JA-57

Environmental Toxicology and Chemistry Vol. 5, pp. 761–768, 1986
Printed in the USA. Pergamon Journals Ltd.

0730-7268/86 $3.00 + .00

PHOTOINDUCED TOXICITY OF ANTHRACENE TO JUVENILE BLUEGILL SUNFISH (*LEPOMIS MACROCHIRUS* RAFINESQUE): PHOTOPERIOD EFFECTS AND PREDICTIVE HAZARD EVALUATION

JAMES T. ORIS* and JOHN P. GIESY, JR.
Pesticide Research Center,
Department of Fisheries and Wildlife and Center for Environmental Toxicology,
Michigan State University, East Lansing, Michigan 48824

(*Received* 10 *October* 1985; *Accepted* 4 *March* 1986)

Abstract—The effect of daily light-cycle duration (photoperiod) on the solar ultraviolet radiation (SUVR) -induced toxicity of anthracene to juvenile bluegill sunfish (*Lepomis macrochirus* R.) in a laboratory system under simulated sunlight was examined. Rates of acute mortality were dependent on both anthracene concentration and photoperiod. Median lethal time values calculated on the basis of accumulated SUVR exposure time (UV-LT50) were compared with LT50 values calculated from real time of exposure (R-LT50) to determine relative rates of phototoxic damage versus physiologic repair during periods of darkness. The comparison of these LT50 values indicated that the photoinduced toxicity of anthracene to fish was slowly repaired during periods of darkness but that enough damage accumulated over several SUVR cycles to cause acute mortality.

The results from these experiments were incorporated into a relationship to predict no-effect anthracene concentrations from the daily light-cycle duration at one SUVR intensity. Information from acute toxicity tests was used to extrapolate to chronic no-effect values. No-effect anthracene concentrations in water were predicted to range from 1.2 μg/L for 24 h light:0 h dark photoperiod to 13.5 μg/L for a 6 h light:18 h dark photoperiod. A no-effect anthracene body burden of 131 μg/kg has been calculated for juvenile bluegill sunfish for a 16 h light:8 h dark photoperiod at an equivalent depth of 3.0 m in a typical eutrophic system. Thus, considering current natural polycyclic aromatic hydrocarbon concentrations in water and in fish tissue, there exists natural waters in which photoinduced PAH toxicity may occur.

Keywords—Anthracene Polycyclic aromatic hydrocarbons *Lepomis macrochirus* Photoinduced toxicity Solar radiation

INTRODUCTION

Solar ultraviolet radiation (SUVR) -induced effects of polycyclic aromatic hydrocarbons (PAH) on mammals [1–4] and microbes [5–7] have been known for many years, but only recently have aquatic ecologists and toxicologists recognized the importance of SUVR in aquatic ecosystems. Concern about the potential degradation of the atmospheric ozone layer and the subsequent increase in global intensities of injurious SUVR has lent impetus to studies on the damaging effects of direct UV irradiation in fish [8] and other aquatic organisms [9–11]. Concern about potential increases of PAH inputs to aquatic systems from nonpoint-source combustion of fossil fuels [12] has resulted in studies on fate and transport mechanisms, long-term chronic effects and the determination of vectors of carcinogen transfer to humans.

PAH are extremely toxic when aquatic organisms are exposed simultaneously to natural or artificial SUVR. This acute, photoinduced toxicity has been observed in a variety of aquatic organisms, including fish [13,14], tadpoles [15], cladocerans [16], mosquito larvae [17] and algae [18]. Reviews of the possible modes of toxic action [19] and potential ecological consequences [17] of the photoinduced toxicity of anthracene and other PAH in the aquatic environment have been presented previously.

To be able to predict the response of an organism to a photodynamic compound one must know

*To whom correspondence may be addressed at: Zoology Department, Miami University, Oxford, OH 45056.

the dose both of the compound and of UV radiation. In addition, the responses of an organism to a toxicant is determined, in part, by the rate of damage caused by the toxicant relative to the rate at which the organism can repair the damage. Previously, we have presented the results of laboratory experiments that investigated the effect of SUVR intensity on the photoinduced toxicity of anthracene to juvenile sunfish (*Lepomis* spp.) under conditions of continuous light [14]. Here we report on a series of laboratory experiments designed to assess the effect of daily light-cycle duration (photoperiod) on the photoinduced toxicity of anthracene to juvenile bluegill sunfish (*Lepomis macrochirus* Rafinesque). We have developed a relationship to predict no-effect anthracene concentrations (∞ LC1) from daily light-cycle duration at one SUVR intensity. This assessment is presented as a preliminary estimate of photoinduced 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.

MATERIALS AND METHODS

Juvenile bluegill sunfish (*Lepomis macrochirus* Rafinesque), weighing 1 to 1.5 g, were obtained from Osage Catfisheries (Osage Beach, MO). Prior to the bioassays, the fish were held for at least 2 weeks in a large flow-through fiberglass tank. Aerated and charcoal-filtered water (22 ± 1°C) [20] was used in holding tanks and in all bioassays. Fish were maintained on an 18 h light:6 h dark photoperiod under a low-pressure sodium lamp; they were fed twice a day with Biodiet-Starter (Bioproducts, Inc., Worrenton, OR).

The laboratory lighting system, the flow-through dosing system and the analytical and bioassay procedures have been described previously in detail [14]. Briefly, sunlight was simulated using a combination of Chroma F40C50 white (General Electric) and FS40 ultraviolet (Westinghouse) fluorescent bulbs filtered with a 5-mil thickness of Kodacel® cellulose triacetate to eliminate radiation of wavelengths shorter than 290 nm. The lights were wired into four independent circuits on separate 24-h time clocks. A 30-min transition period from light to dark and vice versa was achieved by turning on (or off) each circuit at 10-min intervals at the initiation of each light or dark cycle. SUVR intensities for all experiments were as follows: UVB (310 ± 34 nm), 70 $\mu W/cm^2$; UVA (365 ± 36 nm), 100 $\mu W/cm^2$.

Anthracene (mol. wt. 178.23, Sigma grade III) solutions were delivered at aqueous solubility (35 $\mu g/L$ at 22°C) by a once-through aqueous elution column that contained anthracene adsorbed to silica sand at 0.2% (w/w) and diluted to the desired concentrations prior to dosing the aquariums in the flow-through system [14].

The bioassays were conducted in 20-liter glass aquaria. Fish were exposed to anthracene and acclimated for 48 h in the aquaria before SUVR exposure was initiated [14]. During the acclimation period the 18 h light:6 h dark photoperiod was changed (50% per day) to one of four others: 24:0, 18:6, 12:12 or 6:18. Ten or eleven fish per aquarium and two aquaria per anthracene concentration were used in all bioassays. Fish were not fed for 48 h before or for the first 96 h of a bioassay [14]. After 96 h, fish were fed sparingly every other day for the duration of the test.

Mortality, assessed as the lack of opercular movement, was recorded at least twice daily. Concurrent with all anthracene-SUVR exposures, a no-anthracene treatment was performed as an SUVR-only control. Previous studies demonstrated a lack of acute anthracene toxicity in the absence of SUVR [14]. Therefore, an anthracene-no SUVR treatment was not performed in this set of experiments.

Median lethal times (LT50) were calculated from the recorded time-mortality data [21]. Two different types of LT50 estimates were derived for each anthracene concentration for 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 h light: 12 h dark photoperiod, for example, accumulated only 50% of the SUVR exposure time as compared to fish exposed during the same real-time period in the 24 h light:0 h dark photoperiod.

RESULTS

SUVR-anthracene-treated fish exhibited the identical signs of adverse effect observed in a previous study [14]. Affected fish had tremors and showed 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 aquaria along the sides or in the corners; probably to avoid SUVR irradiation.

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 in these fish and they were less prone to bouts of coughing and uncoordinated swimming. However, within 1 h after the initiation of another light cycle, the condition of these fish would regress to that demonstrated before the end of the previous light cycle. Over several photoperiod cycles, the condition of these fish progressively deteriorated until they died.

Time to mortality was dependent both on anthracene concentration and on daily light-cycle length (Fig. 1). Real-time median lethal times (R-LT50) ranged from 20 h at an anthracene concentration of 15 μg/L or greater and a 24 h light:0 h dark photoperiod to 202 h at 15 μg/L and a 12 h light:12 h dark or 6 h light:18 h dark photoperiod. Above 15 μg/L, R-LT50 was independent of anthracene concentration during the continuous photoperiod. Anthracene dose independence of R-LT50 was observed above 20 μg/L during the 18 h light:6 h dark photoperiod. During the 18 h light:6 h dark photoperiod dose independence of mortality rate occurred at greater anthracene concentrations than during the 24 h light:0 h dark photoperiod; however, the maximum rates of mortality between the two photoperiods were not significantly different (Fig. 1).

DISCUSSION

One important objective of our study of photoperiod effects was to examine rates of phototoxic damage as compared with rates of physiologic repair. The simplifying assumption that damage is nonrepairable during periods of darkness allowed for an initial estimate of LT50 values for any given anthracene concentration and SUVR intensity [14]. The present study was conducted, in part, to test the validity of this assumption.

Assessment of the repairability of damage can be accomplished by the examination of the different LT50 types. 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 be a measure of the rate of damage for cumulative SUVR exposure, assuming that little or no repair occurs during SUVR exposure periods. If damage occurs only 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 of the same magnitude, independent of photoperiod. When damage is shown to be cumulative, then only total SUVR dose (dose rate × duration) and PAH dose are needed to predict the photoinduced toxic response.

The results obtained in this study suggest that photoinduced 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 were the R-LT50 values, but they were still dependent on daily light-cycle duration (Fig. 2). This indicates that damage is not entirely cumulative and that damage incurred in the light cycle is partially repaired as a direct function of the length of the dark cycle. The observation of partial recovery after a dark cycle corroborates this evidence.

The achievement of a maximum rate of mortality across anthracene concentrations for the two longest photoperiods also implies that damage involves an equilibrium (reversible) process, as well

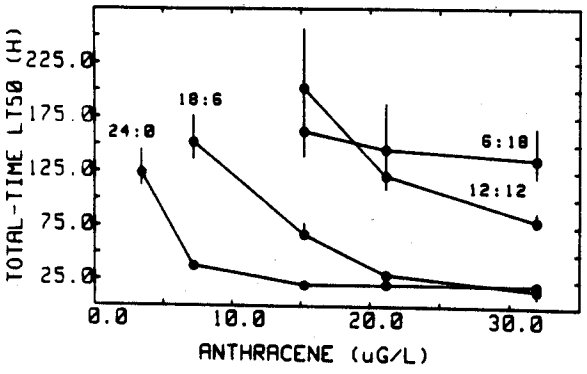

Fig. 1. Plot of real-time median lethal time (R-LT50) versus anthracene concentration in water for (hours light:hours dark), 24:0, 18:6, 12:12 and 6:18 photoperiods. Error bars indicate 95% C.I.

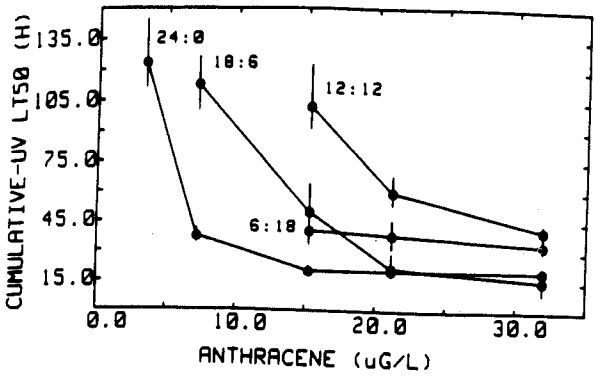

Fig. 2. Plot of median lethal time, calculated based on cumulative light-hours exposure (UV-LT50), versus anthracene concentration in water for (hours light:hours dark) 24:0, 18:6, 12:12 and 6:18 photoperiods. Error bars indicate 95% C.I.

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 h light:18 h dark photoperiod is inverted in position relative to the curve for the 12 h light:12 h dark photoperiod (Fig. 2) when compared with the same dose-response curves for R-LT50 values (Fig. 1). This inversion suggests that at some point between 12 and 6 h of SUVR per day there is a shift from reversible to cumulative damage and that below a threshold length of SUVR exposure anthracene becomes more toxic per SUVR-hour than in longer photoperiods. Because of this discontinuous response across daily light-cycle duration of UV-LT50 values and because there is evidence that damage is only partially cumulative, the length of the daily SUVR exposure period must be known in addition to the total SUVR dose to accurately describe anthracene phototoxicity to juvenile bluegill sunfish.

Predictive hazard assessment

In a previous study [14] it was demonstrated that median lethal time (LT50) values for photoinduced anthracene toxicity to juvenile sunfish could be predicted from total SUVR dose and concentration of anthracene in the water. This prediction was based on the assumption that no repair of photoinduced toxic damage occurred in the absence of SUVR. That is, damage accumulated as a direct function of total SUVR dose. While the previous predictive relationship still represents a sound preliminary estimate of photoinduced anthracene toxicity, this study demonstrated that the assumption of cumulative damage is not necessarily valid.

A predictive relationship that would account for variations due both to SUVR intensity and to daily SUVR exposure time would be the most accurate mechanistic description of photoinduced anthracene toxicity. Also, it is often more desirable to obtain estimates of lethal concentration (LC) values rather than lethal time (LT) values, which require a priori knowledge of toxicant concentrations, especially in determining guidelines and interim water quality criteria.

In developing a predictive hazard assessment for photoinduced anthracene toxicity to bluegill sunfish, therefore, the two independent variables considered were SUVR intensity [14] and photoperiod duration. The dependent variable chosen was that 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 a previously outlined method [22]. The derivation of this chronic toxicity estimate is slightly different from that proposed in the original method [22], which uses the chronic LC0. We chose to use the chronic LC1 because we felt that our estimate was a conservative and acceptable level of risk for fish populations and because there is an extremely large inherent amount of uncertainty involved in extrapolating to LC0 values in a probability relationship.

The predictive relationship for chronic hazard assessment for the photoperiod experiments was developed as follows. Acute LC1 values were estimated [21] 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 (Fig. 3). The estimated ∞LC1 value for each photoperiod was then derived as the least-squares linear-regression estimate of the intercept from each of

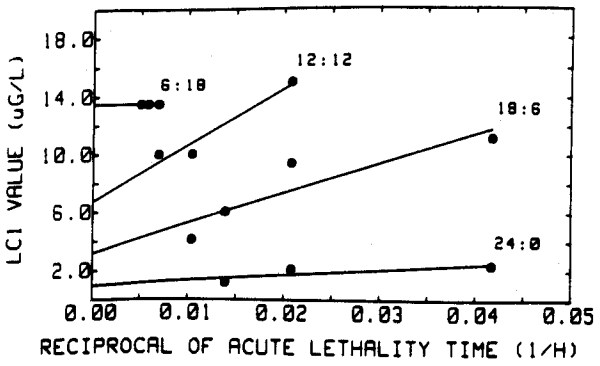

Fig. 3. Relationship between anthracene concentration and acute LC1 values as a function of the reciprocal of acute lethality time period. Plotted points represent estimated LC1 values calculated from log anthracene-probit analysis. Plotted lines are linear least-squares estimates for each photoperiod (hours light:hours dark). Chronic LC1 values are predicted as the y-intercepts from these following regressions: for 6:18, $y = 13.5 + 0.00(x)$; for 12:12, $y = 6.901 + 380.53(x)$; for 18:6, $y = 3.196 + 207.38(x)$; for 24:0, $y = 1.151 + 34.23(x)$.

these plots. This procedure resulted in estimates of ∞LC1 for each photoperiod for a UVA (365 ± 36 nm) intensity of 100 $\mu W/cm^2$.

The incorporation of predictions of chronic LC1 values for all SUVR intensities from the previous study [14] 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, which 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, we did not feel justified in making any adjustments for differential species sensitivities for that or other SUVR intensities. Even if species sensitivities were similar, the data from the SUVR intensity experiments could not be incorporated into 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 [14] do, in fact, intersect (Fig. 4), implying an interaction between anthracene dose and SUVR intensity as they relate to photoinduced 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 as compared with 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 as compared with acute toxicity estimates for each respective SUVR intensity. The simultaneous exposure of fish to both anthracene and SUVR can be considered a multiple toxicant exposure. The method used to derive chronic toxicity predictions apparently cannot account for multiple toxicant exposure when interaction among toxicants occurs. Until a method is developed that will account for these interactions, it is suggested that chronic photoinduced anthracene LC values be predicted on the basis of the intermediate SUVR intensity as a

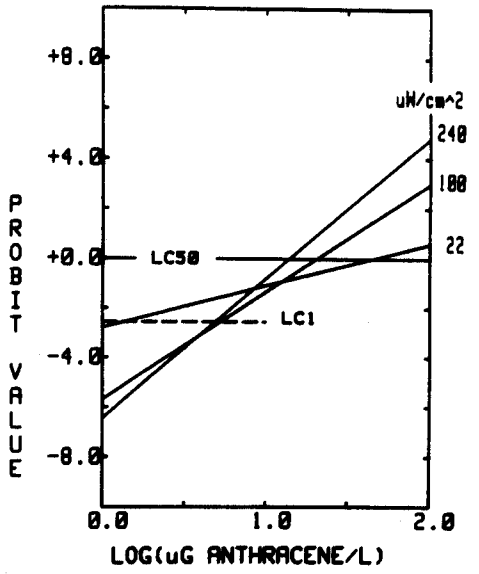

Fig. 4. Plot of calculated probit values for the 96-h acute lethality period versus $\log_{10}$ anthracene concentration, showing the interaction between UVA (365 ± 36 nm) intensity and anthracene concentration.

median value for all SUVR intensities or that LT50 values be predicted on the basis of the simplifying assumption of cumulative damage [14]. These predictions should be considered tentative 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 (Fig. 5). Estimates for 96-h LC50 values ranged from 4.5 μg/L anthracene for the 24 h light:0 h dark photoperiod to 46 μg/L for the 6 h light:18 h dark photoperiod. Predicted ∞LC1 values ranged from 1.2 μg/L anthracene for the 24 h light:0 h dark photoperiod to 13.5 μg/L for the 6 h light:18 h dark photoperiod. Both sets of estimates were negative-exponentially related to the number of daily SUVR exposure hours (Fig. 5). 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, SD 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.

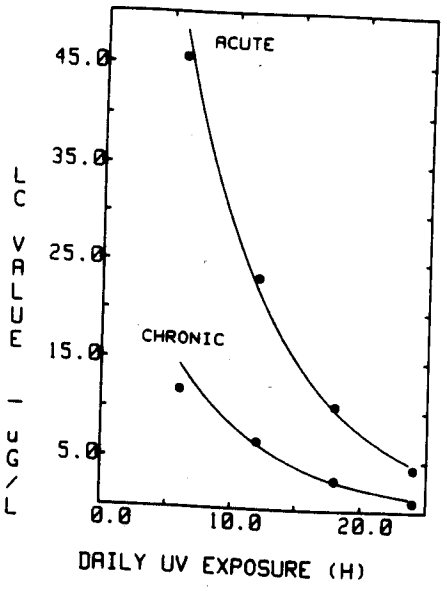

Fig. 5. Relationship between estimated lethal concentration values and the number of daily hours of SUVR exposure (HL/D = hours light/day) for both acute (96-h LC50) and chronic (∞ LC1) toxicity estimates. ln(96-h LC50) = 4.656 − 0.130 (HL/D), $r^2 = 0.997$. ln(∞ LC1) = 3.48 − 0.134(HL/D), $r^2 = 0.992$.

The above estimates were derived for an SUVR intensity 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_0).[1] The equivalent depth for these estimates in offshore Lake Michigan ($K_{UVA} = 0.45\ m^{-1}$; $I_0 = 5{,}000\ \mu W/cm^2$) is 8.7 m, and the equivalent depth in a small local eutrophic impoundment (Fink's Pond, Ingham County, Michigan; $K_{UVA} = 1.32\ m^{-1}$; $I_0 = 5{,}000\ \mu W/cm^2$) is 3.0 m. The water surface UVA intensity used in these equivalent depths is approximately equal to our measured summer maximum in Michigan (43° latitude).

Concentrations of anthracene in most natural waters currently are less than our acute toxicity estimates as well as our predicted chronic no-effect concentrations. However, the input of anthracene from nonpoint sources into the Great Lakes region is considerable [23], and this input is expected to increase as the utilization (combustion) of fossil fuels increases [12]. In addition, anthracene is only one of the many PAH that can cause phototoxicity [4]. Recent findings suggest that anthracene exhibits a median level of phototoxicity compared with other PAH commonly identified in aquatic systems [24, personal communication, J.L. Newsted, Michigan State University]. Therefore the results presented here can be considered to represent an average assessment of total PAH phototoxicity in fish, if simple additivity of toxicity is assumed. In terms of the summed totals of molar PAH concentrations, there are natural waters in which the current concentrations of PAH approach or exceed our estimated chronic toxicity thresholds [25,26].

The hazard assessment presented here is based only on water-borne PAH. Because of the complicating factor of fish deriving a significant PAH body burden from food sources and because body burden has been shown to be a controlling factor

[1] The penetration of solar radiation in a column of water can be described using the negative-exponential relationship:

$$\ln(I_z) = \ln(I_0) - K \cdot (z)$$

where z is depth (m), I_z is intensity at depth z, I_0 is intensity at $z = 0$ and K is the extinction coefficient for a specified wavelength or waveband (fitted parameter).

in photoinduced anthracene toxicity [13], 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 1,367 [14], a UVA intensity equivalent to a 3.0-m depth in a typical eutrophic impoundment (100 $\mu W/cm^2$), a 16 h light:8 h dark photoperiod and a safety factor of 50, an estimated no-effect anthracene body burden of 131 $\mu g/kg$ (0.736 $\mu M/kg$) 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 because 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 less photoinduced toxicity than anthracene on a molar body-burden basis to larvae of the fathead minnow (*Pimephales promelas*) [24]. Worldwide, BAP body burdens for various fish species have been reported to range from 0.0003 to 65 $\mu M/kg$ [25, 27–29]. It is apparent, therefore, that there are some areas where photoinduced PAH toxicity to fish may be of concern.

Additional considerations

Some of the environmental parameters not considered in our hazard assessment may need to be added to experimental designs in future investigations. Lower water temperature, which is integrally linked to photoperiod duration especially in north temperature regions, significantly reduces the solubility of PAH [30], thus reducing the bioavailability of the compounds. In addition, lower water temperature would reduce metabolic, and thus respiratory, demand in poikilothermic organisms and would thus decrease acute anthracene phototoxicity, which has been shown to be partly due to respiratory stress [14]. The presence of suspended and dissolved organic matter in the water column could also ameliorate anthracene phototoxicity by decreasing light penetration [31], decreasing bioavailability [32], or increasing rates of photodecomposition [33].

CONCLUSIONS

In this and other reports, we have stressed the importance of an environmentally realistic assessment of the hazards posed by PAH in the aquatic environment. We have identified an important accessory parameter that in laboratory experiments, as compared with previously published data significantly enhances the toxicity of PAH to fish. Currently, there are only a few areas where photoinduced PAH toxicity is of concern but further increases of PAH loading to aquatic systems should be prevented. There are many other environmental parameters that also may affect PAH toxicity to aquatic organisms that we have not accounted for in our laboratory system, but we feel that SUVR is one of the most important of these. While our experimental design is far from completely realistic, our approach—identifying the key environmental parameters involved in this phenomenon—can be considered a conservative representation of what occurs in natural aquatic systems.

Acknowledgment—Funding for this research was received from the Michigan Agriculture Experiment Station, from which this is publication No. 11946. In addition, portions of this research were funded by the Michigan College Sea Grant Program project R/TS-21 (grant MA-85AA-D-SG-045) from which this is contribution number MICHU-SG-86-302. The authors wish to express their appreciation to Dr. P. F. Landrum and to Mr. W. R. Gala for valuable comments on the manuscript.

REFERENCES

1. **Burkhardt, W.** 1939. Zur Frage der photosensibilisierden Wirkung des Teers. *Schweiz. Med. Wochenschr.* 4:82.
2. **Morimura, Y., P. Kotin** and **H.L. Falk.** 1964. Photodynamic toxicity of polycyclic aromatic hydrocarbons in tissue culture. *Cancer Res.* 24:1249–1255.
3. **Forbes, P.D., R.E. Davies** and **F. Urbach.** 1976. Phototoxicity and photocarcinogenesis: Comparative effects of anthracene and 8-methoxypsoralen in the skin of mice. *Food Cosmet. Toxicol.* 14:303–306.
4. **Kochevar, I.E., R.B. Armstrong, J. Einbinder, R.R. Walther** and **L.C. Harber.** 1982. Coal tar phototoxicity: Active compounds and action spectra. *Photochem. Photobiol.* 36:65–69.
5. **Dworkin, M.** 1958. Endogenous photosensitization in a carotenoidless mutant of *Rhodopseudomonas spheroides*. *J. Gen. Physiol.* 41:1099–1112.
6. **Harrison, A.P.** and **V.E. Raabe.** 1967. Factors influencing the photodynamic action of benzo(a)pyrene on *Escherichia coli*. *J. Bacteriol.* 93:613–636.
7. **Shimizu-Takahama, M., T. Egashira** and **U. Takahama.** 1981. Inhibition of respiration and loss of membrane integrity by singlet oxygen generated by a photosensitized reaction in *Neurospora crassia conidia*. *Photochem. Photobiol.* 33:689–694.
8. **Hunter, J.R., S.E. Kaupp** and **J.H. Taylor.** 1981. Effects of solar and artificial ultraviolet-B radiation on larval northern anchovy, *Engraulis mordax*. *Photochem. Photobiol.* 34:477–486.

9. **Damkaer, D., D. Dey, G. Heron** and **E. Prentice.** 1980. Effects of UV-B radiation on near-surface zooplankton of Puget Sound. *Oecologia* **44**:149–158.
10. **Barcelo, J.A.** and **J. Calkins.** 1979. Positioning of aquatic microorganisms in response to visible light and simulated solar UV-B irradiation. *Photochem. Photobiol.* **29**:75–83.
11. **Damkaer, D., D. Dey** and **G. Heron.** 1981. Dose/dose-rate responses of shrimp larvae to UV-B radiation. *Oecologia* **48**:178–182.
12. **Gehrs, C.W.** 1976. Coal conversion: Description of technologies and necessary biomedical and environmental research. No. 5192. Oak Ridge National Laboratory, Oak Ridge, TN.
13. **Bowling, J.W., G.J. Leversee, P.F. Landrum** and **J.P. Giesy.** 1983. Acute mortality of anthracene-contaminated fish exposed to sunlight. *Aquat. Toxicol.* **3**:79–90.
14. **Oris, J.T.** and **J.P. Giesy.** 1985. The photoenhanced toxicity of anthracene to juvenile sunfish (*Lepomis* spp.). *Aquat. Toxicol.* **6**:133–146.
15. **Kagan, J., P.A. Kagan** and **H.E. Bushe.** 1984. Light dependent toxicity of alpha-terthienyl and anthracene toward late embryonic stages of *Rana pipiens. J. Chem. Ecol.* **10**:1115–1122.
16. **Allred, P.M.** and **J.P. Giesy.** 1985. Solar radiation-induced toxicity of anthracene to *Daphnia pulex. Environ. Toxicol. Chem.* **4**:219–226.
17. **Oris, J.T., J.P. Giesy, P.M. Allred, D.F. Grant** and **P.F. Landrum.** 1984. Photoinduced toxicity of anthracene in aquatic organsims: An environmental perspective. In T.N. Veziroglu, ed., *The Biosphere: Problems and Solutions.* Elsevier Science Publishers, Amsterdam, pp. 639–658.
18. **Cody, T.E., M.J. Radike** and **D. Warshawsky.** 1984. The phototoxicity of benzo[a]pyrene in the green alga *Selenastrum capricornutum. Environ. Res.* **35**:122–132.
19. **Landrum, P.F., J.P. Giesy, J.T. Oris** and **P.M. Allred.** 1985. The photoinduced toxicity of polycyclic aromatic hydrocarbons to aquatic organisms. In J.H. Vandermeulen and S. Hrudey, eds., *Oil and Freshwater: Chemistry, Biology, Technology.* Pergamon Press, New York, NY (in press).
20. **Versteeg, D.J.** and **J.P. Giesy.** 1985. Lysosomal enzyme release in the bluegill sunfish (*Lepomis macrochirus* Rafinesque) exposed to cadmium. *Arch. Environ. Contam. Toxicol.* **14**:631–640.
21. **Finney, D.J.** 1971. *Statistical Methods in Biological Assays*, 2nd ed. Griffith Press, London.
22. **Mayer, F., D. Buckler, M. Ellersieck** and **G. Krause.** 1984. Estimating chronic toxicity of chemicals to fishes from acute toxicity test data: An alternative to the application factor. *Abstracts*, Interface of Laboratory and Field Data: Predicting and Understanding Environmental Effects. Society of Environmental Toxicology and Chemistry, Fifth Annual Meeting, Washington, DC, p. 169.
23. **Strand, J.W.** and **A.W. Andren.** 1980. Polyaromatic hydrocarbons in aerosols over Lake Michigan, fluxes to the lake. In A. Bjorseth and A.J. Dennis, eds., *Polynuclear Aromatic Hydrocarbons, Chemistry and Biological Effects.* Battelle Press, Columbus, OH, pp. 127–137.
24. **Oris, J.T.** 1985. The photo-induced toxicity of polycyclic aromatic hydrocarbons to fish (*Lepomis* spp., *Lepomis macrochirus*, and *Pimephales promelas*). Ph.D. thesis. Michigan State University, East Lansing, MI.
25. **Neff, J.M.** 1979. *Polycyclic Aromatic Hydrocarbons in the Aquatic Environment.* Applied Science Publishers, London.
26. **U.S. Environment Protection Agency.** 1980. Ambient water quality criteria for polynuclear aromatic hydrocarbons. EPA 440/5-80-069. U.S. Environmental Protection Agency, Washington, DC, pp. C-1–C-180.
27. **Braunstein, H.M., E.D. Copenhaver** and **H.A. Pfuderer**, eds. 1977. *Environmental, Health, and Control Aspects of Coal Conversion: An Information Overview*, Vol. 2. ORNL/EIS-95 UC-11, -41, -48, -90i. Oak Ridge National Laboratory, Oak Ridge, TN, pp. 6-1–6-53.
28. **Hallett, D.J.** and **R.W. Brecher.** 1984. Cycling of polynuclear aromatic hydrocarbons in the Great Lakes ecosystem. In J.O. Nriagu and M.S. Simmons, eds., *Toxic Contaminants In the Great Lakes.* John Wiley & Sons, New York, NY, pp. 213–238.
29. **Baumann, P.C., W.D. Smith** and **M. Ribick.** 1982. Hepatic tumor rates and polynuclear aromatic hydrocarbon levels in two populations of brown bullhead (*Ictaluras nebulosus*). In M.W. Cooke, A.J. Dennis, and G.L. Fisher, eds., *Polynuclear Aromatic Hydrocarbons, Physical and Biological Chemistry.* Battelle Press, Columbus, OH, pp. 93–102.
30. **May, W.E., S.N. Chesler, H.S. Hertz** and **S.A. Wise.** 1982. Analytical standards and methods for the determination of polynuclear aromatic hydrocarbons in environmental samples. *Int. J. Environ. Anal. Chem.* **12**:259–275.
31. **Baker, K.S.** and **R.C. Smith.** 1982. Spectral irradiance penetration in natural waters. In J. Calkins, ed., *The Role of Solar Ultraviolet Radiation in Marine Ecosystems.* Plenum Press, New York, NY, pp. 233–246.
32. **Leversee, G.J., P.F. Landrum, J.P. Giesy** and **T. Fannin.** 1983. Humic acids reduce bioaccumulation of some polycyclic aromatic hydrocarbons. *Can. J. Fish. Aquat. Sci.* **40** (suppl. 2):63–69.
33. **Miller, G.C.** and **R.G. Zepp.** 1979. Effects of suspended sediments on photolysis rates of dissolved pollutants. *Water Res.* **13**:453–459.