PHOTOINDUCED TOXICITY OF POLYCYCLIC AROMATIC HYDROCARBONS TO AQUATIC ORGANISMS

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Abstract – Polycyclic aromatic hydrocarbons (PAH) such as anthracene are ubiquitous pollutants that are normally not considered acutely toxic to aquatic organisms because they are only sparingly soluble in water. Thus, bioassays conducted under the usual laboratory conditions have resulted in estimates of acute toxicity that exceed the aqueous solubilities of PAH. However, these studies are usually conducted under conditions that minimize photodegradation, and therefore the potential to observe ecologically relevant photoinduced toxicity is eliminated.

Studies under more ecologically relevant conditions in an illuminated artificial stream microcosm have demonstrated that anthracene is acutely toxic (100% mortality) to juvenile bluegill sunfish at 12 $\mu g \cdot l^{-1}$ in less than 9 h. This toxicity is more than 400 times greater than previously reported no-effect concentrations. *Daphnia pulex* are even more sensitive (LT_{50}, time to 50% immobilization – 13 min at 1.2 $\mu g \cdot l^{-1}$). These dramatic effects occur as a result of the interaction of bioaccumulated parent PAH and light, not the action of externally formed photodegradation products. Preliminary screening has indicated that benzo(a)pyrene is even more toxic than anthracene. We predict that 50% of the aquatic organisms in a lake will be immobilized at a depth of 7.25 m with a 1.2 $\mu g \cdot l^{-1}$ anthracene concentration based on the use of the Bunsen-Roscoe Law of Reciprocity, the measured extinction coefficient for UV-B in Lake Michigan (0.575 m^{-1}), an average summer day length of 14 h, and the LT_{50} for *Daphnia pulex*.

These effects, the depths at which they may be occurring, and the concentrations of PAH in the Great Lakes indicate that acute effects could be occurring at present in the Lakes. With expected increases in PAH load due to increased coal usage and increased ambient levels of solar UV due to depletion of the ozone layer, the potential exists for large effects in the Great Lakes and other aquatic systems.

Keywords – polycyclic aromatic hydrocarbons, phototoxicity, acute toxicity of PAH, anthracene, benzo(a)pyrene, PAH toxicity to *Daphnia*, bluegills, and algae.

INTRODUCTION

Polycyclic aromatic hydrocarbons (PAH) are ubiquitous pollutants that enter the aquatic environment from many anthropogenic sources including oil spills, coking processes, and atmospheric deposition from incomplete combustion of fossil fuels, as well as from natural sources such as forest fires and volcanic activity. Nearly 230,000 metric tons of PAH are expected to enter the oceans and surface waters every year (Neff, 1979). PAH loads to the environment are expected to increase with the proposed increase use of coal as an energy source (Ghers, 1976).

Acute toxicity to aquatic organisms has been demonstrated primarily for the small PAH (up to three rings) at relatively high concentrations (Neff, 1979; Korn and co-workers, 1979; Cairns and Nebeker, 1982; DeGraeve and co-workers, 1982; Eastmond and co-workers, 1984; Edmisten and Bantle, 1982; Giddings, 1979; Govers and co-workers, 1984; Lee and Nicol, 1978a,b; Sabourin, 1982; Soto and co-workers, 1975; Holcombe and co-workers, 1983). Acute toxicity appears to increase as the molecular

weight increases; however, when the molecular weight reaches that of the three-ring compounds, anaqueous concentration equivalent to the solubility in water is required to elicit acute toxicity (LC_{50}) (Neff, 1979). The acute toxicity of the less water soluble PAH (those of higher molecular weight) has been demonstrated for some compounds using carrier solvents at concentrations exceeding their solubility in water (Neff, 1979). Within the aqueous solubility limit, the higher molecular weight compounds have not been shown to be acutely toxic. Because most of the above studies were conducted under gold fluorescent lights so as to avoid photodegradation of the parent compound, possible Photo-induced toxicity was not observed. As a result, most work on PAH effects has focused on chronic toxicity, since many of the homologous series are mutagenic and carcinogenic to organisms, including fish (Schultz and Schultz, 1982; Black, 1983a,b; Baumann and co-workers, 1982). Subacute toxicity, such as egg hatching success and developmental abnormalities in fish and sea urchins, occurs at environmentally relevant concentrations with benzo(a)pyrene (BaP), a high molecular weight compound that is sparingly soluble in water (Hose and co-workers, 1982; Hannah and co-workers, 1982; Hose and co-workers, 1983). Thus, it seemed that acute toxicity of most PAH was not a toxicological problem in aquatic systems because the maximum soluble PAH concentration in water would not cause acute toxicity, but would be sufficient to cause developmental problems. Here we report that, in the presence of natural and simulated sunlight, anthracene and other PAH are acutely toxic to aquatic organisms at concentrations that are well below the PAH aqueous solubility limits.

ACUTE PHOTOTOXICITY TO AQUATIC ANIMALS

The role of ultraviolet (UV) radiation in the induction of toxicity with PAH was observed very early with *Paramecium caudatum* (Mottram and Doniach, 1938; Doniach, 1939) and was confirmed using *Drosophilia melanogaster*, Ciliata, Choretha larvae, daphnia, yeast cells, and tissue cultures as reviewed by Matoltsy and Fabian (1946, 1947). Further work has recently been done with tissue culture (Strniste and co-workers, 1983; Malling and Chu, 1970) and bacteria in culture (Harrison and Raabe, 1967). The phototoxic activity of PAH is currently used in the medical treatment of psoriasis (Tanenbaum and co-workers, 1975; Joshi and Pathak, 1984). Furthermore, UV radiation will activate otherwise noncarcinogenic PAH, such as anthracene, to carcinogens in skin painting experiments with mammals (Heller, 1950). The above evidence indicates that photoinduced changes in PAH can produce acute toxicity as well as alteration of DNA, resulting in mutagenesis and carcinogenesis. The radiation required for these actions is within the solar spectrum reaching earth ($\lambda > 300$ nm) and is ecologically relevant.

Aquatic Vertebrates

During studies of fate and transport of anthracene in a 100 m long and 0.2 m deep outdoor stream microcosm, bluegill sunfish exposed to sunlight in the 20 m just below the anthracene input died within 9 h when exposed to 11.1 ± 1.0 $\mu g \cdot l^{-1}$ anthracene in ethanol carrier (30 $mg \cdot l^{-1}$). The bluegill sunfish in the reach furthest from the anthracene input (80 m downstream) suffered no mortality during the first day of exposure. The anthracene concentration in this reach at noon was 3.9 ± 0.3 $\mu g \cdot l^{-1}$, with the decrease due primarily to photodegradation. At the start of the second day, the same fish were alive even though the early morning anthracene concentration was 10.8 ± 0.8 $\mu g \cdot l^{-1}$ 80 m from the input. All of these fish died by 1100 h despite the decrease in anthracene concentation to 3.4 ± 0.1 $\mu g \cdot l^{-1}$ (Landrum and co-workers, 1984; Bowling and co-workers, 1983). During this time, no mortality was observed in the control channel, which received no anthracene or ethanol, nor was the ethanol carrier found to be toxic in separate experiments. This acute toxicity was at least 400 times greater than any previously reported no-effect concentrations of anthracene to bluegill sunfish (Applegate and co-workers, 1957) or rainbow trout (Verschueren, 1983).

Laboratory studies were conducted to better define the response of bluegills to the phototoxicity of PAH. The LT_{50} (time required to achieve 50% mortality at a specific PAH concentration) for bluegill sunfish collected at Park Lake, MI, exceeded 500 h of continuous exposure to simulated sunlight at anthracene concentrations of 3.5 $\mu g \cdot l^{-1}$ and UV-B intensities up to 75 $\mu W \cdot cm^{-2}$ (310 ± 34 nm; Fig. 1). (Ultraviolet light is divided into three regions for photochemical consideration with the following approximate bands: UV-A, 390-315 nm, UV-B, 315-285 nm, UV-C, 285 nm and lower.) However, even at this low anthracene dose, the LT_{50} was 300 h at 170 $\mu W \cdot cm^{-2}$ (Oris and co-workers, 1984; Oris and Giesy, 1984). This light intensity is approximately equal to the measured intensity at 1 m in offshore

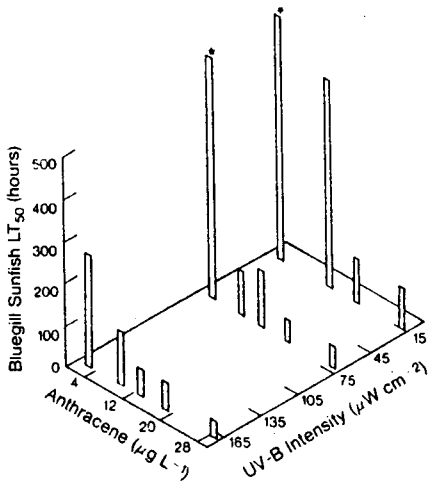

Fig. 1. Response surface for bluegill sunfish phototoxicity to the combination of artificial solar radiation and anthracene expressed as the time required to achieve 50% mortality (LT_{50}).

* No LT_{50} was observed in 500 h for fish exposed to these levels of radiation and anthracene.

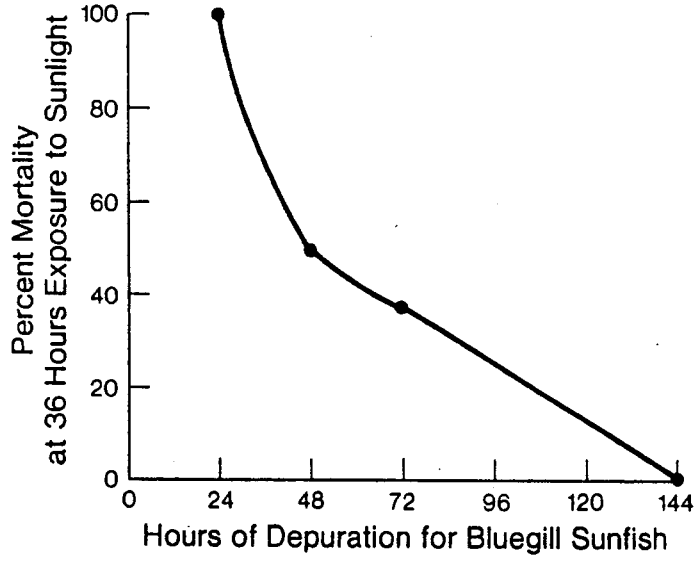

Fig. 2. Elimination of anthracene, represented as hours of depuration, by bluegill sunfish reduces the mortality of the fish pre-exposed to anthracene in the presence of sunlight.

Lake Michigan on a clear day in May. At a higher anthracene concentration (28 $\mu g \cdot l^{-1}$), the LT_{50} was less than 100 h at 15 $\mu W \cdot cm^{-2}$ – the equivalent UV intensity measured between 4 and 5 m depth in offshore Lake Michigan in May.

Toxicokinetic studies with anthracene provide important information on photoinduced toxicity. Fish held in uncontaminated water in the dark for 48, 72, 96, and 144 h after exposure to anthracene, corresponding to 1 to 4 depuration half times (Spacie and co-workers, 1983), showed decreased mortality (Fig. 2) and increased time to onset of mortality upon exposure to sunlight. Thus, the decrease in phototoxicity related directly to the bluegill body burden of the parent anthracene.

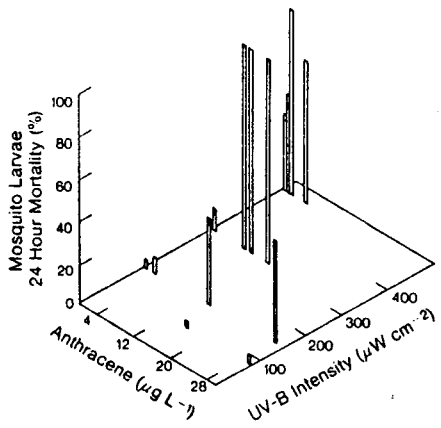

Fig. 3. Response surface for mosquito larvae phototoxicity to the combination of artificial solar radiation and anthracene expressed as cumulative mortality in 24 h.

This corresponded to decreases in measured body burden of anthracene from 318.2 ± 55 $\mu g \cdot g^{-1}$ dry weight (mean ± 2 SE) to background levels with no observed mortality after 144 h depuration and subsequent exposure to sunlight (Bowling and co-workers, 1983).

Anthracene was also phototoxic to tadpoles, *Rana pipiens*, with a 30 min sunlight exposure at LC_{50} was 65 $\mu g \cdot l^{-1}$ and after a 5 h sunlight exposure the LC_{50} was 25 $\mu g \cdot l^{-1}$ (Kagan and co-workers, 1984).

Insects

The LT_{50} for the mosquito larvae, *Aedes aegypti*, exposed to anthracene was 24 h at 14-28 $\mu g \cdot l^{-1}$ and 750 $\mu W \cdot cm^{-2}$ simulated sunlight UV radiation (Fig. 3). Twenty-four hour acute toxicity to mosquitos was not detected for exposures to 75 $\mu W \cdot cm^{-2}$ at < 14 $\mu g \cdot l^{-1}$ anthracene (Oris and co-workers, 1984). Because mosquito larvae live near the surface of the water column, they would be exposed to greater solar radiation fluences (the sum of direct and reflected sunlight impinging on a point). Thus, the potential of the phototoxic effect would be enhanced relative to that of organisms that live deeper in the water column, even if their exposure to PAH and species sensitivity are the same. Furthermore, PAH are enriched in the surface microlayer (Strand and Andren, 1980); therefore, organisms that frequent the surface will be exposed to both greater light influence and perhaps higher PAH concentrations dependent upon the bioavailability of the compounds in the surface microlayer. The response surface for the phototoxicity of anthracene to mosquito larvae and bluegill sunfish (Figs. 1 and 3) suggests that threshold limits exist for acute toxicity for both light and compound concentration.

Microcrustaceans

Daphnia pulex were extremely susceptible to the phototoxic action of anthracene. At a light intensity of 4,245 $\mu W \cdot cm^{-2}$, the LT_{50} was 15 min at 1.2 $\mu g \cdot l^{-1}$ when the organisms remained in the dosing solution containing anthracene during the bioassay. When the animals were transferred to uncontaminated water prior to exposure to sunlight, acute toxicity decreased dramatically, e.g. the LT_{50} increased to 30 min at 32.7 $\mu g \cdot l^{-1}$ (Allred and Giesy, 1984). This is because of the extremely rapid elimination rate of PAH by *D. pulex* (Leversee and co-workers, 1981). Since the parent compound has been implicated as the sensitizer for phototoxicity, the rapid elimination should lead to either reduced mortality, increased time to onset of mortality (as was the case for bluegill sunfish), or both.

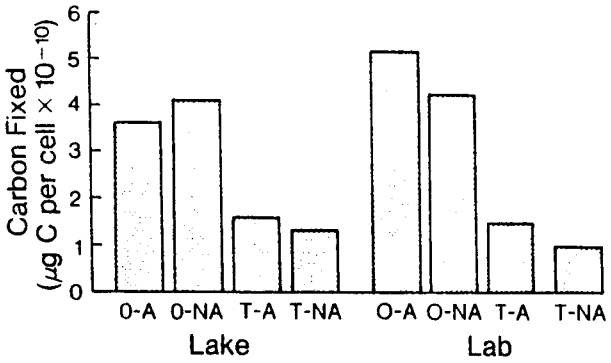

Fig. 4. Response of the primary productivity of the algae, *Chlorella pyrenoidosa*, to anthracene in the presence and absence of the UV portion of the solar spectrum at 1 m in Lake Michigan or with artificial solar radiation in the laboratory. (O-A: UV opaque chambers with algae pre-exposed to anthracene; O-NA: UV opaque chambers with no anthracene preexposure; T-A: UV transparent chambers with algae pre-exposed to anthracene; T-NA: UV transparent-chambers with no anthracene.)

Phytoplankton

The photoxicity of PAH to phytoplankton is markedly different from that of PAH to aquatic animals. Inhibition of ^{14}C-CO_2 incorporation was observed for both naphthalene and benzene at their respective aqueous solubility limits. No effect was observed for anthracene and only a slight inhibition of ^{14}C-CO_2 incorporation was observed for phenanthrene under cool white fluorescent light using *Selenastrum capricornutum* (Giddings, 1979). This work did not determine whether the quality of the light involved was important for the toxic response.

BaP elicited a photoinduced reduction in the growth rate of *S. capricornutum* at concentrations of 5 $\mu g \cdot l^{-1}$ using cool white fluorescent bulbs (General Electric F20T12/CW) compared with algae grown under gold fluorescent light (General Electric F20T12/GO) (Warshawsky and co-workers, 1983). Because these doses exceeded BaP water solubility, growth inhibition by BaP at environmental levels is not certain.

Another green alga, *Chlorella pyrenoidosa*, was pre-exposed to saturated solutions of anthracene in the dark for 24 h prior to transfer into UV transparent (plexiglass II UVT) and UV opaque (Lexan 0P1) chambers. Incorporation of ^{14}C-CO_2 was measured after the algae were exposed both to ecologically relevant wavelengths in the laboratory and to sunlight at a depth of 1 m in offshore Lake Michigan (Fig. 4). The UV opaque chambers eliminated 80% of all radiation shorter than 400 nm. No phototoxic effect was observed. In fact, the anthracene stimulated ^{14}C-CO_2 incorporation by *C. pyrenoidosa*. Perhaps more importantly, UV light alone inhibited the incorporation of ^{14}C-CO_2 by a factor of four whether or not anthracene was present (Oris and co-workers, 1984).

Autotrophic phytoplankton are exposed to both photosynthetically active solar radiation and potentially damaging UV radiation. Also, the nature of the photosynthetic reactions expose phytoplankton to potentially damaging photooxidative chemical reactions. The carotenoid accessory pigments of algae are known to protect them from lethal photooxidation caused by their own chlorophyll (Matheus and Sistrom, 1960). While photosensitizers cause damage through both oxygen-independent (type I) and oxygen-dependent (type II) photochemical mechanisms (see Mechanisms below) (Lockmann and Micheler, 1979), the type II of singlet oxygen pathway is more prevalent (Webb and Lorena, 1970). β-carotene, a normal plant pigment, is an effective singlet oxygen quencher (Anderson and Krinsky, 1973). Thus, carotenoid-containing phytoplankton should reasonably be less sensitive to PAH-photosensitized toxicity than zooplankton or fish larvae. Phototoxicity of BaP to algae may have little environmental significance owing to the low concentrations of BaP in the environment and the protective mechanisms of the algae. Studies to date with algae have been conducted with very hardy species. More fragile species, such as diatoms and microalgae, may be more

susceptible to phototoxicity effects and should be studied.

Bacteria

Incorporation of glucose, amino acids, and thymidine by heterotrophic microorganisms in sea water was reduced in the presence of crude oil and light. The crude oil alone was not sufficient to inhibit the incorporation of the above nutrients by the bacteria (Penegrud and co-workers, 1984). This result for a mixed assemblage is consistent with the reduced viability observed previously for *Escherichia coli* in the presence of BaP and light (Harrison and Raabe, 1967). Oxygen was also required to elicit the phototoxic response with *E. coli*.

RELATIVE PHOTOTOXICITY OF PAH

Examination of the relative phototoxicity of various PAH within a species can be accomplished as the protocols for testing are internally consistent. Examination of the relative sensitivity between species, for the examples below, is more subjective as it requires evaluating both PAH concentration and light quality and quantity as well as the usual differences one should consider in aquatic toxicity testing. Certainly, the establishment of standardized protocols, such as was used for comparison of strains of bluegill sunfish (Oris and co-workers, 1984) will be necessary for thorough comparisons.

Various PAH were screened for phototoxicity in detail with *Paramecium caudatum*, *Tetrahymena pyriformis*, and *Artemia salina nauplii* (Epstein and co-workers, 1964a; Small and co-workers, 1966; Morgan and Warshawsky, 1977). Carcinogenic activity was always correlated with phototoxic activity; however, substantial phototoxic activity did not require the compound to be carcinogenic. Anthracene was very phototoxic in most studies with aquatic organisms discussed previously. However, about 300 times greater concentrations of anthracene were required to produce the same phototoxic effect compared to BaP for *P. caudatum*, and 100 times greater concentration of anthracene was required compared to benzo(a)anthracene (Epstein and co-workers, 1964a).

In some preliminary screening studies with *Daphnia magna*, time to toxicity was examined for 3-methylcholanthrene (3-MC), BaP, dimethylbenzanthracene (DMBA), and anthracene in paired experiments. DMBA was more phototoxic than BaP, while BaP was more or equally as toxic as 3-MC, and both were much more toxic than anthracene (Leversee, 1984). Similar relationships were found with *Paramecium*, *T. pyriformis*, and *A. Salina* (Epstein and co-workers, 1964a; Small and co-workers, 1966; Morgan and Warshawsky, 1977).

Phototoxicity apparently increases with increased PAH conjugation and lipophilicity. The alkylated PAH also exhibit significant phototoxicity. The increasing phototoxicity is probably due in part to the additional aromatic rings that not only increase the light absorption of the compound, but also increase the molecular weight, and concomitantly increase the lipophilicity enhancing the potential penetration of the compound into the organisms. Further, the added aromatic rings would stabilize any charges or radicals induced in the molecules (see Mechanisms) and therefore increase the reactivity of the compounds.

The order of PAH phototoxicity among compounds differs among species. For instance, DMBA was the most toxic of the PAH studied for *D. Magna*, but required a 5 fold greater concentration compared to BaP for toxicity to *Paramecium* (Leversee, 1984; Epstein and co-workers, 1964a). Similarly, benz(a)anthracene was the most phototoxic PAH for *A. salina*, requiring 10 times lower concentration than BaP, while for *T. pyriformis* the toxicities of the two compounds were similar on a concentration basis. Benz(a)anthracene required about three times greater concentration compared to BaP for toxicity to *Paramecium* (Epstein and co-workers, 1964a; Small and co-workers, 1966; Morgan and Warshawsky, 1977).

Rana pipiens, *D. magna*, *Aedes aegypti*, and bluegill sunfish exhibited similar order of magnitude sensitivities to anthracene phototoxicity (Kagan and co-workers, 1984; Leversee, 1984; Oris and co-workers, 1984, Oris and Giesy, 1984; Bowling and co-workers, 1983), but were less sensitive to anthracene phototoxicity than were *D. pulex* (Allred and Giesy, 1984). Even closely related species appear to differ in PAH photoxicity. For example, for anthracene, the LT_{50} for *D. pulex* was 37 min at 7.5 $\mu g \cdot l^{-1}$ (Allred and Giesy, 1984), while for *D. magna* it was 5 h at 106 $\mu g \cdot l^{-1}$ (Leversee,

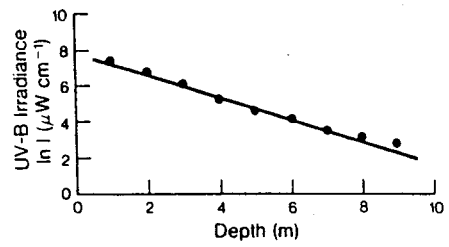

Fig. 5. Natural log of UV-B irradiance (277-344 nm) as a function of depth for a station 12 mi west of Grand Haven, MI, in Lake Michigan at 1,742 h on 23 May 1983, measured with a Macam PhotoMetrics Model UV-103 radiometer equipped with a cosine corrected SD104 photodiode.

1984). Animals in both studies were pre-exposed to anthracene and then tested for phototoxicity in uncontaminated water under natural sunlight. Because *D. pulex* and *D. magna* were studied in Michigan and South Carolina, respectively, there would have been a difference in the angle of incident light as well as difference resulting from season and time of day. Even though there were probably differences in light intensity between the studies, the very large differences in response between the two species implies species differences in susceptibility to PAH phototoxicity.

Sensitivity to phototoxicity also varies within a single species depending on the source of the organisms. These different sources of bluegill sunfish undoubtedly reflect differences in the genetic make up, nutritional state, or other component that may alter the physiological state of the organism under study. Bluegill sunfish from hatchery sources, Osage Catfisheries, MO, and ByBrook Bass Hatchery, CT, were two to four times more sensitive to anthracene and light than were the natural population from Park Lake, MI, based on the LT_{50} measured at the same concentration and light intensity (Oris and co-workers, 1984).

THE ROLE OF LIGHT

Since both compound and light are required for this synergistic action and no mortality occurs in the presence of the PAH in the absence of light (Bowling and co-workers, 1983), the intensity and duration of solar irradiance, as well as the concentration of PAH, required to produce acute toxicity should be determined. The phototoxicity of PAH depends in part on the amount of solar radiation of the active wavelength penetrating the water and is unimportant for aquatic systems with high particulate loads, highly colored waters, or both, that strongly attenuate solar radiation. However, in clear waters, such as the Great Lakes, solar radiation can penetrate to ecologically significant depths. In clear, shallow aquatic systems, such as our artificial streams, microcosms, and some natural streams, all wavelengths of solar radiation penetrate to the bottom of the water column. Furthermore, in deeper systems many aquatic organisms spawn in the shallow areas where sensitive life stages, such as eggs and juveniles, will be exposed to the potential photoinduced toxicity of PAH more than adults.

Light penetration in water is both wavelength- and water-quality-dependent. We measured an extinction coefficient of 0.575 m^{-1} for UV-B radiation using a Macam PhotoMetrics Model UV-103 radiometer equipped with a water-tight model SD104 cosine corrected photodiode in Lake Michigan at a station 12 mi offshore of Grand Haven, MI, in May 1983 (Fig. 5). Thus, only half the radiation intensity remains after penetrating 1.2 m, and 1% of the intensity remains at 8.0 m.

Also, the active waveband for phototoxic activity consists of the shorter wavelengths in the solar spectrum (wavelength cutoff 290 nm) and varies with the absorption spectrum of the compound. The absorption spectra of PAH are "red-shifted" because of hyperconjugation at relatively long UV wavelengths. The absorption spectra of the PAH indicate that many of these compounds will exhibit photoinduced toxicity. For anthracene, the active wavelengths lie in the UV-A region of the solar spectrum (315-380 nm) (Allred and Giesy, 1984).

MECHANISMS

Anthracene and other PAH are readily photolyzed in the aquatic environment (Zepp and Schlotzhauer, 1979; Zadelis and Simmons, 1983). The photooxidation of anthracene proceeds in part through a reactive endoperoxide (Fox and Olive, 1979) that might be partially responsible for the toxicity. In addition, PAH form reactive, potentially toxic epoxides as part of the chemical pathway to diols. Externally generated toxic photoproducts have been observed for aromatic materials in coal liquids (Herbes and Whitley, 1983; Larson and co-workers, 1977). Such externally generated toxic photoproducts have resulted in enhanced mutagenicity of aromatic compounds photolyzed in the presence of nitrate ions (Suzuki and co-workers, 1983) and of nitro-containing PAH photolyzed in sunlight (Okinaka and co-workers, 1983; Strniste and co-workers, 1982). However, additional studies revealed that the proximal toxin was the parent PAH and not a photoproduct generated external to fish (Bowling and co-workers, 1983).

The proposed mechanism for photoinduced toxicity involves the interaction of light with the photosensitizing compound to form a reactive intermediate resulting in a subsequent free radical reaction. The following chemical mechanisms have been suggested:

1. Charge Transfer Complex Formation

$$PAH + B \rightarrow PAH\cdot B \xrightarrow{light} PAH\cdot + B\cdot$$

where B = a biological macromolecule.

Charge transfer complexations have been demonstrated with a wide variety of PAH (Epstein and co-workers, 1964b). These complexes were produced in chloroform solutions, a chemical environment resembling a biological membrane in polarity. The formation of these charge transfer complexes correlated positively but imperfectly with measurements of phototoxicity in *Paramecium caudatum* (Epstein and co-workers, 1964b). The carcinogenic action of PAH was also correlated with its phototoxicity, but carcinogenicity and complexation are not necessarily associated (Epstein and co-workers, 1964a, b).

2. Direct Interaction

$$PAH + R\text{-}SH \xrightarrow{light} PAH\text{-}H\cdot + RS\cdot$$

where R-SH = a sulfhydryl-containing biomolecule.

Direct interaction, a type I photochemical mechanism, can produce photoinduced hemolysis with chlorpromazine and dimethylchlortetracycline (Nilsson and co-workers, 1975). This type of direct interaction of a photosensitizer with biomolecules also occurs in the oxidation of cysteine with crystal violet as the sensitizing agent (Gennari and co-workers, 1974). The above reaction was suggested because of the quinoid structure of the crystal violet and the propensity of quinones to abstract hydrogen. This mechanism may help explain the phototoxicity of PAH, since many of the PAH photooxidation products are quinones. An example is the formation of anthraquinone from anthracene by photooxidation (Fox and Olive, 1979).

PAH may also directly bind covalently to macromolecules, even in the absence of oxygen (Sinha and Chignell, 1983).

$$PAH + B \xrightarrow{light} PAH\text{-}B$$

Binding of PAH to macromolecules in the presence of light contributes to the disruption of cellular processes by cross linking proteins. PAH also bind to liposomes and induce lipid peroxidation. This peroxidation is not affected by superoxide dismutase or catalase, demonstrating that the activity is a direct reaction (Sinha and Chignell, 1983).

3. Hydroperoxide Formation

Another mechanism for initiation of a free radical chain reaction is the formation of PAH hydroperoxide and the subsequent formation of the active hydroxyl radical in aqueous solutions (Cusachs and Steele, 1969).

$$PAH + {}^1O_2 \rightarrow PAHOOH \xrightarrow{light} \cdot OH + \cdot O\text{-}PAH$$

Hydroxyl radical formation results from the above reaction, as well as other singlet oxygen "reactions. (see below.)" Once the reactive species is formed, the free radical reactions are supported primarily by the further formation of hydroxyl radicals. The hydroxyl radical is not as well known for its activity and stability as an oxidizing agent in aqueous systems as singlet oxygen, but it still is well established (Draper and Crosby, 1981). Hydroxyl radicals may also oxidize other PAH to form endoperoxides (Foote and Wexler, 1964) that may then act to bind to macromolecules.

4. Singlet Oxygen Formation

The final mechanism for the phototoxic effect of PAH, and the one often considered most likely, involves the formation of singlet oxygen. This is a type II photochemical mechanism that in turn acts as the reactive species producing biological damage.

$$PAH \xrightarrow{light} {}^1PAH^* \rightarrow {}^3PAH^*$$

$${}^3PAH^* + {}^3O_2 \rightarrow {}^1PAH + {}^1O_2$$

Triplet and singlet excited states of a suitable light absorbing sensitizer are represented by ${}^3PAH^*$ and ${}^1PAH^*$, respectively. The ${}^1O_2$ may then react to form oxidation products with PAH (Cusachs and Steele, 1969) or biomolecules such as methionine (Sysak and co-workers, 1977). These oxidations in turn produce hydrogen peroxide, hydroxyl radicals, and other free radicals that will result in secondary effects (Nilsson and co-workers, 1972). The singlet oxygen may also cause modification of DNA, particularly the destruction of guanine (Gutter and co-workers, 1977).

The potential to produce the above effects depends on the lifetime of singlet oxygen. In aqueous systems, singlet oxygen has a lifetime too short (2 μs) to be effective as an oxidative reactant. In contrast, the reactive lifetime is up to as many as two orders of magnitude greater in organic solvents (Merkel and Kearns, 1972). Similarly long lifetimes would be expected in such biological structures as membranes, where the damage from phototoxicity seems to occur (Oris and Giesy, 1984).

Singlet oxygen leads to lipid peroxidation and causes membrane damage (Cannistraro and Van de Vorst, 1977). Lipid peroxidation also results from direct photolytic interaction without singlet oxygen. (See under Direct Interaction above.) Membrane damage can cause osmoregulatory distress for aquatic organisms, or can significantly alter cellular function. Further evidence of membrane damage by phototoxic action of PAH is an observed change in lysosomal membrane permeability with exposure to PAH and light (Malling and Chu, 1970; Allison and co-workers, 1966).

In addition to membrane damage, the binding of PAH to DNA via a photolytic mechanism may affect other toxic events such as carcinogenicity and mutagenicity (Blackburn and co-workers, 1975). Free radicals (Malins and co-workers, 1983) and free radicals in conjunction with lipid peroxidation (Dix and Marnett, 1983; Corey and Taylor, 1964) are associated with tumors in fish and with formation of the ultimate carcinogen for BaP, respectively. Such free radicals could easily be generated by photolytic events as well as other more classical causes.

ECOLOGICAL SIGNIFICANCE

Aquatic scientists have generally believed that solar radiation in the UV region does not penetrate natural waters more than a few centimeters. However, we as well as others (Calkins, 1982; Smith and Baker, 1979, Damkaer and co-workers, 1980) have shown significant penetration of solar UV to ecologically relevant depths in clear water (Fig. 5). Thus the ecological significance of photoinduced toxicity depends on the habit of the organism, the water quality, and the susceptibility of

the organism as well as the amount of solar radiation and concentration of PAH present. Certainly, clear shallow streams would be a susceptible environment owing to the minimal attenuation of solar radiation. Juveniles of most fish are found in the shallow area of the littoral zone or near the surface as pelagic larvae, and thus would be extremely vulnerable even in lakes.

Photodynamic processes respond to the number of photon events per unit of time and therefore light intensity per unit of time. The time dependence with intensity can be expressed in the Bunsen-Roscoe Law of Reciprocity (Dworkin, 1958):

$$K_r \propto I \cdot T$$

where I = light intensity ($\mu W \cdot cm^{-2}$), T = time (h), and K_r = rate of reaction.

This indicates that rates of reaction will be proportional to the increase in the number of photons per unit of time and that the overall extent of reaction is dependent on the total amount of photons collected, assuming no back reaction. From the Bunsen-Roscoe Law of Reciprocity, the measured extinction coefficient for UV light for Lake Michigan (0.575 m^{-1}), an average summer day length of 14 h, and the LT_{50} for *D. pulex* (Allred and Giesy, 1984), the depth at which 50% of the organisms would be immobilized after 1 d at 1.2 $\mu g \cdot l^{-1}$ would be 7.0 m. This calculation proceeds as follows:

Since the concentration will be assumed constant, changes in the response will only be dependent on changes in the light intensity and time. Assuming the light intensity is constant over the daylight period and no significant biological repair occurs over this time period, the Bunsen-Roscoe Law of Reciprocity states that the amount of photoreaction will be dependent on the total number of photons absorbed. Since the number of photons is the product of intensity and time, an increase of a factor of 56 between the LT_{50} for *D. pulex* (15 min) and the length of daylight (14 h) will permit a reduction in intensity by the same proportion to maintain the total number of photons constant. Then accounting for the extinction of light with depth yields the following:

$$I_d / I_s = e - K_a d$$

where I_d = intensity at depth, I_s = intensity at surface where LT_{50} was determined in light intensity, K_a = extinction coefficient for Lake Michigan (0.575 m^{-1}), and d = depth (m). Then:

$$1/56 = 0.018 = e^{-0.575d}$$

Note that 1/56 represents the relative intensities between the surface and depth of interest and not the absolute intensities which would yield the same ratio. Taking the natural log of both sides of the equation and rearranging terms

$$d = -4.025 / -0.575 = 7.0 \text{ m}$$

This depth includes an important portion of the habitat for both zooplankton and fish larvae (Cole and MacMillan, 1984).

While the current concentrations of PAH in Lake Michigan are lower than 1.2 $\mu g \cdot l^{-1}$ for each compound (individual PAH range from 6 to 24 $ng \cdot l^{-1}$) (Eadie and co-workers, 1983), the compounds may well have additive toxicities in the presence of solar radiation. Some PAH compounds are up to 300 times more phototoxic than anthracene (Epstein and co-workers, 1964a). Therefore, acute effects could be occurring in the Great Lakes.

PAH phototoxicity could potentially further aggravate the effect of UV light on aquatic organisms. Many aquatic organisms, shrimp larvae, crab larvae, and euphausiids are known to live at or near the UV tolerance limit under current irradiance levels (Damkaer and co-workers, 1980). Further, near-surface waters are environmentally important since many zooplankters reside in these upper strata for at least a portion of their life cycle. The ability of the individual species to cope with altered UV exposure or UV sensitivity without requiring modification of physiology or behavior is believed to be small (Calkins and Thordardottir, 1980). For this reason, the added role of PAH phototoxicity may greatly influence aquatic ecosystems, particularly since the mechanisms of action for direct UV toxicity and phototoxicity are similar – a free radical reaction, probably proceeding via singlet oxygen.

Because of the massive sampling regime that would be required, ecological studies that could detect such effects are prohibitively expensive and are not currently being pursued. Further, even if changes in the populations were detected, associating those changes with a particular toxic effect would be impossible at this time.

CONCLUSION

The work summarized here focused on acute lethality from photo-induced toxicity. Because of the proposed mechanisms, free radical reactions and covalent binding to macromolecules, sublethal effects such as alteration of fecundity and hatching success may also result from photoinduced reactions. This sublethal toxicity may also occur at lower concentrations of PAH and lower radiation intensities than those where acute lethality is observed.

For phototoxicity to influence organism health significantly, the damage rate must be balanced against the repair rate for the organism. The rate of damage in this case is subject not only to the toxicokinetics of the compounds in question but also to the flux rate of solar radiation of the appropriate wavelengths. Therefore, photoinduced toxicity, more than other forms of toxicity, is extremely dependent on the kinetics of many processes. All the kinetics constants must be known in order to predict the phototoxicity of any compound under a specified set of environmental conditions.

In conclusion, PAH exerts a photoinduced acute lethal toxicity at concentrations approaching three orders of magnitude lower than that demonstrated previously for acute toxicity in the absence of ecologically significant light intensity at the appropriate wavelengths. Photoinduced toxicity is apparently greater for the larger PAH molecules and for alkylated PAH. Similar species have different sensitivities to photoinduced toxicity, a single compound exerting greater toxicity in one species than in another. Concentrations of phototoxic PAH and light penetration are sufficient in waters such as those of the Great Lakes to predict some phototoxicity, especially among fish larvae and zooplankton that live near the surface. The phototoxic effect does not appear to be as important for phytoplankton, but more research should be conducted with a variety of species before firm conclusions are drawn. The extent, ecological significance, and limitations of the photoinduced toxicity of PAH should be further investigated. Defining this problem is especially important because the proposed increase use of coal as an energy source is expected to increase environmental loads of PAH. Additionally, alteration of the ozone layer from some airborne pollutants is proposed; that would subsequently increase UV light intensity and increase aquatic phototoxicity problems in the future.

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