to be non-phototoxic.

Compound	From:	Classified into:	Posterior probability of membership in: NOTOX	TOXIC
BAN	TOXIC	TOXIC	0.0108	0.9892
PYR	TOXIC	TOXIC	0.0295	0.9705
ACR	TOXIC	TOXIC	0.0002	0.9998
ANT	TOXIC	TOXIC	0.0003	0.9997
BAA	TOXIC	TOXIC	0.0185	0.9815
BAP	TOXIC	TOXIC	0.0621	0.9379
BGP	NOTOX	NOTOX	0.5451	0.4549
DBA	NOTOX	NOTOX	1.000	0.000
BEP	NOTOX	NOTOX	1.000	0.000
PHE	NOTOX	NOTOX	1.000	0.000
PER	NOTOX	NOTOX	1.000	0.000

Table 11. Phosphorescence lifetime (Morgan et al. 1977) and first order molecular connectivity (Koch 1983) values for compounds added to test classification. T_p = phosphorescence lifetime, MCl = first order molecular connectivity.

Compound	T_p (s)	MCl
Fluoranthene	0.99	5.5654
Benzo(a)fluorene	2.61	6.0225
Benzo(b)fluorene	2.24	6.0166
Benzo(b)anthracene	0.01	6.2141
Chrysene	2.54	6.2261
Benzo(k)fluoranthene	0.83	6.9701
Fluorene	5.00	4.5118
Dibenz(a,h)acridine	2.31	7.5535
Benz(a)acridine	0.41	5.8541
Benz(c)acridine	0.28	5.8541
Carbazole	8.04	4.4046
Coronene	9.50	8.2142
Phenazine	0.08	4.6546
Dibenz(a,j)anthracene	2.51	7.3421
Benzo(b)chrysene	0.18	7.6308
Dibenz(a,c)phenazine	0.29	7.4819
Benz(b)triphenylene	0.80	7.3867

Table 12. Results of discriminant analysis test classification for some selected polycyclic aromatic hydrocarbons. TOXIC = Predicted to be phototoxic, NOTOX = predicted to be non-phototoxic.

Compound	Classified Into:	Posterior Probability of Membership in: NOTOX	TOXIC
Fluoranthene	NOTOX	0.794	0.206
Benzo(a)fluorene	NOTOX	1.000	0.000
Benzo(b)fluorene	NOTOX	1.000	0.000
Benzo(b)anthracene	TOXIC	0.000	1.000
Chrysene	NOTOX	1.000	0.000
Benzo(k)fluoranthene	NOTOX	0.890	0.110
Fluorene	NOTOX	1.000	0.000
Dibenz(a,h)acridine	NOTOX	1.000	0.000
Benz(a)acridine	TOXIC	0.010	0.990
Benz(c)acridine	TOXIC	0.005	0.995
Carbazole	NOTOX	1.000	0.000
Coronene	NOTOX	1.000	0.000
Phenazine	TOXIC	0.000	1.000
Dibenz(a,j)anthracene	NOTOX	1.000	0.000
Benzo(b)chrysene	TOXIC	0.280	0.720
Dibenz(a,c)phenazine	TOXIC	0.252	0.748
Benz(b)triphenylene	NOTOX	0.935	0.065

2.4 DISCUSSION

The results of these experiments demonstrate that PAH other than anthracene are acutely phototoxic to fish. Based on RPF, anthracene ranked fourth out of 12 among compounds tested, and exhibited a median level of toxicity among the compounds that were toxic. From the point of view of relative toxicities, therefore, anthracene appears to be an adequate model compound for the examination of photo-induced PAH toxicity to fish. Anthracene also exhibits a median level of photo-induced toxicity to Daphnia magna (Newsted and Giesy 1985) and with few exceptions, the relative toxicities of the various compounds were very similar between fish larvae and zooplankton. The relative photodynamic activities of PAH that were tested for toxicity against brine shrimp nauplii (Morgan and Warshawsky 1977) do not correlate well with the RPF values obtained for the same compounds in the present study. The lack of correlation was not unexpected, however, since Morgan and Warshawsky (1977) did not account for molar body-burdens of the compounds. The importance of obtaining direct measurements of tissue PAH concentrations can be illustrated by the fact that the selected nominal concentrations of PAH in fish were not

accurately obtained (Table 5). These data indicate that using only nominal concentrations and assuming equimolar body-burdens based on literature-derived bioconcentration factors is unacceptable. Therefore, it is felt that the present study provides a more accurate assessment of the true relative activities of these phototoxic compounds.

The development of a classification scheme that can determine whether or not a PAH has the potential to cause photo-induced toxicity is significant. While the discriminant analysis presented here can only classify a compound as being toxic or non-toxic and has no predictive capabilities with regard to relative levels of toxicity, it may prove to possess power in assessing potential for environmental impact and in identifying geographic areas of environmental concern. Validation of the current classification scheme will be difficult. However, comparisons among other studies show that most compounds examined in the test classification were designated correctly. All compounds considered in Morgan and Warshawsky (1977) which were common to the test classification were classified correctly. All but two compounds tested against D. magna by Newsted and Giesy (1985) which were common to the test classification (fluoranthene and benzo(k)fluoranthene) matched the designated categories (Table 12). These compounds were of intermediate toxicity to D. magna with LT50 values of 10.8

and 13.0 h for fluoranthene and benzo(k)fluoranthene, respectively. Although these compounds appear to have been misclassified, comparisons of other phototoxic compounds in Newsted and Giesy (1985) indicate that PAH causing photo-induced toxicity to D. magna with estimated LT50 values greater than 8 to 9 h are not phototoxic to fish within 96 h. These resluts indicate that the use of fish or fish larvae in determining the environmental hazard and structure-activity relationships of phototoxic compounds may not be entirely appropriate. More work is needed in this area of study before a more general assessment of PAH photo-induced toxicity to aquatic organisms can be accomplished.

The derivation of a classification criterion based on molecular connectivity and phosphorescence lifetime lends important insight into the molecular mechanism of action in the photo-induced toxicity of PAH. The molecular connectivity index is a novel descriptor of the structure of a molecule (Koch 1983) and has been successfully used in quantitative comparisons with physico-chemical and biological properties of molecules (Koch 1983). The significance of molecular connectivity in the phototoxicity classification criterion is relatively minor, however, upon comparison of the results in Tables 9 and 10. The major factor determining whether or not a compound is classified as being phototoxic is the phosphorescence lifetime, although molecular connectivity

appears to act as a significant covariate in the classification. Phosphorescence lifetime is a direct measurement of the radiative energy dissipation of a molecule from the excited triplet state to the singlet ground state (Wells 1972). Molecules such as PAH in excited states may return to the ground state in a complex number of pathways, including radiative processes in which energy is dissipated in the form of light and heat. Non-radiative processes may also occur, where energy from a photosensitized molecule is passed to other molecules leading to the formation of excited states in, and reactions with, these other molecules. Radiative and non-radiative processes operate simultaneously and the net dissipation of energy from an excited state molecule is an integration of these competing processes. The probability of collisions with, and hence reactions with, excited state molecules is directly proportional to the lifetime of the excited state (Wells 1972). It could be assumed that PAH with longer phosphorescence lifetimes would be more photo-reactive than PAH with shorter phosphorescence lifetimes. However, the results of these experiments demonstrate that PAH with short phosphorescence lifetimes were more phototoxic to fish larvae, indicating that the photo-induced toxicity of PAH is inversely proportional to the length of existance of the excited triplet state. The rate of energy transfer in non-radiative processes has

also been shown to be inversely proportional to the radiative lifetime of excited states (Bennett and Kellogg 1968). These facts lead to the conclusion that the photo-induced toxicity of PAH to fish is probably determined by the rate of non-radiative energy transfer from the excited state of a particular compound. Therefore, mechanisms which depend on the lifetime of excited states, such as direct interaction (Landrum et al. 1985), most likely do not predominate, and reactions which depend on the rate and efficiency of energy transfer, such as the formation of reactive singlet oxygen (Bennett and Kellogg 1968), are more probable.

2.5 CONCLUSIONS

The studies presented in this chapter have demonstrated that the potential exists for many PAH other than anthracene to cause photo-induced toxicity to fish, but that anthracene has been a good overall model compound for the study of this phenomenon. The development of a classification criterion, based on easily obtained information about the structure and photochemistry of a compound, to predict whether a PAH has the potential to cause photo-induced toxicity to fish has major significance in the assessment of the possible environmental impact of these compounds.

GENERAL DISCUSSION

Potential Ecological Consequences

The laboratory studies presented here have demonstrated a potential for detrimental environmental impact due to the photo-induced toxicity of polycyclic aromatic hydrocarbons (PAH) to fish. The question remains, however, as to what are these potential impacts and how might photo-induced toxicity affect an aquatic system on the community and ecosystem level of organization? The following discussion focuses on these questions from an ecological point of view, and presents evidence for potential impacts on many aquatic organisms, including fish, zooplankton, phytoplankton and microbes.

For largely unknown reasons, but perhaps due to the lack of instrumentation, aquatic biologists historically have assumed that SUVR does not penetrate natural waters to significant depth, and have discounted the importance of SUVR in the aquatic environment. Solar UV does penetrate surface waters to a considerable extent and this

penetration has been observed by numerous authors (Smith and Baker 1979; Calkins 1982). The depth of UV penetration is dependent on the productivity and turbidity of a particular body of water (Smith and Baker 1979; Smith et al. 1983). For example, in eutrophic Park Lake, MI, 99% of incident UV-B is attenuated in the upper 2 meters (attenuation coefficient (K) = 2.504 m^{-1}), while in offshore Lake Michigan 1% of incident UV-B penetrates to about 10 meters (K = 0.496 m^{-1}). From these measurements, it is evident that solar ultraviolet radiation is present at ecologically significant depths and may play an important interactive role in the toxicity of PAH in the aquatic environment.

The impacts of phototoxic compounds in aquatic systems are unknown since research to present has not made explicit tests on an ecosystem-wide basis. It is instructive, however, to examine the direct effects of SUVR on aquatic organisms since UV exposure is necessary to elicit the phototoxic phenomenon due to PAH. In addition, photosensitized PAH toxicity may be considered to enhance the damaging potential of SUVR to aquatic organisms. It is common to describe this toxic response as the photo-induced toxicity of PAH to aquatic organisms, but there are no compelling reasons why the converse cannot be true (i.e. PAH enhanced toxicity of UV radiation).

The impact of direct UV irradiation on planktonic organisms has been the focus of many studies. Shrimp larvae, crab larvae, and euphasids are known to be living at or near their UV-B tolerance under current irradiance conditions (Damkaer et al. 1980). These animals have a threshold UV-B tolerance level, below which little or no effect occurs and above which a strong dose/dose-rate response is observed. Damkaer et al. (1980) suggest that near surface waters are environmentally important since many zooplankters have their center of abundance in these strata or are found exclusively there for at least part of their life cycle. These authors calculate that a 20% reduction in global ozone could significantly shorten the larval season of these species, and suggest that natural intensities of UV-B have had a selective role in the seasonal adaptation and community structure of zooplankton species. At slightly enhanced UV-B intensities, seasonal or geographical restrictions could occur in shrimp populations if reproductive success is dependent on late season (i.e. summer-fall) larvae (Damkaer et al. 1981). Tolerance of exposure to solar UV of many aquatic microorganisms (e.g. bacteria, yeast, algae, protozoa and arthropods) and current environmental UV intensities are approximately equal, and SUVR has been implicated as being a major ecological factor controlling the distribution of these organisms (Calkins and Thordardottir 1980). It has been suggested that there may be no large reserve of

organismal resistance which could cope with altered solar UV exposure, or UV sensitivity, without requiring modification of physiology or behavior (Calkins and Thordardottir 1980).

The vertical migrations of marine and freshwater zooplankton and phytoplankton have been variously interpreted as a maximization of resources such as food or nutrients, photosynthetically active radiation, and avoidance of predation and/or of injurous UV radiation (Bainbridge 1961; McNaught 1966; Klugh 1929). In many species of zooplankton, significant negative correlations between incident radiation and the pattern of vertical migration have been observed (Wilson and Roff 1973). In addition, seasonal changes in the vertical distribution and migration patterns correlate significantly with incident radiation, among other factors (Wilson and Roff 1973). In perhaps one of the earliest studies to recognize the ecological importance of SUVR, Klugh (1929) addressed the evolutionary significance of vertical migration of marine copepods relative to exposure to damaging UV irradiation. A close relationship was found between the depth of daylight occurrence and the susceptability to UV of these organisms. These results further indicate that the differential sensitivity of organisms to SUVR may play an important role in determining planktonic community structure. More recently, it has been observed that the

habitat of a wide variety of planktonic organisms is behaviorally determined by avoidance of SUVR on the basis of differential species sensitivity (Barcelo and Calkins 1979; Barcelo 1980; Barcelo and Calkins 1980). For instance, of the many factors considered by Barcelo and Calkins (1980), including wind, cloud cover, temperature gradients and food gradients, only UV-B and total solar irradiance were significantly correlated with vertical distribution.

The effects of SUVR exposure on marine and freshwater fish have also been examined. Larvae of the northern anchovy are very sensitive to UV-B (Hunter et al. 1981). Anchovy and other species of clupeiod fish spawn only during seasons when UV-B irradiance is low or utilize habitats where solar UV-B is strongly attenuated (e.g. productive, turbid inshore waters). There are exceptions to the spawning seasonality pattern, such as the pacific mackeral which spawns in June, but these species are more tolerant to UV-B exposure. These seasonal and locational patterns probably evolved with co-occurring periods or areas of optimal food density, but since the food organisms may also exhibit sensitivity to UV irradiation, one should exercise caution in interpreting the evolutionary significance of direct effects of UV irradiation on spawning behavior of fishes (Hunter et al. 1981). Solar UV has been shown to influence periods of optimal food density, and anchovy as well as other fish

species are known to be currently existing near their tolerance threshold for UV exposure (Hunter et al. 1982). Thus, the effect of SUVR on seasonal occurrence and habitat utilization remains an important factor.

Sockeye salmon eggs irradiated with UV-B exhibit a high rate of mortality compared to non-irradiated controls (Bell and Hoar 1950). Hatching among irradiated eggs occurred approximately 1 month prematurely, and alevins from these eggs suffered significant developmental abnormalities. Decreased hatching success due to SUVR stress could severely alter the population dynamics of fish. The eggs and larvae of fish are not the only life stages that can be deleteriously affected by solar UV exposure. There are many accounts of 'sunburn' in juvenile and adult fishes in the literature (Bullock 1982; Dunbar 1951; Crowell and McCay 1930; Allison 1960; Bullock and Roberts 1981), and it is common knowledge among hatchery workers that juvenile fish are sensitive to exposure to bright sunlight (Rucker 1957).

Worrest et al. (1981) investigated the impact of UV-B on estuarine microcosms in one of the few studies of the community level effects of SUVR. Elevated UV-B exposure resulted in altered phytoplankton community structure, lesser community biomass, less total chlorophyl-a concentration, and lesser radiocarbon assimilation. These authors speculated that altered species composition could

affect the quality and quantity of food for primary consumption and that organic carbon exchange between trophic levels could be affected. This impact would be significant if organisms selected by UV were of lower nutritional value. In addition, a decrease in size of repesentative diatoms upon which consumers could graze was observed, thereby possibly increasing the energy allotment required for grazing and reducing the feeding efficiency of the consumers (Worrest et al. 1981). In another community level study, the possible effects of SUVR on the competition among species of coral reef epifauna was examined (Jokiel 1980). It was hypothesized that organisms could gain a selective advantage by developing UV tolerance to avoid competition for space in shaded areas, but that these species would be inferior competitors in the absence of UV since the metabolic burden of maintaining enzyme systems required for UV protection would reduce growth and reproductive potential. This possibility was investigated using two closely related species of sponges, differing mainly in their UV tolerances, and it was found that the shade-adapted species was a better competitor when UV was removed by selective filters, and vice versa when UV was present (Jokiel 1980).

From the above discussion, it is apparent that a wide array of aquatic organisms are sensitive to SUVR, and that many are living close to their tolerance threshold or are

currently under UV related stress. Any mitigating factor such as increased PAH loading that would either increase the effect of UV or decrease tolerance of aquatic organisms to UV irradiation even slightly could cause significant short and long term ecological consequences through species and size specific mortality, habitat limitations, seasonal tolerance restrictions, reduced reproductive success, or altered energy-flow dynamics.

Future Needs and Conclusions

The present study has encompassed several aspects of photo-induced PAH toxicity to fish, but there are many more questions to be answered. This study is considered as a stepping-stone for the design of future studies. The questions which remain include 1) the examination of the effects that dissolved and particulate organic materials may have on photo-induced PAH toxicity, 2) the examination of possible synergistic relationships within and among mixtures of phototoxic PAH, 3) the determination of biochemical and molecular modes of toxic action, 4) the development and performance of comprehensive field and validation studies, and 5) the examination of long-term, chronic effects.

These studies have demonstrated that anthracene and other PAH are acutely toxic to fish at concentrations within aqueous solubility in the presence of SUVR.

Anthracene exhibits a median level of photo-induced toxicity compared to other PAH and can be considered to be an adequate model compound in the study of this phenomenon. The rate of photo-induced anthracene mortality is dependent on UV intensity, on daily photoperiod length and on anthracene body-burden. Acute sites of toxic action include the gills and epidermis, and there is evidence that the acute mechanism(s) of toxicity is related to non-radiative energy transfer from excited state molecules.

The results of the present study may impact several areas of concern in the sciences of environmental toxicology and hazard assessment by creating an expanded interest in the study of the potential for toxicity of PAH in the aquatic environment. The focus of concern over acid precipitation can now possibly be expanded to cover aspects of "toxic precipitation" since the formation and transport of acid rain and non-point source PAH inputs into aquatic systems are integrally related. These studies have provided a more realistic approach to aquatic hazard assessment than traditional methods, and have demonstrated the need to consider important environmental accessory parameters in laboratory toxicity testing.

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