Assessment of the Risk of Solar Ultraviolet Radiation to Amphibians. II. In Situ Characterization of Exposure in Amphibian Habitats

ARTICLE *in* ENVIRONMENTAL SCIENCE AND TECHNOLOGY · AUGUST 2002

Impact Factor: 5.33 · DOI: 10.1021/es0306157 · Source: PubMed

4 AUTHORS, INCLUDING:

Lucinda Johnson
University of Minnesota Duluth

Richard P Axler
University of Minnesota Duluth

Stephen A Diamond
NanoSafe, Inc.

Environ. Sci. Technol. **2002**, *36,* 2859−2865

Assessment of the Risk of Solar Ultraviolet Radiation to Amphibians. II. In Situ Characterization of Exposure in Amphibian Habitats

GREGORY S. PETERSON,[†]
LUCINDA B. JOHNSON,[*,‡]
RICHARD P. AXLER,[‡] AND
STEPHEN A. DIAMOND[†]

U.S. Environmental Protection Agency, National Health and Environmental Effects Research Laboratory, Mid-Continent Ecology Division, 6201 Congdon Boulevard, Duluth, Minnesota 55804, and Natural Resources Research Institute, University of Minnesota, Duluth, 5013 Miller Trunk Highway, Duluth, Minnesota 55811

Ultraviolet B (UVB) radiation has been hypothesized as a potential cause of amphibian population declines and increased incidence of malformations. Realistic studies documenting UV irradiance or dose have rarely been conducted in wetlands used by amphibians. Our data indicates that 99% of UVB is attenuated in the top 5−20 cm of wetlands in our study region (northern Minnesota and Wisconsin). Furthermore, vegetation and other habitat features have substantial impacts on local UVB irradiance levels and dose. UVB attenuation in the water columns of our wetlands is controlled by the specific absorption of dissolved organic carbon (DOC), and consequently, UVB attenuation is best predicted by simple laboratory absorbance measurements such as bulk water color (absorbance at 440 nm) or wavelength-specific absorbance coefficients. Seasonal data indicate that the UVB absorption by early and mid-season DOC is higher than that of late summer and fall DOC, suggesting increased protection from UVB during the potentially sensitive stages of amphibian development. In addition to dissolved components, our model indicates that suspended solids play a small role in UVB attenuation in our wetlands but apparently only at high concentrations. Models predicting UV attenuation in wetlands should be used cautiously and should consider temporal variability, given the volatility and dynamic nature of water column characteristics in wetlands. Organism behavior is a critical but poorly understood phenomenon that must be addressed for development of an accurate UV exposure risk model for amphibians.

Introduction

Declining amphibian populations have been documented on both global and regional scales, and reports of unusually high rates of malformed amphibians are increasingly common. Among the current hypotheses regarding the cause of these trends is increased exposure to solar ultraviolet B (UVB; 290−320 nm) radiation. The UVB hypothesis is based, in part, on experimental evidence demonstrating damage to cellular DNA from exposure to UVB radiation (*1*). Further, the largest relative increases in UVB flux appear to occur in the early spring and summer (*2*), corresponding to the period of reproduction and early development for many amphibian species. The ubiquitous nature of amphibian malformations and declines reported in both pristine and impacted environments also is consistent with a global stressor such as UVB.

Empirical evidence implicating UVB in deleterious effects on amphibian survival and development has been reported in several recent laboratory studies using artificial lighting (UV lamps), as well as field studies conducted under natural sunlight (e.g., refs *3*−*6*). In addition, a recent survey of amphibian distributions in montane ponds in Olympic National Park, WA, reported that breeding populations of *Rana cascadae* were negatively associated with UVB transmission (*7*). Other field studies conducted under natural sunlight, however, were unable to produce effects (*8*−*10*). These exposures often did not include the full range of potentially sensitive developmental stages or reflect realistic field or exposure conditions. Further, critical information lacking in most of these field studies was in situ measurements of UVB exposure. As a result, the significance of most of these studies is unclear, due to uncertainties in the doses received by developing amphibians under natural field conditions.

In aquatic environments, exposure to incident UVA (320−400 nm) and UVB is controlled by a complex interaction between organism behavior and water column characteristics that affect spectral attenuation. Dissolved organic carbon (DOC) has been demonstrated to be the principal factor controlling UV attenuation in temperate lakes (*11*, *12*). In these studies, suspended particulates and chlorophyll (i.e., phytoplankton algae) did not contribute significantly to UV attenuation. This notion, however, has been a point of contention in more recent lake studies, which showed a lesser contribution from DOC and suggested that chlorophyll *a* (chl-*a*) and particulate measurements may also attenuate UV (*13*−*15*). Ponds and wetlands where amphibians spend the early portion of their development often differ from typical lakes in their limnological, hydrological, and watershed characteristics. High productivity and inputs of allochthonous organic material, relatively small sizes, and shallow depths of wetlands result in different water column optical properties compared to lakes. UV attenuation has recently been studied in Ontario wetlands on the edge of the Canadian shield (*16*) and in freshwater and saline lakes and wetlands in the prairie pothole region of Saskatchewan (*17*). Both studies reported that DOC was not a reliable predictor of UVB attenuation in wetlands; however, the range of DOC concentrations and the resulting models for freshwater systems differed considerably between the two studies.

The present study was designed to assess the range and variability of UV attenuation in northern Minnesota and Wisconsin wetlands that support Northern Leopard Frog (*Rana pipiens*) populations. We present in situ vertical attenuation coefficients for wetlands across various wavebands within the UV spectrum and predict the depth of UVB penetration from water column characteristics. In addition, we explore the influence of habitat type on the flux (irradiance) and cumulative exposure (dose) received at depth. Finally, the consequences and relevance of these data in the context of UV exposure of developing amphibians are discussed.

* Corresponding author phone: 218-720-4251; fax: 218-720-4328; e-mail: ljohnson@d.umn.edu.
[†] U.S. Environmental Protection Agency.
[‡] University of Minnesota.

10.1021/es011196l CCC: $22.00
Published on Web 05/30/2002

Materials and Methods

Site Selection. Thirty emergent and aquatic bed wetlands located throughout central and northeastern Minnesota and northwestern Wisconsin with established populations of *R. pipiens* were studied. These sites encompassed a wide range of water column properties potentially influencing UV attenuation (e.g., DOC, turbidity, chlorophyll *a*).

Radiometry. In situ radiometric measurements were made using an Ocean Optics linear CCD array dual-channel fiber optics spectrometer (S2000; Ocean Optics, Dunedin, FL) capable of collecting simultaneous intensity measurements from 179 to 878 nm. One channel was configured using a selective grating with maximum efficiency at 300 nm and a UV band-pass filter that effectively blocked wavelengths >375 nm, to isolate and maximize UV sensitivity. The second channel exhibited a maximum sensitivity at 400 nm and encompassed both UV and photosynthetically active radiation (PAR) producing spectral measurements from <200 to >800 nm. Each channel was calibrated using a NIST traceable lamp at 1-nm increments (*18*).

The spectrometer was controlled by a minilaptop computer. Two 2-m fiber optic cables, each with a submersible terminal probe containing a Teflon cosine corrector, allowed for direct in situ underwater measurements. Measurements at frequently visited sites were made from a small boat tethered to anchor posts. The sensor was positioned at depth using a graduated, vertical slide mounted on a post. The cosine corrector was maintained horizontally using a bubble level. At sites visited infrequently, a portable floating platform was carried/towed by researchers. A 1.5 m aluminum pole with a 12 cm adjustable-angle terminal arm served to position the sensor at the desired depth, read from a graduated rod perpendicular to its plane. All measurements were made facing due south within 3 h of solar noon under a clear sky to ensure a relatively high and consistent solar angle and to minimize cloud effects. A typical profile of vertical extinction measurements included 5−10 depths and was obtained in about 5 min. The speed and portability of the Ocean Optics system facilitated measuring multiple sites on the same day and allowed for extinction measurements on days when clear sky was only available for short periods.

Following the Beer−Lambert equation, diffuse vertical attenuation coefficients (K_d) were determined from the slope of the linear regression of the natural logarithm of downwelling irradiance (E_d) versus depth (z). Irradiance values used in these calculations were means of 5−10 replicate spectra from a given depth. Individual K_d were determined for five selected portions of the UV and visible spectrum by integrating mean spectral irradiance across the waveband of interest. Because the action spectrum for observed or hypothesized effects in amphibians is not known and the UVA band is three times wider than UVB (with intensity rapidly increasing with wavelength), we arbitrarily defined five spectral wavebands for our suite of K_d measurements to better characterize the overall UV spectrum: K_dB, 290−320 nm; K_dA$_1$, 320−345 nm; K_dA$_2$, 345−370 nm; K_dA$_3$, 370−400 nm; K_dPAR, 400−700 nm.

Water Chemistry. Subsurface grab samples of water were collected concurrent with the field radiometry. DOC was determined by UV−persulfate oxidation (Dohrmann TOC analyzer), chlorophyll *a* by spectrophotometry on 90% acetone extracts, and total suspended solids (TSS) by gravimetry (*19*, *20*). True color (color) was measured spectrophotometrically at 440 nm on 0.45 μm filtered samples and quantified against a platinum−cobalt standard curve. Absorbance coefficients (K_a) were calculated for specific wavelengths by multiplying absorbance values by 2.303 and then dividing by the path length in meters. In addition, full spectrum absorbance scans were performed on filtered and unfiltered samples at 5−10 nm intervals by spectrophotometry using 1 cm Suprasil (quartz) cuvettes and a deionized water blank.

Relative Dose Estimation. In the absence of an action spectrum for the effects on amphibians, biological weighting functions (BWF) based on published action spectra for DNA damage (*1*) and inhibition of photosynthesis (*21*) were applied to spectral data from each wetland. The BWF for each effect consists of wavelength-specific weights on a scale of 0−1, calculated by normalizing individual wavelength intensities to the 280 nm value (*22*). The resulting wavelength-specific relative weights were used to multiply laboratory measured spectral transmittance data (1 cm path length) from wetland water samples. The weighted spectral data were then summed, generating a relative dose for that wetland sampling event.

Habitat-Specific UV Attenuation. In August 1999, we conducted a pilot experiment to compare the UVB irradiance regime in three vegetated habitats: emergent, submergent, and floating leaf. The site (WI-116) was chosen for its accessibility, the presence of well-developed macrophyte beds with adjacent open water, and large populations of tadpoles. Emergent vegetation was comprised of *Typha* spp. (cattail) while both the submergent and floating leaf vegetation were *Potomageton* spp. Because UV profiles were not feasible within the vegetation because of inconsistencies in shading with depth, we selected a single depth at which the sensor was slowly moved through a small patch of vegetation (~50 cm diameter). The spectrometer was programmed to take 10 readings at 2 s intervals. This technique reduced bias because the researcher did not know when individual readings were occurring. This procedure was performed 6 days apart at 5 and 9 cm water depths, respectively. For each depth, measurements were made at five different locations within each vegetation type, as well as in adjacent open waters.

Dosimetry. In addition to spectrometer readings, polysulfone film UVB dosimeters were deployed in each of the three vegetative habitat types and in open water. Dosimeters were developed to integrate diurnal fluctuations to determine a realistic daily dose of UVB (*23*). The dosimeters consist of strips of polysulfone plastic film (0.010 in. thick) whose absorbency increases proportionally with UVB exposure. Units were suspended horizontally from a floating support rack anchored facing south to avoid shading effects. Dosimeters were deployed for several days to provide sufficient dose for each habitat. The difference in pre- and postexposure absorbance at 330 nm was determined using a standard spectrometer; the resulting absorbance change was then compared to a sunlight-generated calibration curve to determine the cumulative UVB dose (*23*). Twelve dosimeters were deployed in each habitat at depths of 5 and 9 cm.

Statistical Analysis. Multiple linear regression analysis was performed using a stepwise selection method (*24*) to assess the power of environmental variables in predicting UV attenuation. All models were evaluated using the Cp statistic and r^2 values (*25*, *26*). Standard techniques for evaluating model fit were applied (*25*). The standardized partial regression coefficient was used to assess the relative importance of each independent variable in the final model (*24*). An α of 0.05 was used throughout to assess significance.

Results

Incident UVB irradiance measurements across all wetland sites and sampling periods ranged from 1.19 to 4.07 W·m^{-2} (mean = 2.81 W·m^{-2}), reflecting typical mid-day clear sky surface intensities measured at these latitudes. As expected, UV radiation was attenuated rapidly with depth and a pattern of decreasing K_d with increasing wavelength was observed. K_dB ranged from 5.6 to 136 m^{-1} across the study wetlands

TABLE 1. Multiple Linear Regression Analysis Results (Best Model) from SAS Stepwise Procedure for Predicting UV and PAR Attenuation in Wetlands[a]

parameter	K_dB (290−320 nm) coeff	K_dB (290−320 nm) std coeff	K_dA_1 (320−345 nm) coeff	K_dA_1 (320−345 nm) std coeff	K_dA_2 (345−370 nm) coeff	K_dA_2 (345−370 nm) std coeff	K_dA_3 (370−400 nm) coeff	K_dA_3 (370−400 nm) std coeff	K_dPAR (400−800 nm) coeff	K_dPAR (400−800 nm) std coeff
intercept	0.0742		0.0162		−0.0032		0.0361		0.0167	
DOC	0.0074	0.225	0.0058	0.160	0.0035	0.122				
color at 440 nm	0.0024	0.683	0.0023	0.598	0.0019	0.643	0.0011	0.535	0.0002	0.396
TSS	0.0042	0.166	0.0126	0.448	0.0096	0.443	0.0085	0.568	0.0007	0.278
chl-*a*			−0.0031	−0.210	−0.0022	−0.190	−0.0019	−0.251		
overall model		$r^2 = 0.81$[b]		$r^2 = 0.83$[c]		$r^2 = 0.86$[d]		$r^2 = 0.79$[e]		$r^2 = 0.34$[f]

[a] All parameters included in models are significant at $p \leq 0.05$. The standardized partial regression coefficient (std coeff) is used to compare the relative importance of each independent variable within a model (*24*). [b] $K_dB_{(290-320\ nm)} = 0.0742 + 0.0024[color] + 0.0074[DOC] + 0.0042[TSS]$. [c] $K_dA_{(320-345\ nm)} = 0.0162 + 0.0023[color] + 0.0058[DOC] + 0.0126[TSS] - 0.0031[chl\text{-}a]$. [d] $K_dA_{(345-370\ nm)} = -0.0032 + 0.0019[color] + 0.0035[DOC] + 0.0096[TSS] - 0.0022[chl\text{-}a]$. [e] $K_dA_{(370-400\ nm)} = 0.0361 + 0.0011[color] + 0.0085[TSS] - 0.0019[chl\text{-}a]$. [f] $K_dPAR_{(400-700\ nm)} = 0.0167 + 0.0002[color] + 0.0007[TSS]$.

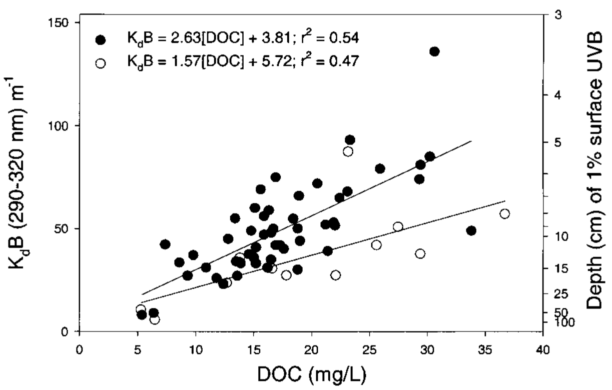

FIGURE 1. Vertical attenuation coefficients (K_dB) and depth of 1% surface UVB irradiation as a function of DOC (filled circles = early season (March−July); open circles = late season (August−October)).

(Table A, Supporting Information). In all cases, the data sets were well-described by the exponential attenuation model with regression coefficients (r^2) typically >0.98 and always >0.90.

DOC concentrations ranged from 5.3 to 36.7 mg·L^{-1} (Table A, Supporting Information). The mean versus median values for both TSS and chlorophyll *a* indicate that the data are skewed toward the lower end of their respective ranges. The occasional high TSS values generally coincided with high chlorophyll *a* values. A significant, but weak, positive linear relationship (overall model $r^2 = 0.43$) was found between the integrated UVB (290−320 nm) attenuation coefficients and DOC (Figure 1). Variability increased with increasing DOC. To evaluate whether this variability was a function of error in K_d estimates, r^2 values for individual K_dB measurements were regressed against DOC concentration. No significant relationship was apparent, and residual analysis showed little justification for exploring nonlinear models. However, a seasonal trend was evident (Figure 1). Wetlands sampled from August through October ("late" season) consistently had a pattern of lower UVB attenuation for a given level of DOC as compared to wetlands sampled in March through July ("early" season). A comparison of the early and late season regressions (ANCOVA, $p < 0.05$) showed that K_dB was more sensitive to changes in DOC in the early season. K_dB was much more strongly dependent upon the optically defined parameter, color, measured at 440 nm ($r^2 = 0.75$), which did not exhibit seasonal trends (Figure 2). The relationship between color and DOC (Figure 3) also had higher variability with increasing DOC and exhibited a late season decrease in color relative to DOC.

Color, DOC, and TSS were significant parameters in the model of UVB attenuation (Table 1). Color was the best

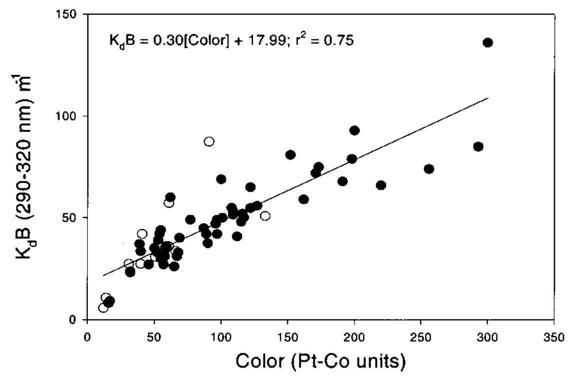

FIGURE 2. Vertical attenuation coefficients (K_dB) as a function of color (filled circles = early season (March−July); open circles = late season (August−October)).

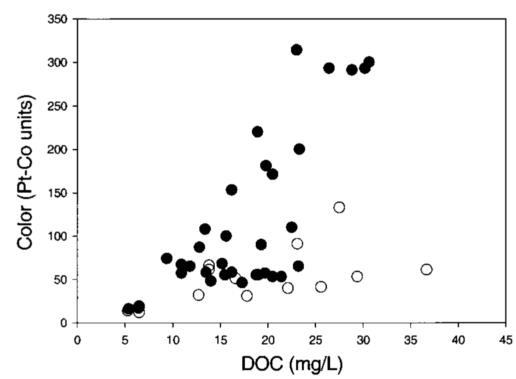

FIGURE 3. Seasonal relationship of DOC and color at 440 nm in northern wetlands (dark circles = early season (March−July); open circles = late season (August−October)).

predictor of UVB attenuation, with the standardized regression coefficients 3−4 times higher than those for DOC and TSS, respectively. The relative importance of both DOC and TSS in the UV attenuation models, however, was wavelength-dependent. Relative to color, DOC becomes increasingly less important in the model as wavelength increases and is not included as a significant parameter in the $K_dA_{3(370-400\ nm)}$ model. Conversely, TSS becomes more important relative to both DOC and color as wavelength increases throughout the UV range. Chlorophyll *a* is a significant parameter in all UVA models (Table 1). The overall PAR model that included only color and TSS provided a poor fit to the data ($r^2 = 0.34$). This is likely the result of a narrow range of absorbance values across sites and minimal attenuation of PAR occurring over the relatively shallow water columns measured.

The estimated depth to which 1% of the subsurface integrated UVB (290−320 nm) intensity would penetrate is

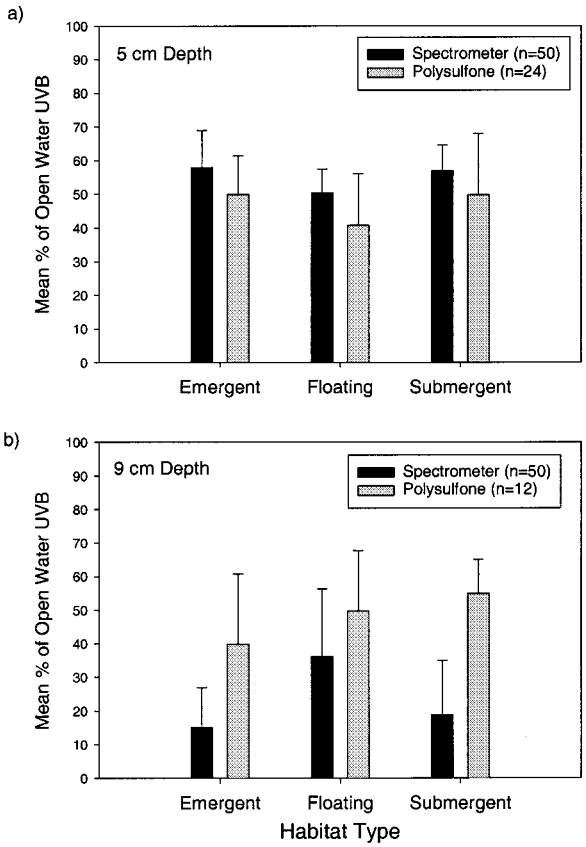

FIGURE 4. Percent of open water UVB for (a) 5 cm and (b) 9 cm depths for three vegetative habitat types (mean ± SD), as measured by spectrometer (instantaneous mid-day irradiance) and polysulfone dosimeters (96-h cumulative dose).

shown in Figure 1. These 1% attenuation depths ($Z_{1\%}$) were calculated from the attenuation coefficients as $Z_{1\%} = 4.605/K_dB$ and provide an intuitive measure of actual UV penetration in wetlands. For most wetlands, 99% of UVB was attenuated at depths of 5−20 cm; however, two sites exhibited a $Z_{1\%} >$ 45 cm. These two "clearwater" sites were different from the others in that they were small spring-fed trout ponds, with substrates low in organic matter content, and were more similar to lakes than to "typical" frog breeding ponds.

The proportion of incident irradiation reflected or scattered due to surface effects for each waveband was determined by calculating the ratio of the intercept of the K_d regression to the corresponding incident irradiation. Surface effects were wavelength dependent, with shorter wavelengths being affected most. Mean percent reductions for all sites were 11.9 ($UVB_{290-320\ nm}$), 4.8 ($UVA_{320-345\ nm}$), 4.0 ($UVA_{345-370\ nm}$), 4.0 ($UVA_{370-400\ nm}$), and 2.8 ($PAR_{400-800\ nm}$).

At the wetland where habitat differences in UVB attenuation were assessed, spectrometer data showed a consistent 40−50% reduction in UVB for all vegetation types at 5 cm and a 60−80% reduction at 9 cm, relative to the adjacent open water (Figure 4). Dosimeter results supported the spectrometer data for the various habitats, with overall reductions ranging from 35−60% for both the 5 and 9 cm depths. DOC measurements in water samples collected from different vegetative habitats and open water were relatively constant (13−16 mg·L^{-1}), ruling out a differential response due to water chemistry.

Discussion

As expected, UV was attenuated rapidly with depth in wetlands utilized for amphibian reproduction, with 99% of the UVB typically attenuated in the upper 5−20 cm (Figure 1).

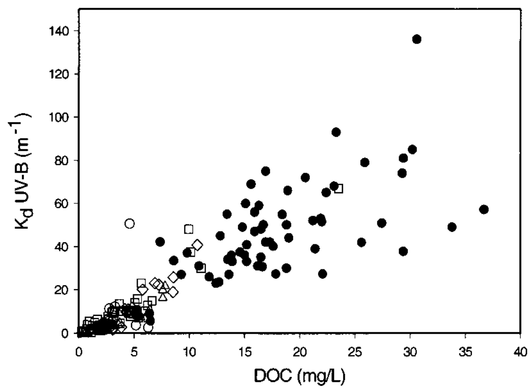

FIGURE 5. Comparison of UVB vertical attenuation coefficients, in this study, versus recent lakes studies as a function of DOC concentration (open squares = Morris et al. (*12*), 320 nm, $r^2 = 0.87$; open triangles = Scully and Lean (*11*), UVB, $r^2 = 0.97$; open diamonds = Laurion et al. (*28*), 320 nm, $r^2 = 0.90$; open circles = Smith et al. (*14*), UVB, $r^2 = 0.77$; filled circles = Peterson et al. (this study), UVB, $r^2 = 0.43$).

The use of 1% level of surface irradiance, however, is purely convention based on the photic zone for PAR and, therefore, is not necessarily applicable to UVB dose estimation. Alternatively, a more ecologically relevant depth may be a 40% attenuation depth based on the occurrence of malformations observed in developing *R. pipiens* at 60% of ambient UV (*4, 27*). If the 40% attenuation depth is calculated for the same wetland data, $Z_{40\%}$ values range from 0.55 to 2.2 cm, which further limits the potential zone of risk (i.e., "impact") in wetlands.

Relationship of DOC and UV Attenuation. Attenuation coefficients for both UVB and UVA were typically much greater than those observed by Scully and Lean (*11*), Morris et al. (*12*), Smith et al. (*14*), and Laurion et al. (*28*) in lakes, due to the much higher DOC concentrations in wetlands (Figure 5). The K_dB at our wetland sites with low DOC concentrations overlapped the ranges reported in those studies. However, most K_dB values for our wetlands were 2−5 times higher than those reported for lakes. In addition, increased variability in K_dB with respect to DOC was observed at higher DOC concentrations. This results in a weaker K_dB versus DOC relationship ($r^2 = 0.43$) for wetlands as compared to those reported in the lake studies (e.g., $r^2 = 0.97, 0.87, 0.90$ (*11, 12, 28*)). This variability, however, does not appear to be related to accuracy of K_d measurement, as correlations of ln(irradiance) with depth for individual wetlands were as strong as those reported in the lakes studies.

Our wetland K_dB values were similar to those measured in Ontario wetlands on the edge of the Canadian shield (*16*), although our sites encompassed a larger range of DOC concentrations (Figure 6). Both our wetlands and the Ontario wetlands had higher K_dB values than those from prairie pothole lakes in Saskatchewan (*17*; freshwater data only) for a similar range of DOC. However, no statistical difference was observed between the slopes of the K_dB−DOC linear regression models for the three studies. Similarly, intercepts for our model of upper Midwest wetlands and that of Crump et al. (*16*) for Canadian shield wetlands were not different, but both models differed significantly from Arts and Robarts (*17*) freshwater prairie pothole lakes and wetlands (DOC < 45 mg·L^{-1}). One explanation is that the DOC-specific absorbance in freshwater Saskatchewan wetlands is lower than that of Midwestern and Ontario wetlands. Another possibility is that there is a methodological difference in the measurement of DOC or UV attenuation between studies. In any case, the negative intercept in the Arts and Robarts (*17*) model suggests a problem with the linear model. They

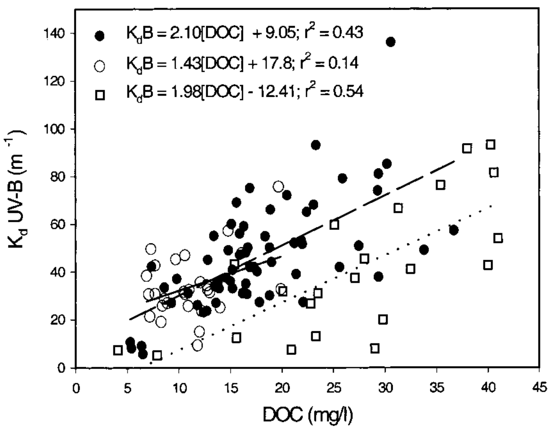

FIGURE 6. Comparison of UVB vertical attenuation coefficients for wetlands in the present study versus other wetland studies as a function of DOC (<45 mg·L^{-1}) concentration (filled circles/dashed line = Peterson et al. (this study); open circles/solid line = Crump et al. (*16*); open squares/dotted line = Arts and Robarts (*17*) (freshwater)).

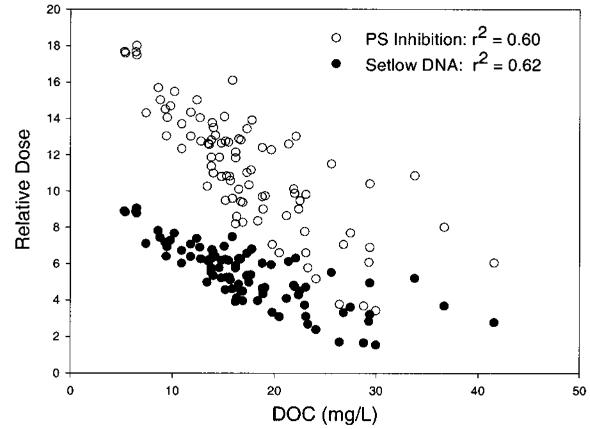

FIGURE 7. Relative dose calculated using selected biological weighting functions (BWFs) for Setlow DNA damage (1) and inhibition of photosynthesis (20), versus DOC concentration.

addressed this issue by fitting a power equation, which remedied the intercept issue and increased the correlation coefficient from 0.53 to 0.76. However, we found no justification for exploring nonlinear models. Despite slight differences in the models, our study supports the findings of both the Ontario and Saskatchewan studies that DOC is not as robust a predictor of UVB attenuation in wetlands as seen in clearwater lakes.

Temporal and Spatial Variability. Seasonal differences in the relationship between K_d and DOC were observed (Figure 1) and were especially evident at sites with high DOC. K_d decreased from spring to late summer, while DOC remained relatively consistent. The magnitude of the seasonal effects appears to be positively related to DOC concentration. These observations are consistent with the pattern of increasing fraction of colored DOC at higher DOC levels observed for lakes and streams in Ontario (*29*) and Nova Scotia (*30*), as well as observed increases in the specific absorbance of DOC occurring following spring melt in the Shingobee River in northwestern Minnesota (*31*). The observed variability in the relationship of K_dB and DOC both within and between wetland studies suggests that wetland DOC is neither spatially nor temporally consistent in its UV absorbing properties.

Unfortunately, DOC source and composition is not explicitly characterized in most UV studies. The amount and chemical composition of allochthonous DOC input to an individual wetland are determined by the land cover and soil characteristics (*32*), as well as relative size (*30*) of the surrounding watershed, and may differ considerably between wetlands. In addition, the aromaticity and C/N ratio of organic matter produced within aquatic systems are lower than that of terrestrial DOC (*33*), resulting in a lower UV absorption by autochthonous DOC (*34*). Further, the relative proportions of these two sources varies seasonally resulting in temporal differences in the UV absorptive properties of wetlands (*35*). This spatial and temporal variability in DOC composition ultimately manifests itself in the observed dynamic relationship between DOC and UV attenuation in wetlands, including the pattern of elevated UVB attenuation in the spring.

Photobleaching of DOC by solar UVB (*36*) can also lead to lower UV absorbance (*37*) and substantial temporal variability. Photobleaching is controlled by the intensity and duration of solar radiation, as well as the mixing regime and chemical properties of the water body (*38, 39*). Further, it is the colored portion of DOC, and therefore the main UV absorbing constituent, which is preferentially photooxidized (*40, 41*). Consequently, under conditions of prolonged water column stability, DOC photobleaching may extend the depth of UVB penetration or produce a surface microhabitat layer with higher than expected UV radiation. Indeed, many of our K_dB plots showed a marked deviation in the upper 2 cm of water, indicating a surficial layer with lower UVB attenuation. Likewise, local variations in DOC concentration and type, as a result of climate warming, drought, or acidification (*42*−*45*), may also dramatically increase UV penetration in wetlands. Because wetlands have seasonally variable sources of DOC, complex mixing regimes, and are particularly sensitive to local climate changes, it is not surprising that UV attenuation in wetlands is more variable and, therefore, more difficult to predict than in most lakes.

Relative Dose Estimation. No action spectra currently exist for demonstrated or hypothesized amphibian effects. Instead, action spectra for two known biological effects that emphasize different areas of the UV spectrum were applied to spectral transmittance data for individual wetlands to evaluate the variability in the biologically effective dose that could result from differences in DOC composition. The DNA damage (Setlow DNA) action spectrum (*1*) emphasizes wavelengths in the UVB, while the action spectrum for inhibition of photosynthesis (PS) includes much of the UVA spectrum in addition to UVB (*21*). Plots of these relative doses for our wetlands (Figure 7) suggest that effects based on action spectra that include significant amounts of UVA are likely to extend to greater depths than those based only on UVB (e.g., DNA damage). This observation could be significant if biomacromolecules important in amphibian development other than DNA (e.g., proteins, lipids) are shown to be sensitive to wavelengths longer than UVB (*27*). These figures also reveal a 2-fold or greater range in the effective Setlow DNA and PS inhibition doses for wetlands with DOC concentrations >15 mg·L^{-1}. Given that these data were based on spectral attenuation at 1 cm, this variability in dose would most likely be magnified at greater depths.

Comparing the same relative dose calculations to color values for wetlands (data not shown) yielded a tighter relationship ($r^2 = 0.78$ versus 0.60 for PS inhibition and 0.76 versus 0.62 for Setlow DNA damage, respectively). These differences in the relationship of DOC and color with relative dose further support the need to account for the specific absorbance properties of DOC to characterize the full spectral attenuation properties of water bodies.

DOC−Specific Absorbance Measures. In our upper Midwest wetlands, color was a more robust predictor of K_dB ($r^2 = 0.75$) than DOC ($r^2 = 0.47$; Figure 2) and did not vary seasonally with K_dB. Color is optically measured and, therefore, incorporates the specific absorbance characteristics of DOC and other dissolved compounds. Color is a simple, commonly measured limnological parameter for which considerable archival data exist for wetlands that could be used retrospectively to predict K_dB. In addition to color, absorbance coefficients (K_a) were calculated for our wetland data at a UVB wavelength (i.e., K_a 310 nm) and showed a slightly higher correlation with K_dB ($r^2 = 0.77$) than color at 440 nm.

In lakes, color is controlled by allochthonous inputs, which are a function of catchment vegetation and hydrology (*46*–*48*). Further, these allochthonous humics are generally more highly colored than humics produced in situ (*49*). However, because wetlands are typically shallow highly productive water bodies, their color may be significantly influenced by autochthonous DOC contributions as well. Thus, it is conceivable that allochthonous sources of DOC predominate during early high water periods, while autochthonous sources become increasingly important during late summer periods corresponding to algal senescence. These patterns are consistent with the temporal patterns observed between color and DOC (Figure 3), as well as the seasonal trends between K_dB and DOC. Likewise, Crump et al. (*16*) reported temporal differences in the relationship between DOC and another optically defined parameter, DOC fluorescence. Unlike color in the present study, however, they reported that fluorescence did not reliably predict either UVB or UVA attenuation. It is clear from our data that both DOC concentration and its specific absorbance properties in water influence UVB attenuation in wetlands.

Modeling UV Attenuation in Wetlands. Multiple regression analysis corroborated the importance of DOC-specific absorbance by including both DOC and color in the final model (Table 1), with color explaining most of the variability in K_dB. In fact, standardized regression coefficients showed that color was three times more important than DOC in predicting K_dB in the overall model. Diagnostics indicated that collinearity of color and DOC was not significant in this model (see also Figure 3).

In addition to DOC and color, the predictive model of UVB attenuation included TSS. Although of relatively minor importance, this result demonstrates the potential for TSS to play a role in UVB attenuation in wetlands. A comparison of linear regression models of K_a310 versus K_dB for filtered and unfiltered samples (not shown) also suggests that particulates can play a role in controlling K_dB in wetlands. These results differ from most lake studies (e.g., refs *11* and *12*) that show TSS to be unimportant for predicting UVB attenuation, although TSS recently was shown to control 70% of the K_dB variation in Lake Erie (*14*). TSS effects on UV in wetlands would most likely be episodic, resulting from resuspension of bottom sediments, allochthonous inputs following rainstorms, or phytoplankton blooms. Phytoplankton or algal biomass is typically estimated by chlorophyll *a* and, like DOC and color, is seasonably variable. Chlorophyll *a* can effect UV attenuation in some lakes (*13*, *15*); however, it was not included in our final UVB model and was of only minor importance for predicting UVA.

Overall, the high temporal and spatial variability in wetland water chemistry make it difficult to assign a single UV attenuation or risk value to a given wetland. For example, two of our study sites, EPA-13.3A and EPA-13.3B, were actually part of the same wetland. Site EPA-13.3B was essentially a shallow channel extending from the main pond (site EPA-13.3A) and was sampled separately because it contained large numbers of tadpoles and appeared to differ in water clarity from the main pond. In May and June of 1999, these sites were sampled within minutes of one another. The pond had lower DOC concentrations (15.9 and 17.3 mg·L^{-1}) as compared to the channel (23.1 and 29.4 mg·L^{-1}) during May and June, respectively. The UVB attenuation in these two habitats differed as well, with K_dB values in the pond ~52–69% lower than that in the channel. Given the dynamic nature of water column characteristics in wetlands, models predicting UV attenuation should be used cautiously and should consider temporal and spatial variability.

Other Factors That Influence UV Exposure. The combined interaction of many factors ultimately determines the UVB dose received by wetland biota. Latitude, atmospheric conditions, and solar angle determine the incident level of radiation (cf. Diamond et al. (*50*)). Surface reflection of incident radiation further eliminates a small to moderate percentage of UVB. Because of the diffuse nature of UVB, models of surface reflection have shown this effect to be relatively constant between 5% and 8% regardless of season, solar zenith angle, or surface waves for latitudes between 45° N and 75° N (*51*). Surface reflection calculated for our wetlands was somewhat higher, accounting for an average 11.9% reduction of incident UVB irradiation.

Extinction measurements are necessarily conducted in relatively deep, open water portions of lakes and wetlands. However, tadpoles often reside in shallow or vegetated habitats. Within a wetland, UV exposure is spatially and temporally heterogeneous due to differences in overhead cover, flow regime, and internal habitat structure. Vegetation, for example, can affect incident UV levels as well as water column attenuation. Relative to open water, vegetation considerably reduced both the instantaneous flux (>40% at 5 cm) as well as cumulative UVB dose (>50% at 5 cm) at depth, in our study wetland (Figure 4). These preliminary findings are interesting because there was no statistically significant difference in the degree of UVB protection afforded by each habitat type, despite considerable morphological differences in the three types of vegetation sampled. This is likely due to the diffuse nature of UVB in these partially shaded habitats. Interestingly, the reduction of UVB in the macrophyte beds, relative to open water, was greater in deeper water for the instantaneous flux, whereas cumulative dose remained relatively consistent with depth (Figure 4). It is clear that habitat plays a significant role in determining the UV exposure of amphibians in wetlands.

Behavior. The low UVB penetration predicted for wetlands suggests that developing amphibians must be confined to extremely shallow environments to receive a biologically significant dose. It has been suggested that climate-induced reductions in water level can cause high mortality of amphibian embryos through synergistic effects of increased exposure to UVB and fungal disease (*52*). Additionally, tadpoles that seek the warmer O_2-rich upper layers of the water column may consequently be exposed to higher doses of UVB as a result of their behavior. Other factors such as thermoregulation, predator avoidance, social interactions, and interspecific competition also influence specific habitat selection and, therefore, UVB exposure. Likewise, the existence and use of light refugia by developing amphibians is undoubtedly of critical importance. Behavioral studies of larval amphibian phototaxis in relation to UV radiation, however, are generally absent from the literature. We have found only two such studies, and the implications of their results are unclear. One study (*53*) suggested that Alpine Newt larvae preferred a shady environment to a UV-enhanced or sunlight-exposed environment, while exhibiting a positive phototactic response to visible light. The second study (*54*) tested three species of Australian frogs and reported that tadpoles of two of the three species were observed more frequently in a UVB-free environment than in a high-level UVB enhanced environment. However, this study also suggested that observed differences in population stability between these species were unlikely due to the differential ability to detect and avoid UVB radiation.

It is clear that many factors influence UVB attenuation in wetlands resulting in a heterogeneous light environment in which organism behavior will ultimately determine the dose received. Consequently, we stress that behavior is a critical component that must be better understood for the development of accurate UV exposure risk models for amphibians in wetlands.

Acknowledgments

Elaine Ruzycki and Lindsay Anderson provided critical support during field sampling, as well as important feedback in the planning and execution of this project. We would like to thank Gerald Ankley and Edward Little for their critical review of this manuscript. Gerald Ankley and Joseph Tietge were instrumental in the conception and implementation of this project, and, along with Edward Hammer and Charles Maurice from EPA-Region 5, successfully obtained funding to complete this work. Funding for this project was provided through a cooperative agreement with the U.S. EPA Mid-Continent Ecology Division with funding from the RARE program (Grant CR 826590-01-1). This is Publication Number 310 for the Center for Water and Environment. This document has been subjected to review by the National Health and Environmental Effects Research Laboratory and approved for publication. Approval does not signify that the contents reflect views of the Agency, nor does mention of trade names or commercial products constitute endorsement or recommendation for use.

Supporting Information Available

K_d values for four bandwidths, DOC, color at 440 nm, total suspended solids, and chlorophyll *a* concentrations for wetland sampling events. This material is available free of charge via the Internet at http://pubs.acs.org.

Literature Cited

(1) Setlow, R. B. *Proc. Natl. Acad. Sci. U.S.A.* **1974**, *71*, 3363−3365.
(2) Herman, J. R.; Bhartia, P. K.; Ziemke, J.; Ahmad, Z.; Larko, D. *Geophys. Res. Lett.* **1996**, *23*, 2117−2120.
(3) Ankley, G. T.; Tietge, J. E.; Holcombe, G. W.; DeFoe, D. L.; Diamond, S. A.; Jensen, K. M.; Degitz, S. J. *Can. J. Zool.* **2000**, *78*, 1092−1100.
(4) Blaustein, A. R.; Hoffman, P. D.; Hokit, D. G.; Keisecker, J. M.; Wells, S. C.; Hays, J. B. *Proc. Natl. Acad. Sci. U.S.A.* **1994**, *91*, 1791−1795.
(5) Blaustein, A. R.; Keisecker, J. M.; Chivers, D. P.; Anthony, R. G. *Proc. Natl. Acad. Sci. U.S.A.* **1997**, *94*, 13735−13737.
(6) Hays, J. B.; Blaustein, A. R.; Keisecker, J. M.; Hoffman, P. D.; Pandelova, I.; Cooyle, D.; Richardson, T. *Photochem. Photobiol.* **1996**, *64*, 449−456.
(7) Adams, M. J.; Schindler, D. E.; Bury, R. B. *Oecologia* **2001**, 128, 519−525.
(8) Grant, K. P.; Licht, L. E. *Can. J. Zool.* **1995**, *73*, 2292−2301.
(9) Ovaska, K.; Cavis, T. M.; Novles-Flamarique, I. *Can. J. Zool.* **1997**, *75*, 1081−1088.
(10) Corn, P. S. *Ecol. Appl.* **1998**, *8*, 18−26.
(11) Scully, N. M.; Lean, D. R. S. *Ergeb. Limnol.* **1994**, *43*, 135−144.
(12) Morris, D. P.; Zagarese, H.; Williamson, C. E.; Balseiro, E. G.; Hargreaves, B. R.; Modenutti, B.; Moeller, R.; Queimalinos, C. *Limnol. Oceanogr.* **1995**, *40*, 1381−1391.
(13) Hodoki, Y.; Watanabe, Y. *Jpn. J. Limnol.* **1998**, *59*, 27−37.
(14) Smith, R. E. H.; Furgal, J. A.; Charlton, M. N.; Greenberg, B. M.; Hiriart, V.; Marwood, C. *Can. J. Fish. Aquat. Sci.* **1999**, *56*, 1351−1361.
(15) Laurion, I.; Ventura, M.; Catalan, J.; Psenner, R.; Sommaruga, R. *Limnol. Oceanogr.* **2000**, *45*, 1274−1288.
(16) Crump, D.; Lean, D.; Berrill, M.; Coulson, D.; Toy, L. *Photochem. Photobiol.* **1999**, *70*, 893−901.
(17) Arts, M. T.; Robarts, R. D. *Limnol. Oceanogr.* **2000**, *45*, 292−299.
(18) Kostkowski, H. J. *Reliable Spectroradiometry*; Spectroradiometry Consulting: La Plata, MD, 1997.
(19) American Public Health Association. *Standard Methods for the Examination of Water and Wastewater*, 18th ed., Washington, DC, 1992.
(20) Ameel, J. J.; Ruzycki, E. R.; Owen, C. J.; Axler, R. P. *Analytical Chemistry and Quality Assurance Procedures for Natural Water, Wastewater, and Sediment Samples*; NRRI/TR-98/28; Duluth, MN, 1998.
(21) Boucher, N. P.; Prezelin, B. B. *Mar. Ecol. Prog. Ser.* **1996**, *144*, 223−236.
(22) Smith, R. C.; Baker, K. S. *Photochem. Photobiol.* **1979**, *29*, 311−323.
(23) Diamond, S. A.; Peterson, G. S.; Axler, R. P.; Johnson, L. B. Unpublished data.
(24) SAS/STAT. *Users Guide*; SAS Institute Inc.: Cary, NC, 1998.
(25) Draper, N. R.; H. Smith. In *Applied Regression Analysis*; John Wiley and Sons: New York, 1981.
(26) Belsley, D. A.; Kuh, E.; Welsch, R. E. In *Regression Diagnostics*; John Wiley and Sons: New York, 1980.
(27) Ankley, G. T.; Diamond, S. A.; Tietge, J. E.; Holcombe, G. W.; Jensen, K. M.; DeFoe, D. L.; Peterson, R. *Environ. Sci. Technol.* **2002**, *36*, 2853−2858.
(28) Laurion, I.; Warwick, F. V.; Lean, D. R. S. *Photochem. Photobiol.* **1997**, *65*, 107−114.
(29) Mollot, L. A.; Dillon, P. J. *Can. J. Fish. Aquat. Sci.* **1997**, *54*, 2789−2795.
(30) Gorham, E.; Underwood, J. K.; Janssens, J. A.; Freedman, B.; Maass, W.; Waller, D. H.; Ogden, J. G., III. *Wetlands* **1998**, *18*, 115−132.
(31) Aiken, G. R.; McKnight, D. M. *Effects of Hydrological Factors on Organic Matter in the Williams and Shingobee Lake systems*; USGS Water-Resources Investigations Report 96-4215, 1997; pp 71−76.
(32) Rasmussen, J. B.; Godbout, L.; Schallenberg, M. *Limnol. Oceanogr.* **1989**, *34*, 1336−1343.
(33) Carder, K.; Steward, R.; Harvey, G.; Ortner, P. *Limnol. Oceanogr.* **1989**, *34*, 68−81.
(34) McKnight, D. M.; Harnish, R.; Wershaw, R. L.; Baron, J. S.; Schiff, S. *Biogeochemistry* **1997**, *36*, 99−124.
(35) Clair, T. A.; Sayer, B. G. *Biogeochemistry* **1997**, *36*, 89−97.
(36) Allard, B.; Boren, H.; Petterson, C.; Zhang, G. *Environ. Int.* **1994**, *20*, 97−101.
(37) Morris, D. P.; Hargreaves, B. R. *Limnol. Oceanogr.* **1997**, *42*, 239−249.
(38) Reche, I.; Pace, M. L.; Cole, J. J. *Biogeochemistry* **1999**, *44*, 259−280.
(39) Whitehead, R. F.; de Mora, S.; Demers, S.; Gosselin, M. *Limnol. Oceanogr.* **2000**, *45*, 278−291.
(40) Curtis, P. J.; Schindler, D. W. *Biogeochemistry* **1997**, *36*, 125−138.
(41) Dillon, P. J.; Mollot, L. A. *Water Resour. Res.* **1997**, *33*, 2591−2600.
(42) Schindler, D. W.; Curtis, P. J. *Biogeochemistry* **1997**, *36*, 1−8.
(43) Schindler, D. W.; Curtis, P. J.; Parker, B. R.; Stainton, M. P. *Nature* **1996**, *379*, 705−708.
(44) Yan, N. D.; Keller, W.; Scully, N. M.; Lean, D. R. S.; Dillon, P. J. *Nature* **1996**, *381*, 141−143.
(45) Williamson, C. E.; Stemberger, R. S.; Morris, D. P.; Frost, T. M.; Paulsen, S. G. *Limnol. Oceanogr.* **1996**, *41* (*5*), 1024−1034.
(46) Kerekes, J. J. *J. Fish. Res. Board. Can.* **1974**, *31*, 555−583.
(47) Cronan, C. S.; Aiken, G. R. *Geochim. Cosmochim. Acta* **1985**, *49*, 1697−1705.
(48) Engstrom, D. R. *Can. J. Fish. Aquat. Sci.* **1987**, *44*, 1306−1314.
(49) Visser, S. A. *Freshwater Biol.* **1984**, *14*, 79−87.
(50) Diamond, S. A.; Peterson, G. S.; Tietge, J. E.; Ankley, G. T. *Environ. Sci. Technol.* **2002**, *36*, 2866−2874.
(51) Jerome, J. H.; Bukata, R. P. *J. Great Lakes Res.* **1998**, *24*, 666−680.
(52) Kiesecker, J. M.; Blaustein, A. R.; Belden, L. K. *Nature* **2001**, *410*, 681−683.
(53) Nagl, A. M.; Hofer, R. *Oecologia* **1997**, *110*, 514−519.
(54) Van De Mortel, T. F.; Buttemer, W. A. *Proc. Linn. Soc. N.S.W.* **1998**, *119*, 173−179.

Received for review August 15, 2001. Revised manuscript received February 18, 2002. Accepted February 18, 2002.

ES011196L