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Near UV quantum yields for rotenone and piperonyl butoxide

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Rotenone and piperonyl butoxide (PBO) mixtures, so-called "synergized" rotenone, are invaluable in fisheries management where they are used to protect the habitat of endangered, native species and promote desirable gamefish populations. Continued use of synergized rotenone is threatened by inadequate control of persistence in surface water, especially where drinking water supplies are impacted. The photochemical kinetics of these chemicals were studied in the laboratory with a goal to better understand their fate in natural water. Disappearance quantum yields (Φ) were determined in polychromatic light from fluorescent lamps emitting maximally at 350 nm. Rotenone, PBO and trifluralin, an actinometer, were irradiated as aqueous solutions at 25 or 50 μg L^{-1} and the piscicides were determined by electrospray-liquid chromatography-mass spectrometry (ESI-LC-MS). In the photoreactor rotenone and PBO photodegraded with first-order half-lives of 500 and 220 min, respectively, and corresponding quantum yields of 0.00015 and 0.034. Rotenone absorbs sunlight strongly, while PBO does not. Differences in spectal overlap tended to counteract the disparities in Φ and, in general, mathematical modeling indicates moderately rapid direct photolysis rates for both substances in surface water.

Introduction

Rotenone is a naturally-occurring insecticide and piscicide (fish poison) found in many legumes including plants of the genera *Derris*, *Lonchocarpus*, *Amorpha* and *Tephrosia*. Rotenone was first isolated in 1895, its structure elucidated in 1933 (Fig. 1) and total synthesis accomplished by 1965.[1] Rotenone continues to be obtained by extracting *Derris* roots and related plants,[2] and dusts and resins derived from botanical extracts are marketed today as insecticides and fish poisons.

Rotenone inhibits mitochondrial respiration and is exceptionally toxic to gill breathing organisms. Rotenoids are detoxified by cytochrome P-450 mixed function oxidases, enzymes inhibited by the aromatic aliphatic polyether piperonyl butoxide (Fig. 2). Rotenone formulated with PBO (so-called 'synergized' rotenone) has increased potency and residual activity.

Rotenone is a valuable tool in gamefish management,[3] in part, because it degrades rapidly.[4] Sunlight is believed to play a key role in its degradation. Rotenone photochemistry has been studied for over 70 years in an effort to improve its residual

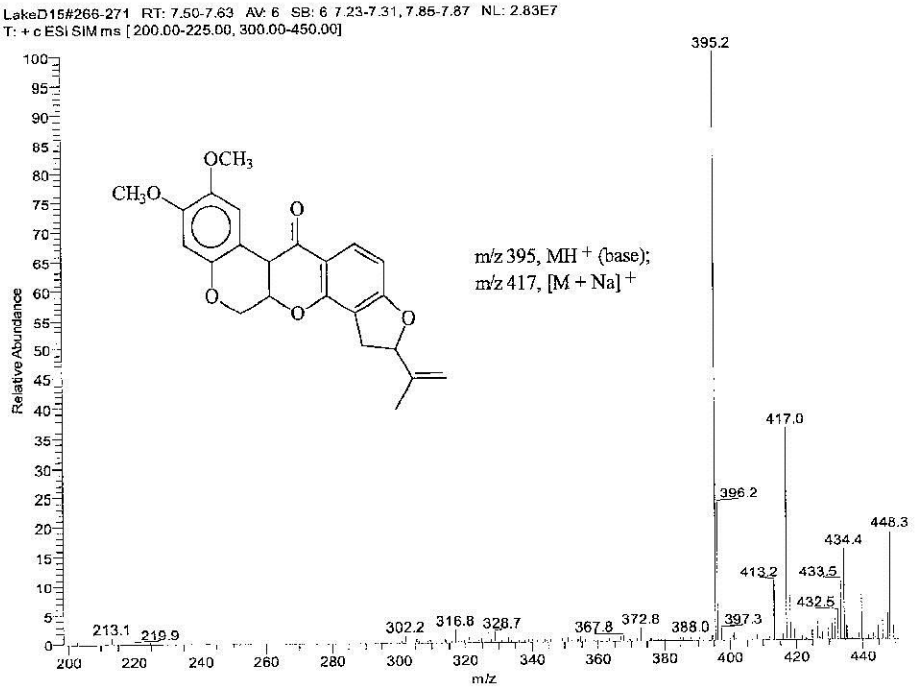

Fig. 1 Positive ion electrospray mass spectrum of rotenone with sample introduction by liquid chromatography using a methanol–water mobile phase.

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insecticidal activity. Rotenone undergoes photochemical *O*-demethylation, epimerization, epoxidation, hydroxylation, and dehydration.[5]

The available information on PBO environmental photochemistry is somewhat contradictory. PBO is reported to be photochemically unstable undergoing conversion to 3,4-methylenedioxy-6-propyl benzyl alcohol (MDB alcohol) and MDB aldehyde.[6] Yet PBO has been found to be much more persistent than rotenone in one surface water application studied in detail.[3]

The objective of this work was to study rotenone and PBO photochemistry under conditions simulating natural aquatic systems. Very low concentrations of the substrates were irradiated in water to minimize photosensitization or autocatalysis.[7] Aqueous solutions of the test compounds were exposed to near UV, polychromatic light and wavelength-averaged quantum yields (Φ) were measured. Quantum yields, needed for environmental modeling, vary over many orders of magnitude and cannot be predicted based on molecular structure.

Methods and materials

Chemicals

Rotenone (97%) and PBO (technical grade, ~90% purity) were obtained from Aldrich (Milwaukee, WI, USA) and were used as received. Trifluralin was an analytical reference standard provided by the US Environmental Protection Agency (USEPA, Research Triangle Park, NC, USA). Methanol used in mobile phases and preparation of standards was purchased from EM Science (Merck, Darmstadt, Germany). Water used in mobile phases and photochemistry experiments was produced by a Nanopure water purifier (Barnstead/Thermolyne, Dubuque, IA, USA) that treated distilled feed water with ion exchange and charcoal resins and UV light.

Instruments

A Finnigan LCQ-Deca mass spectrometer (Finnigan MAT, San Jose, CA, USA) equipped with an electrospray ionization (ESI) probe and fused silica-lined ESI needle was used. The HPLC was an HP 1100 (Agilent, Wilmington, DE, USA) with an autosampler, solvent degasser, binary pump and heated/cooled column compartment. The mass spectrometer and HPLC were controlled by Xcalibur software, also used for data acquisition and processing.

LC-MS operation

The mass spectrometer was tuned with a metobromuron solution as reported previously.[8] The heated capillary temperature was 350 °C, the nitrogen sheath and auxiliary gas flows were 80 and 20 units, respectively, and the spray voltage was +5 kV. All voltages and offsets were determined by autotune software.

A 2.1 mm × 15 cm Supelcosil LC-18 column with 3 μm packing was used exclusively (Supelco, Bellefonte, PA, USA). The HPLC separation was a 10 min methanol–water gradient from 50 to 100% methanol with a total flow rate of 0.5 mL min^{-1} and column temperature of 40 °C. The injection volume was 10 μL.

The mass spectrometer scanned two mass ranges, 200–225 amu and 300–450 amu, and acquired +ve ions after a 0.5 min start delay. The quadrupole ion trap mass analyzer was operated with automatic gain control with 3 total microscans, a 50 ms maximum injection time, and an electron multiplier voltage of 880 V. Monolinuron (*m/z* 215, MH^+), rotenone (*m/z* 395, MH^+) and PBO (*m/z* 356, $[M + H_2O]^+$) eluted at 2.62, 6.64 and 8.78 min, respectively.

Water analysis

Water samples (5 mL) were spiked with 2.0 mg L^{-1} of monolinuron, a quantitation internal standard, by adding 10 μL of a 1.0 mg mL^{-1} methanol solution, and subsamples were transferred to amber autosampler vials for LC-MS analysis. Trifluralin was extracted from water by partitioning 5 mL samples with 2 mL of toluene. Trifluralin in the extract was determined by electron capture detector-gas chromatography (ECD-GC) using an instrument equipped with a J&W Scientific 30 m × 0.25 mm (id) fused silica column with an 0.25 μm DB-1 bonded phase (Rancho Cordova, CA, USA) and a splitless injection port. GC conditions were the following: inlet, 250 °C; detector, 300 °C; carrier gas, 18 psi He; oven temperature program, 80 °C (2 min), 40 °C min^{-1} to 185 °C; 185 °C (15 min), trifluralin t_R, 16.9 min.

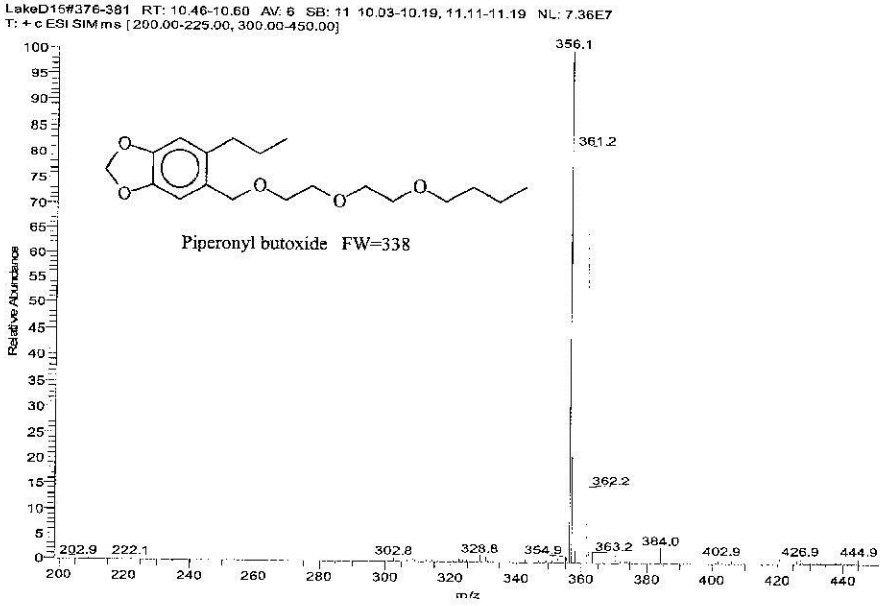

Fig. 2 Positive ion electrospray LC-MS spectrum of piperonyl butoxide.

Quantum yield determination

Rotenone and piperonyl butoxide were irradiated in a laboratory photoreactor to determine wavelength-averaged, near-UV quantum yields.[9,10] Such quantum yields are needed to estimate direct photolysis rates using mathematical environmental fate models such as those developed by the USEPA.[11,12] All calculations, linear regressions and plotting used Excel software (Microsoft, Redmond, WA, USA).

Stock solutions (1.0 mg mL^{-1}) of rotenone, PBO or trifluralin in acetonitrile or methanol were diluted to 20 μg mL^{-1} in acetonitrile. The secondary acetonitrile solutions were further diluted to 25 or 50 μg L^{-1} in laboratory reagent water. Water samples in borosilicate glass test tubes (UV cutoff, ~ 280 nm) were held in a merry-go-round apparatus and irradiated with Rayonet RPR-3500 fluorescent blacklight lamps in a Rayonet RPR-100 photoreactor (Southern New England Ultraviolet Co., Hamden, CT, USA). The lamps emit in the UVA region between 310 and 410 nm and have maximal output at 350 nm. Light intensity was measured by simultaneous irradiation of an actinometer, a 25 μg L^{-1} aqueous trifluralin solution.[10] In sunlight the trifluralin Φ is 0.002[11] and trifluralin in this solvent has a typical half-life of ~ 1 h in the photoreactor.

Absorption spectra were recorded over the range 200–500 nm in quartz cuvettes. Technical piperonyl butoxide (~ 90%) was dissolved in acetonitrile or methanol at 0.2 g L^{-1} (5.3×10^{-4} M). Rotenone's spectrum was recorded in methanol (0.02 g L^{-1}, 5.1×10^{-5} M).

Results and discussion

LC-MS determination of rotenone and PBO

Rotenone's ESI mass spectrum includes two major +ve ions, *m/z* 395 (base) and *m/z* 417 (~ 35%) believed to be proton (MH^+) and sodium adducts (MNa^+), respectively. Piperonyl butoxide's major ESI ions are $[M + H_2O]^+$ or MNH_4^+ (*m/z* 356, base) and MNa^+ (*m/z* 361, variable intensity). ESI spectra appear in Figs. 1 and 2.

Piperonyl butoxide has been determined as a semivolatile by GC-MS. In electron ionization MS, however, little or no M^+ ion is observed and PBO is detected as an odd electron fragment ion, *m/z* 176 ($C_{11}H_{12}O_2$) formed by neutral loss of diethyleneglycolmonobutyl ether from the molecular ion.[6] An even electron fragment ion, *m/z* 177 ($C_{11}H_{13}O_2$), also is present. GC-MS analysis of rotenone is unsatisfactory due to its low vapor pressure and thermal instability. HPLC is preferred for simultaneous rotenone/PBO determination and, when used in conjunction with solid phase extraction (SPE), reporting limits in surface water are < 10 μg L^{-1}.[13] Humic materials such as those occurring in sediments can interfere with trace HPLC-UV determination of these analytes.

ESI mass spectrometry is two- to three-orders of magnitude more sensitive than HPLC with UV or diode array detection. LC-MS detection limits are sufficiently low that the piscicides can be determined at low ppb concentrations without SPE. Detection limits for direct LC-MS determination of rotenone and PBO were 5 and 2 μg L^{-1}, respectively, based on a 3:1 signal-to-noise. Reconstructed ion chromatograms are shown for water spiked at 25 μg L^{-1} (Fig. 3). At this concentration recoveries of rotenone and PBO were 98 and 70%, respectively, laboratory reagent water was free of interferences and calibration was linear.

Electrospray LC-MS analysis of the actinometer, trifluralin, also was investigated. Trifluralin ionizes efficiently giving $[M-H]^-$ (*m/z* 334, 25%), M (*m/z* 335, 40%) and $[M-H + CH_3OH]^-$ (*m/z* 366, base) ions. Thus, all 4 compounds of interest in this study, monolinuron, PBO, rotenone and trifluralin, could be determined simultaneously by monitoring the column effluent with +ve and −ve ion segments. Trifluralin, however, could only be determined with sufficiently low detection limits using the solvent extraction GC-ECD method.

Absorption spectra

Piperonyl butoxide, unlike rotenone, absorbs very weakly in the UVA region where the fluorescent lamps have their principal output. Rotenone has a much stronger chromophore with extinction coefficients of ~ 1000 L (mol cm)$^{-1}$ at the lamp emission maximum. In the UVB rotenone absorbance is over 10^4 L (mol cm)$^{-1}$. The λ_{max} values for rotenone and PBO were 294 nm (methanol) and 290 nm (acetonitrile or methanol), respectively, with extinction coefficients of 18,000 L (mol cm)$^{-1}$ (methanol) and 4,800 L (mol cm)$^{-1}$ (acetonitrile or methanol). Detailed information on both the absorption spectra and lamp emission spectrum appear in Table 1.

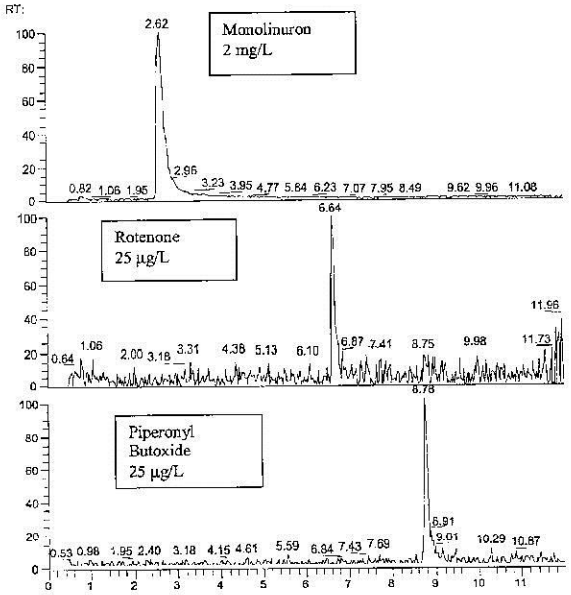

Fig. 3 Recontructed ion chromatograms for monolinuron internal standard (*m/z* 215, MH^+), rotenone (*m/z* 395, MH^+) and PBO (*m/z* 356, $[M+H_2O]^+$). Chromatograms represent 250 pg injected of each piscicide.

Table 1 Typical spectral irradiance in the photoreactor and piscicide extinction coefficients

Wavelength/ nm	Photon irradiance/ cm s^{-1}	Extinction coefficients/L (mol cm)$^{-1}$	
		Rotenone	Piperonyl butoxide
297.5		17819	3199
300		17139	2181
302.5		16174	1163
305		15156	554
307.5		13849	231
310	3.9E13	12838	118
312.5	7.5E13	11958	71
315	1.2E14	11071	51.5
317.5	1.9E14	10264	44.3
320	2.6E14	9249	41.3
323.1	6.3E14	7801	38.7
330	1.6E15	4743	32.1
340	3.1E15	1911	22.8
350	4E15	832	13.4
360	3.6E15	448	10.9
370	2.4E15	249	9.6
380	1.4E15	155	7.7
390	6.3E14	117	6.5
400	2.8E14	107	6.7
410	9.6E13	93.1	5.9

Photodecomposition rates in the photoreactor

Trifluralin is a useful actinometer owing to ease of analysis, high extinction coefficients across a wide wavelength range, reproducible first-order kinetics over multiple half-lives and stability in the dark.[9] In this study trifluralin photodecomposed with a half-life of 33.8 min and a first-order rate constant of 0.0205 min^{-1} (Fig. 4). The estimated light intensity in the reactor was 13,400 $\mu W\ cm^{-2}$, higher than in previous studies presumably because new lamps were used. The analytical recovery of trifluralin was 98% and there was no detectable change (<0.1%) in the trifluralin concentration in a foil-wrapped dark control solution held in the reactor.

Preliminary photochemistry experiment

In a preliminary experiment both rotenone and PBO (25 $\mu g\ L^{-1}$ each) were irradiated as a single solution. This experiment established that both compounds are relatively stable in the dark over a period of days. PBO photodegraded more rapidly than rotenone with ~56% of the compound consumed after 150 min of irradiation. After 19 h PBO was not detected, while 31% of the rotenone remained. The experiment further established the approximate exposure times needed to measure several photochemical half-lives as well as the need for higher initial concentrations to improve accuracy, especially in the case of rotenone.

Rotenone photodecomposition

In a subsequent experiment rotenone was irradiated for 3 days with an initial concentration of 50 $\mu g\ L^{-1}$. After 57 h in the dark <5% loss of rotenone was apparent, *e.g.*, 60 $\mu g\ L^{-1}$ dropping to 57 $\mu g\ L^{-1}$. In the photoreactor the compound was below the detection limit in samples taken after 45 h, but 5 data points were obtained up to 24 h (Fig. 5). From these points a half-life of 8.2 h and a first-order rate constant of 0.0836 h^{-1} were estimated. Owing to rotenone's large extinction coefficients, the rate of light absorption in the photoreactor was very high, ~5×10^{19} photons $(mol\ s)^{-1}$. Rotenone's experimental quantum yield was 0.00015, very low compared to many other aromatic pollutants.

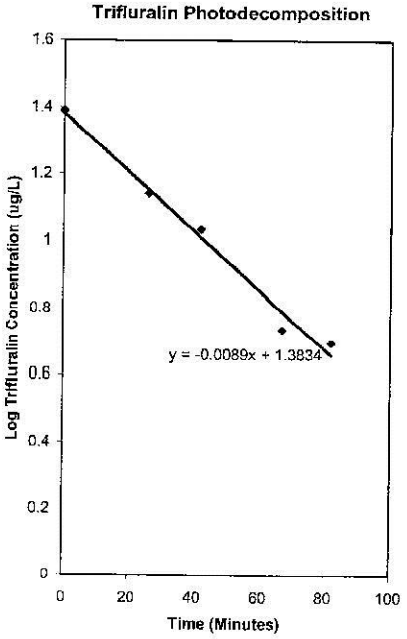

Fig. 4 Photodecomposition of the trifluralin actinometer in the laboratory photoreactor. The initial trifluralin concentration was 25 $\mu g\ L^{-1}$ and the herbicide was determined by solvent extraction followed by ECD-GC.

Piperonyl butoxide photodecomposition

A 50 $\mu g\ L^{-1}$ piperonyl butoxide solution was irradiated for 20.5 h. In the dark control there was no evidence of breakdown with 115% PBO recovered. Very low concentrations of PBO (~1.5 $\mu g\ L^{-1}$) remained after 20.5 h of irradiation. Quantifiable concentrations of PBO were present in solutions irradiated between 68 and 210 min (Fig. 6) when ~50 to 80% of the

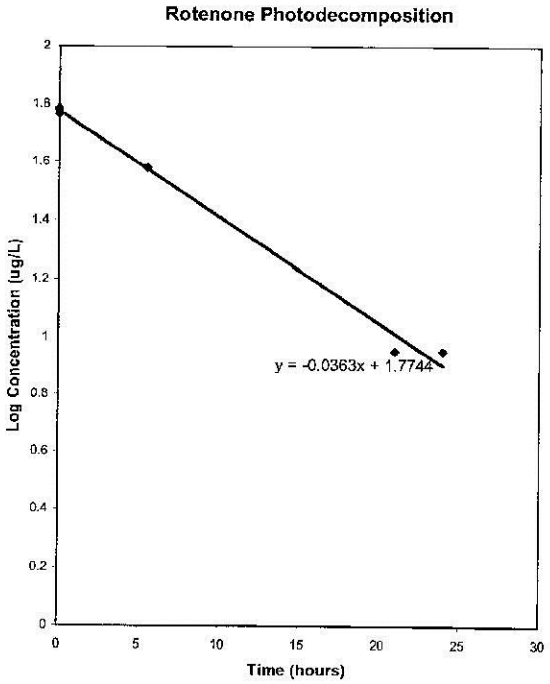

Fig. 5 Semi-logarithmic transformation of rotenone photodecomposition data. The piscicide was irradiated in near-UV light in the laboratory photoreactor and determined by ESI-LC-MS analysis.

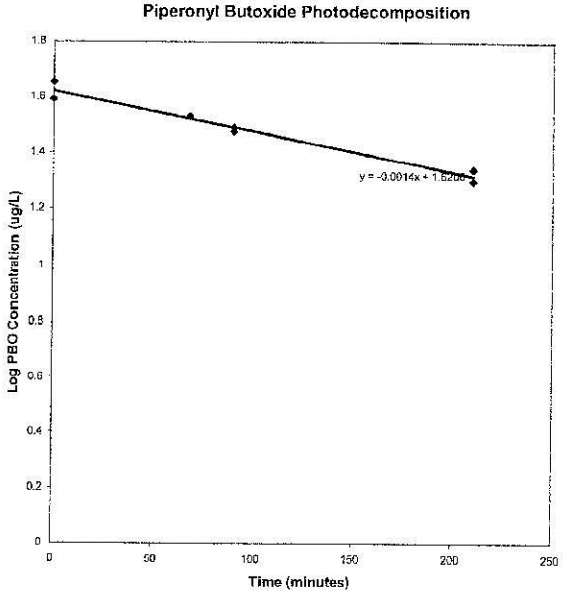

Fig. 6 Photochemical decomposition of PBO in near-UV light with the best-fit line plotted.

compound remained. PBO photodecomposed with a half-life of 220 min and a first-order rate constant of 0.0032 min^{-1}.

While PBO photodecomposed more rapidly than rotenone in the photoreactor, the rate of light absorption was much lower, $\sim 5 \times 10^{17}$ photons (mole s)$^{-1}$ or about 1% that of rotenone. The experimental quantum yield for PBO was 0.034, typical for moderately efficient photochemical substrates.

Hypothetical surface water application

Direct photolysis rates in natural water were estimated using a mathematical model developed and validated by Zepp and coworkers. This model is accurate because the rate of sunlight absorption, the integrated overlap of the solar emission spectrum with the absorption spectrum, is carefully calculated. The model assumes that quantum yields are invariant with wavelength, a satisfactory assumption if Φ is determined in the near UV region of the spectrum where most environmental pollutants photodegrade.

Direct photolysis rates were estimated for a hypothetical piscicide application of 50 $\mu g\ L^{-1}$ each of rotenone and PBO to a moderately deep (500 cm) body of water. Rotenone is a far better sunlight chromophore absorbing ~50 times the sunlight energy of PBO. PBO, however, is ~200 times more efficient as a photochemical substrate and quantum yields are the dominant variable in this simulation. Predicted summertime, direct photolysis half-lives were ~3 months for PBO and ~13 months for rotenone. In winter the sunlight spectrum is reduced in overall intensity and is particularly diminished in UVB radiation resulting in even slower direct photolysis rates (Table 2).

Photochemical reactions are sensitive to water depth and here the model predicts a direct relationship between depth and half-life. Thus, dimensions of the water body affect actual rates. Other physical factors such as shading by suspended particles (or a winter ice cover) or attenuation by dissolved organic matter would limit sunlight penetrating into the water column and reduce photodecomposition rates.

While the goal of this work was to determine rate constants under controlled laboratory conditions, the findings reveal additional details of the mechanisms of piscicide removal from natural waters. Detailed monitoring of a recent synergized rotenone application to a Sierra Nevada (California) mountain lake revealed rapid removal of rotenone ($t_{1/2}$, 8 days).[3] In contrast, PBO persisted many months after the application. Direct photolysis may account for the protracted removal of PBO, but it cannot explain rotenone's rapid disappearance.

In addition to direct photolysis other photochemical reactions occur in natural water. Such processes are referred to collectively as indirect photolysis and include oxidation by photochemical oxidants such as hydroxyl free radical[14] and other radicals and singlet molecular oxygen. Metabolic transformations, of course, also are important for some substrates and each of these processes serves to accelerate contaminant removal. Further research on rotenone is needed to understand which of the well established physical, chemical and biological processes including indirect photolysis are driving its rapid removal from natural water.

Table 2 Hypothetic application and predicted direct photolysis half lives

				Direct photolysis half-life/years	
Pesticide	Φ	Concentation/M	Depth/cm	Summer	Winter
Rotenone	0.00015	1.3×10^{-7}	500	1.1	3.1
PBO	0.034	1.5×10^{-7}	500	0.22	0.58

Summary and conclusions

Rotenone and piperonyl butoxide are photochemically unstable in near UV light and are anticipated to undergo direct photolysis at moderate rates in natural water. The present study establishes that ESI-LC-MS is useful for specific determination of these piscicides at low parts-per-billion concentrations without sample preconcentration. Electrospray mass spectrometry, used here to study photochemical kinetics in the laboratory, also is useful for analysis of surface waters and bottom sediments where interferences have been encountered with other LC detectors.

Rotenone has an extended π-bond system and absorbs sunlight efficiently. Its direct photolysis rate, however, is limited by a low quantum efficiency, Φ = 0.00015. Piperonyl butoxide absorption drops off abruptly in the near UV and it absorbs less incident sunlight, but a higher quantum yield (Φ = 0.034) leads to greater photochemical instability in sunlight. Better understanding of both the rates and mechanisms of piscicide degradation in natural water may help identify formulations and application practices giving superior performance and acceptable environmental residues.

Acknowledgements

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