

Environmental Chemistry

ROTENONE FORMULATION FATE IN LAKE DAVIS FOLLOWING THE 2007 TREATMENT

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Abstract—In September 2007, Lake Davis (near Portola, California) was treated by the California Department of Fish and Game with CFT Legumine, a rotenone formulation, to eradicate the invasive northern pike (*Esox lucius*). The objective of this report is to describe the fate of the five major formulation constituents—rotenone, rotenolone, methyl pyrrolidone (MP), diethylene glycol monethyl ether (DEGEE), and Fenmedefo 99—in water, sediment, and brown bullhead catfish (*Ameiurus nebulosus*; a rotenone-resistant species) by determination of their half-lives ($t_{1/2}$) and pseudo first-order dissipation rate constants (k). The respective $t_{1/2}$ values in water for rotenone, rotenolone, MP, DEGEE, and Fenmedefo 99 were 5.6, 11.1, 4.6, 7.7, and 13.5 d; in sediments they were 31.1, 31.8, 10.0, not able to calculate, and 48.5 d; and in tissues were 6.1, 12.7, 3.7, 3.2, and 10.4 d, respectively. Components possessing low water solubility values (rotenone and rotenolone) persisted longer in sediments (not detectable after 157 d) and tissues (<212 d) compared with water, whereas the water-miscible components (MP and DEGEE) dissipated more quickly from all matrices, except for Fenmedefo 99, which was the most persistent in water (83 d). None of the constituents was found to bioaccumulate in tissues as a result of treatment. In essence, the physicochemical properties of the chemical constituents effectively dictated their fate in the lake following treatment. Environ. Toxicol. Chem. 2012;31:xxx–xxx. © 2012 SETAC

Keywords—Rotenone Environmental fate Dissipation rate

INTRODUCTION

Lake Davis, a reservoir (41,800 acre-feet) situated north of the town of Portola, California (Plumas County; Fig. 1), USA, was treated on September 25, 2007 by the California Department of Fish and Game with the rotenone formulation CFT Legumine™ (16,830 gallons) to eradicate the invasive northern pike (*Esox lucius*); most of the formulation was dispensed within the first 2 d. Rotenone, a natural compound derived from the roots of some members of the bean family (Leguminosae), has been used for centuries by fishermen worldwide to stun fish. It has also been a valuable tool for fisheries managers to maintain diverse and productive aquatic ecosystems [1].

The ecological risk assessment conducted as part of the Department of Fish and Game Environmental Impact Report/Environmental Impact Statement (<http://www.dfg.ca.gov/lakedavis/EIR-EIS/>) process reported that some formulation constituents had been previously found to bioconcentrate (e.g., rotenone, naphthalene), but none had been found to be subject to significant bioaccumulation (i.e., via biomagnification). Numerous public comments and questions were received expressing concern about the potential of constituents to accumulate either in fishes surviving in Lake Davis or in the food web of organisms potentially feeding on rotenone-killed fish. In response, the Department of Fish and Game conducted a posttreatment sampling of sediments, water, and surviving fish for residues of the formulation constituents.

The rotenone formulation CFT Legumine is comprised of five major constituents possessing the following average concentrations: rotenone (5.12%), rotenolone (0.718%), methyl pyrrolidone (MP; 9.8%), diethylene glycol monethyl ether (DEGEE; 61.1%), and Fenmedefo 99 (17.1%) [2]. Rotenolone is a degradation product of rotenone, whereas MP, DEGEE, and Fenmedefo 99 are used as solvents and surfactants to aid in the dissolution of rotenone. Rotenone is the active ingredient of CFT Legumine. Biological effects concentrations, median lethal concentration (LC50) and median effective concentration (EC50), for rainbow trout (*Oncorhynchus mykiss*) and the water flea (*Daphnia magna*) are provided in the Supplemental Data for the major components in the rotenone formulation. None of the constituents identified have been considered persistent in the environment nor subject to bioaccumulation in the screening-level risk analysis (<http://www.dfg.ca.gov/lakedavis/enviro-docs/ScreeningLevelAnalysis/ScreeningLevelAnalysis.pdf>).

Live, caged fish were deployed throughout the lake to verify the effectiveness of the treatment. Postproject monitoring indicated that the treatment eradicated both northern pike and rainbow trout (*O. mykiss*) from the reservoir and its tributary streams, but some species resistant to rotenone, including brown bullhead catfish (*Ameiurus nebulosus*), survived. Because the brown bullhead is also a sport fish caught for human consumption at Lake Davis, it was the species selected for tissue monitoring.

The objective of this investigation was to characterize the dissipation of the rotenone formulation in Lake Davis by determining the pseudo first-order dissipation rate constants (k) and half lives ($t_{1/2}$) for rotenone and its metabolite rotenolone, MP, DEGEE, and Fenmedefo 99 in water, sediment, and

All Supplemental Data may be found in the online version of this article.

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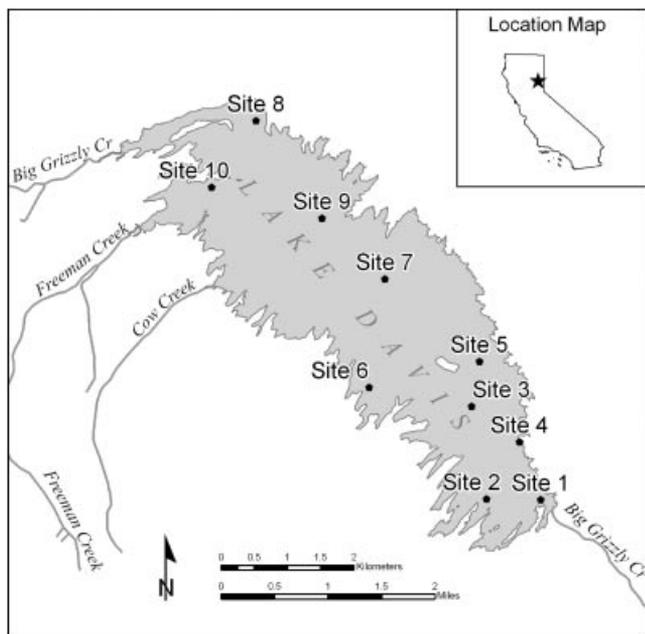


Fig. 1. Lake Davis map with water, sediment, and tissue sampling locations [2].

tissue. It was hypothesized that the physicochemical properties of each compound would dictate relative persistence and localization in environmental compartments (water, sediment, and tissue) after treatment.

MATERIALS AND METHODS

Study area

Lake Davis is an impoundment of Big Grizzly Creek, a tributary of the Middle Fork Feather River (Fig. 1). The reservoir has a maximum capacity of 84,371 acre-feet and contained 41,800 acre-feet during treatment. The valve delivering water to Big Grizzly Creek from Grizzly Valley Dam was closed when the Lake Davis Pike Eradication Project was implemented on September 25, 2007. Minimal flows in the Big Grizzly Creek below Grizzly Valley Dam were because of seepage around Grizzly Valley Dam. These minimal flows were collected in a sump at the base of the dam and trucked back to the reservoir to allow the chemicals to degrade naturally in Lake Davis. Normal flows from Grizzly Valley Dam to Big Grizzly Creek were restored on February 11, 2008.

Water sampling

Water samples were collected from 10 sites (1–10) as described previously [2]. Odd-numbered sites were sampled at the surface, midpoint, and bottom of the reservoir, and even-numbered sites were sampled at the surface and bottom (because of shallow depths; Fig. 1). Water samples from different depths were analyzed separately. Water was collected 2, 6, 13, 20, 27, 34, 41, 62, 70, 83, and 111 d posttreatment in 250-ml amber glass bottles (with Teflon caps), without headspace, and stored on ice for transport to the laboratory.

Sediment sampling

Sediments were also collected as described previously [2]. Briefly, samples (100 ml, $n = 1$ per site) were collected at days 6, 20, 34, 62, 70, 83, 104, 111, 112, 119, 130, 156, and 157

posttreatment in 500-ml glass jars (with Teflon lids) at sites 2, 4, 6, 8, and 10 (Fig. 1). The jars were dipped into the sediment and covered with overlying water, then stored on ice for transport to the laboratory.

Tissue sampling

Brown bullhead catfish (referred to as bullhead) were sampled by using an electrofishing boat. Capture efforts were distributed along the shallow coves in the vicinity of the four sites below; the locations are similar to, but may not be exactly the same as, the following water and sediment collection sites (Fig. 1): Mosquito Slough, Lake Davis (site 8); Freeman Cove, Lake Davis (site 10); South Camp 5, Lake Davis (site 6); and Dam area, Lake Davis (site 1). Electrofishing is useful for the collection of fish located along embankments but may carry a sampling bias. Samples were collected at 3, 10, and 30 d posttreatment. Generally four fish per site were collected by electrofishing for each sampling event, with the exception of two instances: once because of blowing snow and once when only two bullhead could be captured in the dam area, so the remaining fish were collected at nearby Catfish Cove. Pretreatment baseline samples were collected on August 9, 2007. A final round of sampling, for rotenone and rotenolone only, was conducted 212 d posttreatment (April 24, 2008) because they were detected in the 30 d posttreatment bullhead sampling; both bullhead and rainbow trout were collected 212 d posttreatment because trout restocking began in December 2007, and trout are commonly consumed by humans. The target size of fish sampled was a minimum of 150 mm fork length. The mean fork length and standard deviation of fish collected at each site is presented in the Supplemental Data. However, if electrofishing did not yield target-size fish within a reasonable amount of time, smaller fish were utilized. Fish size, date, and location were documented, and the fish were then humanely dispatched, number tagged, wrapped in aluminum foil (dull side to skin), and frozen on dry ice for transport to the laboratory. Pretreatment fish were composited by location, whereas posttreatment fish (3–30 d) were analyzed individually, according to the screening-level methods given in the General Protocol for Sport Fish Sampling and Analysis (<http://oehha.ca.gov/fish/pdf/fishsampling121406.pdf>). Samples collected 212 d posttreatment were composited for common location and species. Collection yielded two rainbow trout from Coot Bay, one bullhead from site 10, four bullhead and two trout from site 6, and one trout and four bullhead from site 8. The fish were dissected, and skin-off fillets were analyzed for all five formulation constituents.

Chemical extraction and analysis

Chemicals. Rotenone (CAS no. 83-79-4) was purchased from ChemService; 1-MP (CAS no. 872-50-4) and DEGEE (CAS no. 111-90-0) were purchased from Sigma-Aldrich. Rotenolone from a degraded rotenone standard was used because no commercial sources are available. A synthesized rotenolone standard (University of California, Berkeley) was used to validate the rotenolone fraction in the degraded standard. Fennefede 99 was provided by the manufacturer of Legumine (Prentiss). The internal standards, *N*-methyl-2-pyrrolidinone-d9 (CAS no. 185964-60-7) and 1-monolinurin-2 (CAS no. 1746-81-2), were purchased from Cambridge Isotopes and Sigma-Aldrich, respectively. The surrogates, diethylene glycol monomethyl ether kit (P/N PS-160C-SET) and 5-methyl-2-pyrrolidinone (CAS no. 108-27-0) were purchased from AccuStandard and Sigma-Aldrich, respectively. The

surrogate compounds were used to account for analyte loss during extraction. Pesticide-grade acetonitrile, methanol, high-performance liquid chromatography-grade water, and formic acid were purchased from VWR.

Water. At room temperature, each water sample was brought to a 10% methanol concentration (v/v), surrogate was added, and each sample was vortexed and filtered (0.45 μm) into a vial (2 ml). Internal standard (10 μl) was added to each vial and vortexed before chemical analysis by direct-injection liquid chromatography–mass spectrometry (LC–MS) or LC–MS–MS analysis. If matrix interferences prohibited direct injection, solid-phase extraction was used as a cleanup procedure to remove interference. Briefly, prefiltered water samples (200 ml) were loaded on a preconditioned C_{18} solid-phase extraction cartridge (500 mg, 6 ml) at a flow rate of 5 ml/min using a vacuum manifold (Resprep; Restek Corp.), not exceeding 20 psi. Dried cartridges were eluted with methanol (2 ml) and filtered (0.45 μm) before internal standard addition (10 μl) and analysis.

Rotenone and rotenolone were analyzed by using LC–MS and LC–MS–MS, whereas MP, DEGEE, and Fenmedefo 99 analyses used only LC–MS [3]. Chemical analysis was performed with an Agilent 1100 high-performance liquid chromatography coupled to either a single-quadrapole (MS) or triple-quadrapole (MS–MS) mass spectrometer using atmospheric pressure electrospray ionization in positive mode. For the LC–MS analysis of rotenone and rotenolone, a ZORBAX Eclipse XDB- C_8 analytical column (150 \times 4.6 mm inside diameter, 5 μm ; Agilent) was used, with a flow rate of 0.5 ml/min, a

column temperature of 40°C, and a 60- μl injection volume. The solvent gradient was as follows: 0 to 8 min, 25:75 acetonitrile (0.1% formic acid):water (0.1% formic acid); 8 to 20 min, 75:25 acetonitrile (0.1% formic acid):water (0.1% formic acid); and 20 min, 25:75 acetonitrile (0.1% formic acid):water (0.1% formic acid). Selected ion monitoring was used for rotenone (m/z 395) and rotenolone (m/z 393) quantification by LC–MS. Ionization source parameters were optimized for each compound.

For analysis of rotenone and rotenolone by LC–MS–MS, the chromatographic column and flow rate were identical, but the following isocratic gradient was used instead: 75:25 acetonitrile (0.1% formic acid):water (0.1% formic acid), a column temperature of 40°C, and a 20- μl injection volume. Analytes were quantified in multiple reaction monitoring mode using the following mass transitions: m/z 395.5 \rightarrow 213.1, 203 for rotenone, and m/z 393.1 \rightarrow 365.1, 335.1 for rotenolone. Ionization source parameters and collision cell energies and potentials were optimized for each compound.

Analysis of MP and DEGEE was performed via LC–MS with a Waters Atlantis d C_{18} analytical column (100 \times 2.1 mm inside diameter, 3 μm), a flow rate of 0.190 ml/min, a column temperature of 38°C, and a 20- μl injection volume. The solvent gradient was as follows: 0 to 10 min, 2.5:97.5 acetonitrile (0.1% formic acid):water (0.1% formic acid); 10 to 10.5 min, 10:90 acetonitrile (0.1% formic acid):water (0.1% formic acid); and 10.5 min, 2.5:97.5 acetonitrile (0.1% formic acid):water (0.1% formic acid). Selected ion monitoring was used for MP (m/z 100 and m/z 122) and DEGEE (m/z 135 and m/z 157) quantification

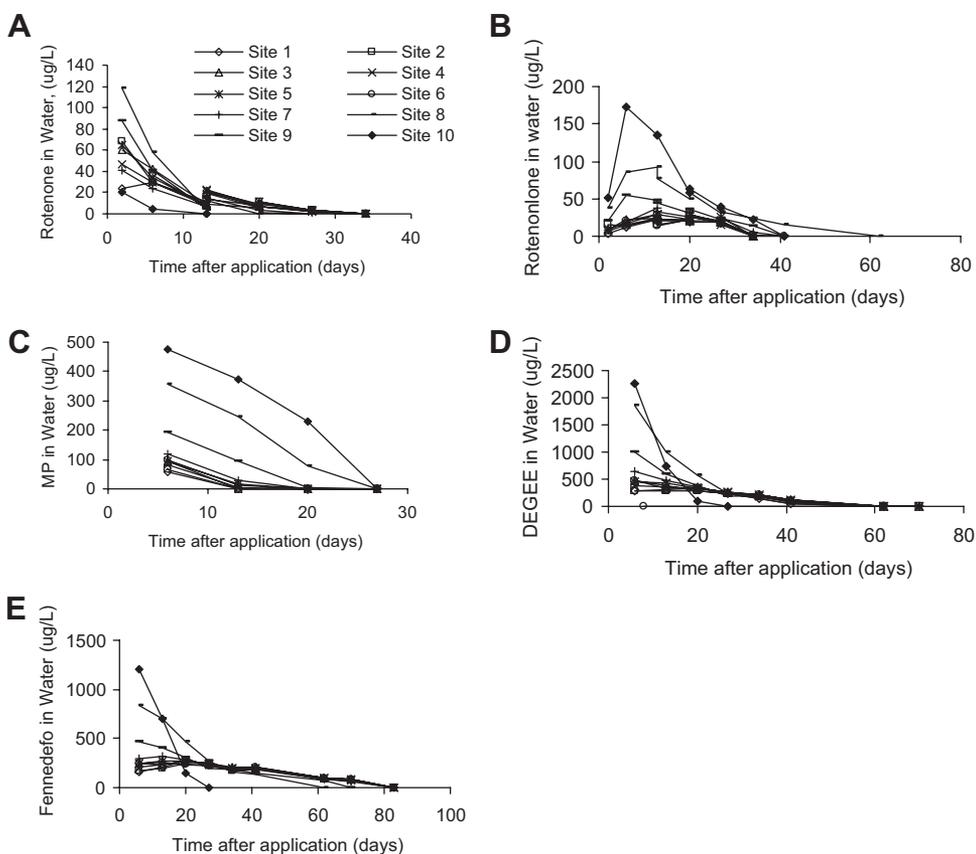


Fig. 2. Dissipation profiles of rotenone (A), rotenolone (B), methyl pyrrolidone (MP) (C), diethylene glycol monethyl ether (DEGEE) (D), and Fenmedefo (E) in water following treatment of Lake Davis with CFT Legumine. Points represent means ($n = 3$ at odd-numbered sites; $n = 2$ at even-numbered sites), and standard deviations are not shown for clarity (see Supplemental Data).

by LC–MS. Ionization source parameters were optimized for each compound.

Fennedefo 99 was also analyzed via LC–MS using a ZORBAX Eclipse XDB-C₈ analytical column (150 × 4.6 mm inside diameter, 5 μm; Agilent), with a flow rate of 0.5 ml/min, column temperature of 40°C, and 80-μl injection volume. The solvent gradient was as follows: 0 to 10 min, 12:88 acetonitrile (0.1% formic acid):water (0.1% formic acid); 10 to 20 min, 80:20 acetonitrile (0.1% formic acid):water (0.1% formic acid); and 20 min, 12:88 acetonitrile (0.1% formic acid):water (0.1% formic acid). Fennedefo 99 is itself a complex formulation that includes a number of polyethylene glycols, so the tetra- through decaethylene glycols were quantified individually and then summed to report Fennedefo 99 concentrations. Selected ions monitored for each glycol ether, corresponding to the [M + H]⁺, [M + Na]⁺, and [M + NH₄]⁺ ions were as follows: tetraethylene glycol (*m/z* 195.2, 217.3, 212.2), pentaethylene glycol (*m/z* 239.3, 261.2, 256.2), hexaethylene glycol (*m/z* 283.3, 305.2, 300.3), heptaethylene glycol (*m/z* 327.5, 349.5, 344.5), octaethylene glycol (*m/z* 371.5 [393.5, 437.5; 388.5, 432.5]), nonaethylene glycol (*m/z* 371.5, 415.5 [393.5, 437.5; 388.5, 432.5]), and decaethylene glycol (*m/z* 459.5, 481.5, 467.5).

The limit of quantification (LOQ) was 2 μg/L, and the method detection limit (MDL) was 1 μg/L rotenone and rotenolone in water. The LOQ was 5 μg/L for MP and DEGEE in water and 50 μg/L for Fennedefo 99. The MDL for MP and DEGEE was 2 μg/L and 20 μg/L for Fennedefo 99. Average

recoveries for all analytes ranged from 79 to 116% (SD ≤ 10% for each individual analyte). Volatile and semivolatile compounds were analyzed in water over the course of treatment but have already been reported [2]. Water quality parameters were measured before and after the treatment and were reported previously [2].

Sediment. Sediment samples (10 g dry wt) were extracted using accelerated solvent extraction with acetonitrile:methylene chloride (50:50 [v/v]; 1,500 psi at 100°C for 5 min and 60 ml flush volume). Sediment acetonitrile:methylene chloride extracts were loaded onto a preconditioned C₁₈ column, and analytes were eluted from the column with methanol (2 ml), as described for the cleanup procedure used for water samples. Rotenone, rotenolone, MP, DEGEE, and Fennedefo 99 were analyzed in sediments by using the LC–MS method as described for the water samples [3]. The LOQs (dry wt) for both rotenone and rotenolone were 10 ng/g, 5 ng/g for MP and DEGEE, and 50 ng/g for Fennedefo 99. Average recoveries for all analytes ranged from 83 to 91% (SD < 4% for each individual analyte). Volatile and semivolatile compounds were analyzed in sediments over the course of treatment but have been reported elsewhere [2].

Tissues. Tissue samples (10 g) were extracted and cleaned using the sediment extraction procedure described previously. Tissue extracts were then filtered before analysis by LC–MS according to the same method used for water analysis. The LOQ (fresh wt) was 10.0 ng/g in tissue for all compounds except for Fennedefo 99 (100 ng/g), and the MDL (fresh wt) was 5.00 ng/g

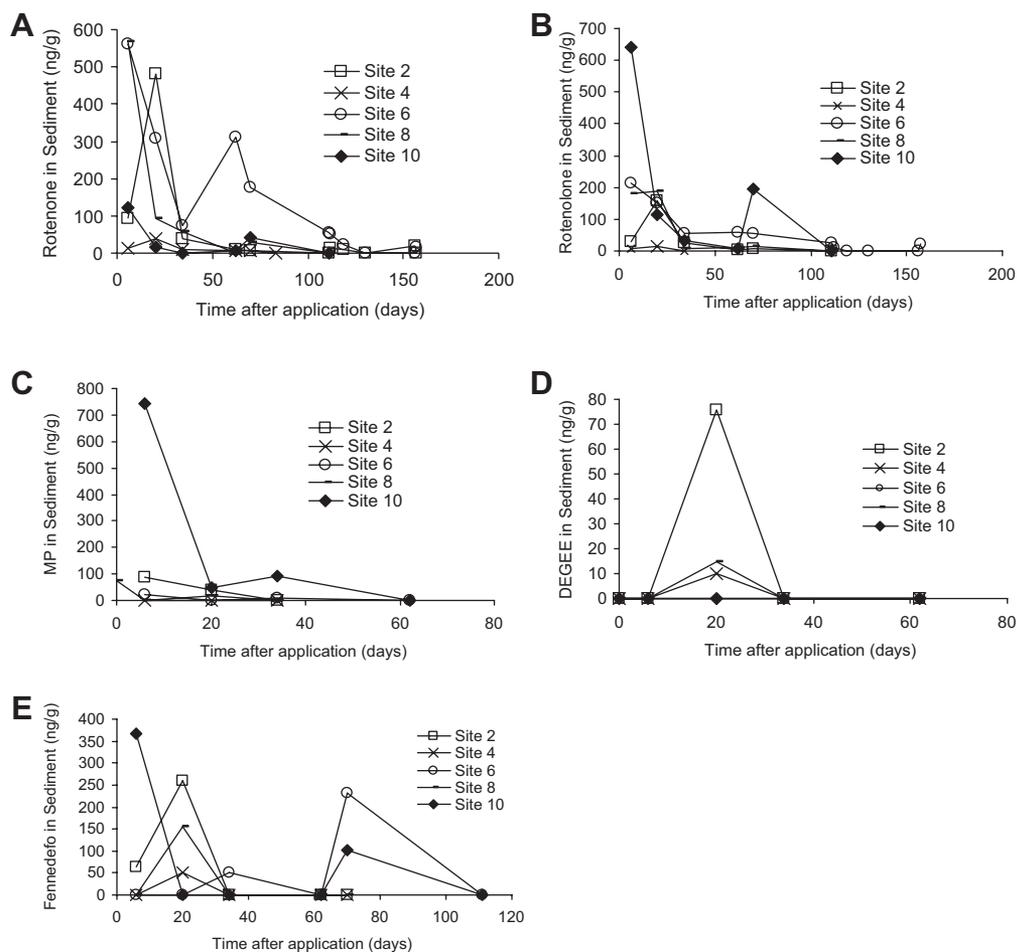


Fig. 3. Dissipation profiles of rotenone (A), rotenolone (B), methyl pyrrolidone (MP) (C), diethylene glycol monethyl ether (DEGEE) (D), and Fennedefo (E) in sediments following treatment of Lake Davis with CFT Legumine. Points represent individual sediment sample concentrations ($n = 1$).

for all compounds except for Fennedefo 99 (50.0 ng/g). Average recoveries for all analytes ranged from 77 to 92% (SD < 30% for each individual analyte).

Data analysis

Dissipation rate constants were calculated based on pseudo first-order kinetics in which the rate constant (k) is calculated from the equation

$$C_t = C_o e^{-kt} \quad (1)$$

where C_o represents the initial analyte concentration (ppb), C_t is the concentration (ppb) at time t (day), and k (day^{-1}) is the first-order dissipation rate constant. The half-life ($t_{1/2}$) is calculated according to the equation

$$t_{1/2} = -(\ln 2)/k \quad (2)$$

Dissipation kinetics were modeled for sites 6, 8, and 10 because these sites were represented with all three sample matrices: water, sediment, and tissue. The dissipation kinetics of all five constituents were also described for the lake overall, using all data collected to represent each matrix over time after application.

For statistical purposes, sample values reported below the LOQ were taken as one-half the LOQ unless the MDL was determined. Lake and site average sample concentrations represent the average concentrations in a particular matrix at all sites and depths available.

RESULTS

Pretreatment and target concentrations

All pretreatment samples (water, sediment, and tissue) had undetectable levels of rotenone, rotenolone, MP, DEGEE, and Fennedefo 99, except for the detection of MP (72.9 ng/g) in sediment at site 8. The pretreatment MP detection was considered an artifact. The target concentration of CFT Legumine for treatment of the lake and tributaries was 1 ppm, or 50 $\mu\text{g/L}$, of rotenone. Results of water sampling, 2 d posttreatment, indicated that the maximum lake average for rotenone was $58.4 \pm 30.6 \mu\text{g/L}$; thus, the target was achieved.

Posttreatment

Average water, sediment, and tissue concentrations at various days posttreatment are shown by sampling site in Figures 2, 3, and 4, respectively. The dissipation profile of

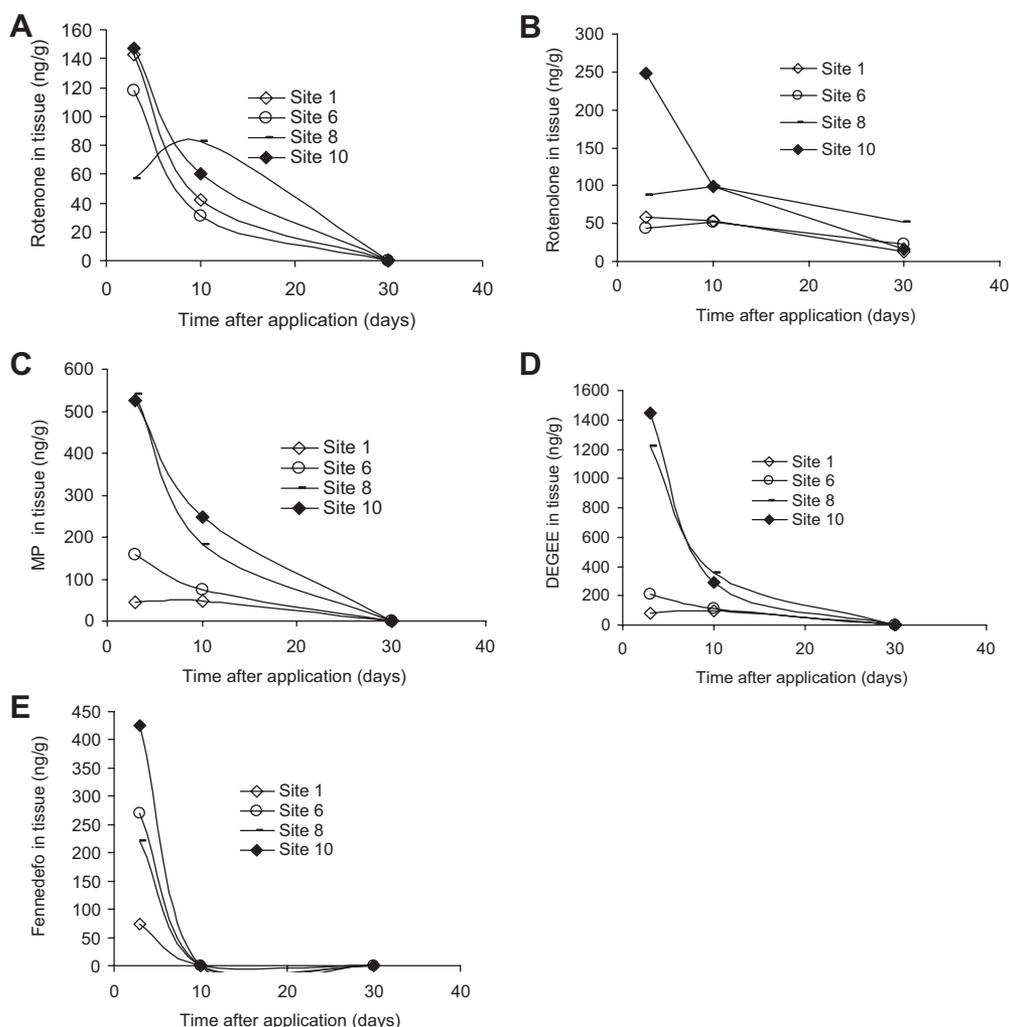


Fig. 4. Dissipation profiles of rotenone (A), rotenolone (B), methyl pyrrolidone (MP) (C), diethylene glycol monethyl ether (DEGEE) (D), and Fennedefo (E) in bullhead tissues 3, 10, and 30 d after treatment of Lake Davis with CFT Legumine. Points represent mean/average, where $n = 4$ on day 3 for all sites, $n = 2$ on day 10 for all sites, and $n = 1$ on day 30 for site 1 ($n = 2$ for other sites). Standard deviations not shown for clarity (see Supplemental Data).

each constituent followed a first-order kinetic model, which was used to calculate pseudo first-order rate constants and half-lives with each matrix (Table 1) for the lake (Fig. 5) and sites 6, 8, and 10 (Fig. 6).

Water. Dissipation profiles of the Legumine formulation constituents in Lake Davis water are shown in Figure 3. A maximum aqueous rotenone concentration of $111 \pm 42 \mu\text{g/L}$ was attained 2 d posttreatment at site 8, and the maximum rotenolone concentration was $174 \pm 4 \mu\text{g/L}$ attained 6 d posttreatment at site 10. The maximum average lake concentration of rotenone was $58.4 \pm 30.6 \mu\text{g/L}$ attained 2 d posttreatment, while the maximum average rotenolone concentration was $39.4 \pm 46.0 \mu\text{g/L}$ attained 6 d posttreatment (Fig. 5). The overall $t_{1/2}$ in lake water was 5.6 d for rotenone and 11.1 d for rotenolone (Table 1), and both rotenone and rotenolone dissipated to below analytical reporting limits ($2 \mu\text{g/L}$) by 34 and 62 d posttreatment, respectively (online supporting information).

Maximum aqueous concentrations (Fig. 2) of MP ($474 \pm 52 \mu\text{g/L}$), DEGEE ($2,260 \pm 283 \mu\text{g/L}$), and Fenedefo 99 ($1,200 \pm 0 \mu\text{g/L}$) were detected at site 10, 6 d posttreatment. The maximum average lake concentrations of MP, DEGEE, and Fenedefo 99 were $156 \pm 127 \mu\text{g/L}$, $779 \pm 632 \mu\text{g/L}$, and $389 \pm 310 \mu\text{g/L}$ at 6 d posttreatment, respectively. The $t_{1/2}$ of MP in water was 4.6 d, whereas it was 7.7 d for DEGEE and 13.5 d for Fenedefo 99 (Table 1). Concentrations of MP, DEGEE, and Fenedefo 99 dissipated to below reporting limits (5, 5, and $50 \mu\text{g/L}$, respectively) at all sites within 34, 70, and 83 d, respectively (see Supplemental Data). The CFT Legumine

constituent ranking for persistence in water would be as follows: Fenedefo 99 > rotenolone > DEGEE > rotenone > MP.

Sediments. Rotenone and rotenolone reached maximum sediment concentrations of 568 ng/g (site 8) and 640 ng/g (site 10), respectively, 6 d posttreatment (Fig. 3). The lake average maximum rotenone and rotenolone sediment concentrations of 272 ng/g and 266 ng/g, respectively, were attained 6 d posttreatment (Fig. 5). The average $t_{1/2}$ values for rotenone and rotenolone in sediment were 31.1 d and 31.8 d, respectively (Table 1). At 157 d posttreatment only two sites were sampled for rotenone and rotenolone: site 2 was below the reporting limit of 10 ng/g for both analytes, and site 6 had concentrations just above 10 ng/g (15.6 and 20.8 ng/g, respectively).

The maximum sediment concentrations of MP and Fenedefo 99 (742 and 367 ng/g, respectively; Fig. 3) were at site 10, 6 d posttreatment, whereas DEGEE levels reached a maximum of 75.7 ng/g in sediment at site 2, 20 d posttreatment (Fig. 3). Maximum lake average sediment concentrations of MP reached 172 ng/g, 6 d posttreatment (Fig. 5), whereas those of DEGEE (21.1 ng/g) and Fenedefo 99 (94.3 ng/g) were attained at 20 d posttreatment (Fig. 5). It should be noted that both rotenone and DEGEE were elevated in sediment at site 2, 20 d following application, without a clear correlation to the corresponding water concentrations (Fig. 3). It is difficult to determine whether these points are true outliers because the sediment concentrations were based on only one replicate sample.

The $t_{1/2}$ values for MP and Fenedefo 99 in sediment were 10 and 48.5 d, respectively (Table 1). The $t_{1/2}$ for DEGEE in lake

Table 1. Pseudo first-order dissipation kinetics (half-life in days [$t_{1/2}$], rate constant in days⁻¹ [k], and correlation coefficient [r^2]) of rotenone, rotenolone, methyl pyrrolidone (MP), diethylene glycol monethyl ether (DEGEE), and Fenedefo following CFT Legumine treatment of Lake Davis, California, USA

Matrix		Rotenone	Rotenolone	MP	DEGEE	Fenedefo
Site 6 water	$t_{1/2}$	5.9	7.7	2.6	8.6	27.3
	k	-0.1169	-0.0895	-0.2631	-0.0808	-0.0254
	r^2	0.954	0.7233	0.9926	0.9128	0.823
Sediment	$t_{1/2}$	21.6	21.1	24.7	NA	NA
	k	-0.0321	-0.0329	-0.0281	NA	NA
	r^2	0.6124	0.7251	0.4484	NA	NA
Tissue	$t_{1/2}$	8.8	24.4	4.4	4.1	10.1
	k	-0.079	-0.0284	-0.1571	-0.1705	-0.0686
	r^2	0.8813	0.8087	0.9935	0.9845	0.4907
Site 8 water	$t_{1/2}$	4.3	8.7	3.1	6.0	10.9
	k	-0.1623	-0.0799	-0.2268	-0.1164	-0.0637
	r^2	0.8958	0.5659	0.8376	0.9815	0.9865
Sediment	$t_{1/2}$	16.7	20.4	NA	NA	NA
	k	-0.0415	-0.034	NA	NA	NA
	r^2	0.7671	0.6581	NA	NA	NA
Tissue	$t_{1/2}$	5.4	30.1	3.4	3	11
	k	-0.1289	-0.023	-0.2024	-0.2333	-0.063
	r^2	0.8832	0.826	0.9965	0.9961	0.4907
Site 10 water	$t_{1/2}$	2.6	7.3	3.4	1.9	2.1
	k	-0.2699	-0.0955	-0.175	-0.3213	-0.1844
	r^2	0.9773	0.6445	0.8071	0.9403	0.9542
Sediment	$t_{1/2}$	38.1	19.3	7.5	NA	NA
	k	-0.0182	-0.0359	-0.0921	NA	NA
	r^2	0.286	0.5379	0.8774	NA	NA
Tissue	$t_{1/2}$	4.5	7.0	3.4	2.9	8.5
	k	-0.1526	-0.0997	-0.205	-0.2362	-0.0818
	r^2	0.9984	0.9938	0.9851	0.9999	0.4907
Lake average water	$t_{1/2}$	5.6	11.1	4.6	7.7	13.5
	k	-0.1247	-0.0623	-0.1498	-0.0903	-0.0513
	r^2	0.9959	0.7818	0.9933	0.9626	0.7745
Sediment	$t_{1/2}$	31.1	31.8	10.0	—	48.5
	k	-0.0223	-0.0218	-0.0693	—	-0.0143
	r^2	0.6588	0.6631	0.9225	—	0.3774
Tissue	$t_{1/2}$	6.1	12.7	3.7	3.2	10.4
	k	-0.1142	-0.0544	-0.185	-0.2143	-0.0669
	r^2	0.9996	0.9982	0.9881	0.9967	0.5171

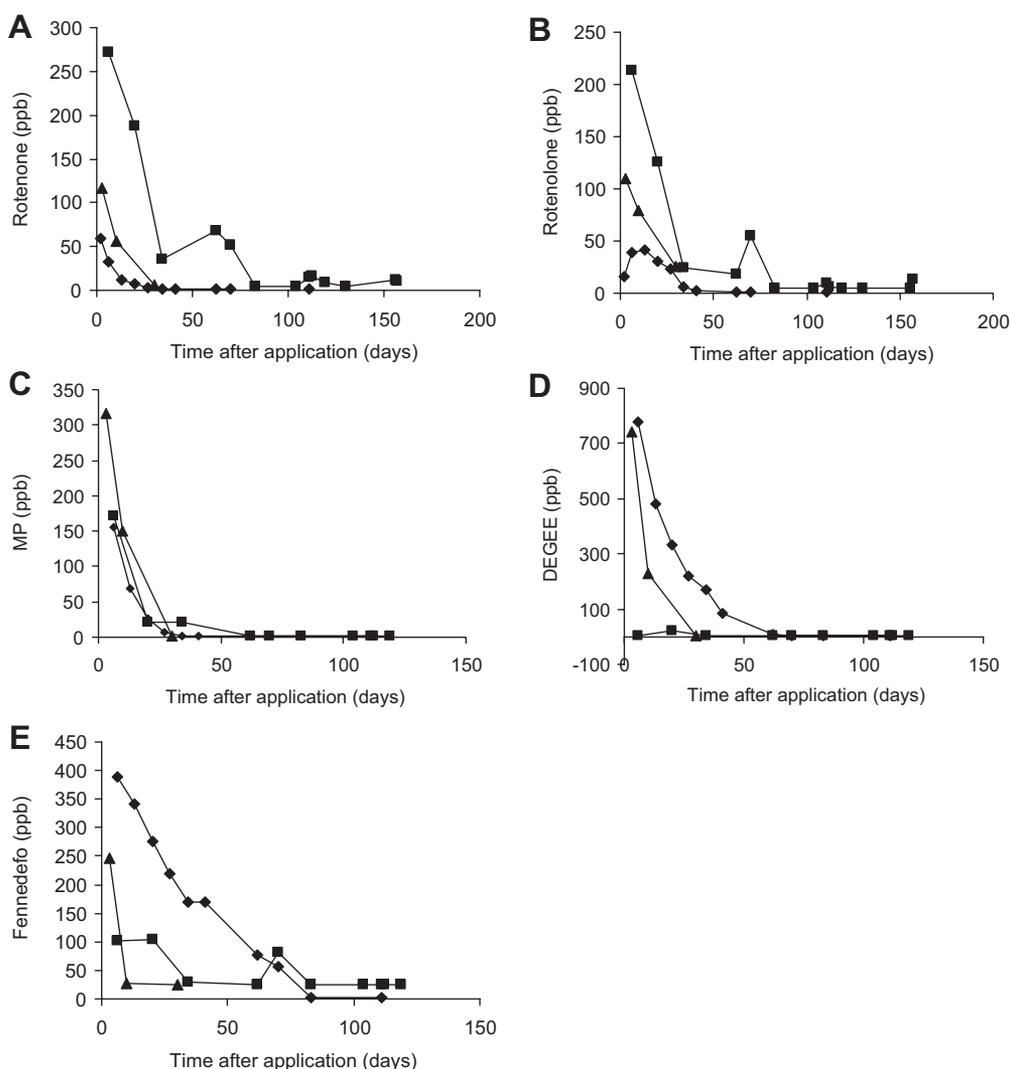


Fig. 5. Whole lake averages (all sites) for water (◆), sediment (■), and tissue (▲) concentrations of rotenone (A), rotenolone (B), methyl pyrrolidone (MP) (C), diethylene glycol monethyl ether (DEGEE) (D), and Fennedefo (E) following CFT Legumine treatment. Points represent the mean site concentrations for water ($n = 10$), sediment ($n = 5$), and tissue ($n = 4$) at each time point. Standard deviations not shown for clarity (see Supplemental Data).

sediment could not be calculated because DEGEE was detected during only one sampling event 20 d posttreatment. Concentrations of MP, DEGEE, and Fennedefo 99 dissipated to below reporting limits at all sites (5, 5, and 50 ng/g) within 62, 34, and 111 d posttreatment, respectively. In sediment, comparative chemical persistence was as follows: Fennedefo 99 > rotenolone > MP > DEGEE.

Tissues. Maximum rotenone and rotenolone tissue concentrations (fresh wt) of 147 ± 99 ng/g and 248 ± 23 ng/g, respectively, were found at site 10, 3 d posttreatment (Fig. 4). Lake average rotenone tissue concentrations in bullhead reached undetectable levels by 30 d posttreatment in all sites sampled except for site 6, which had a 30 d posttreatment average tissue concentration of 11.3 ± 27.9 ng/g (fresh wt; Fig. 5). Although the average value is above the reporting limit (10 ng/g), two of the four replicates were above the reporting limit (20.4, 19.7 ng/g) and two were undetectable (<5.00 ng/g).

The average rotenone $t_{1/2}$ in bullhead tissue was 6.1 d for all sites and 12.7 d for rotenolone (Table 1). Quantifiable levels of rotenone remained in tissue 30 d posttreatment at all sites (Fig. 5). Neither rotenone nor rotenolone was detected in either rainbow trout or bullhead (composite) tissue 212 d posttreatment at any site.

A maximum concentration of MP (540 ± 48 ng/g) was attained at site 8, 3 d posttreatment (Fig. 4), whereas maximal DEGEE ($1,450 \pm 70$ ng/g) and Fennedefo 99 (425 ± 280 ng/g) concentrations were reached at site 10, 3 d posttreatment (Fig. 4). The maximum lake average concentrations of MP (317 ± 230 ng/g), DEGEE (742 ± 622 ng/g), and Fennedefo 99 (247 ± 190 ng/g) were attained 3 d posttreatment (Fig. 5).

The respective $t_{1/2}$ values for MP, DEGEE, and Fennedefo 99 in bullhead tissue were 3.7, 3.2, and 10.4 d, respectively (Table 1). Concentrations of MP and DEGEE decreased to below detection limits (5.00 ng/g for both) within 30 d posttreatment, and Fennedefo 99 decreased to below detection limits (25 ng/g) within 10 d posttreatment (see Supplemental Data). Persistence in tissue compared as follows: rotenolone > Fennedefo 99 > rotenone > MP > DEGEE.

DISCUSSION

Rotenone and rotenolone

The physicochemical properties of a compound can predict how a chemical will partition and dissipate in the environment [4]. The properties of the five major constituents in the CFT Legumine formulation are presented in Table 2. The low water

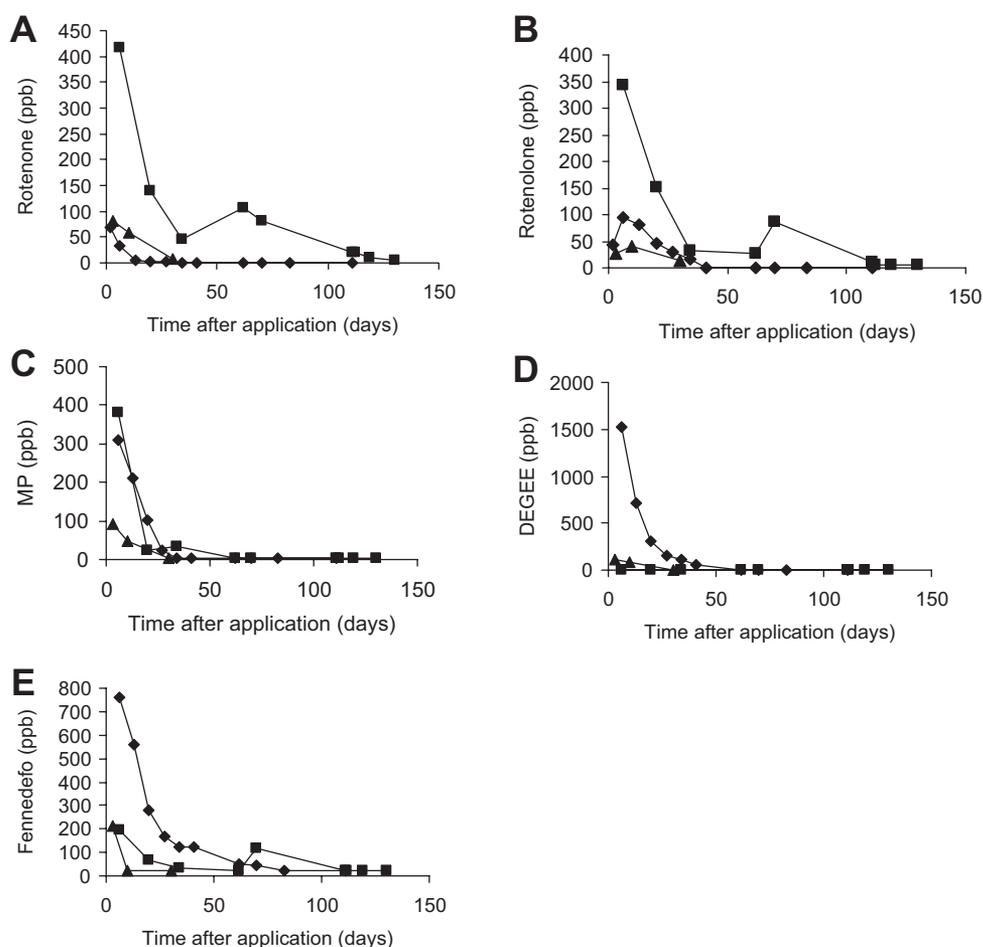


Fig. 6. Sites 6, 8, and 10 average water (◆), sediment (■), and tissue (▲) concentrations of rotenone (A), rotenolone (B), methyl pyrrolidone (MP) (C), diethylene glycol monethyl ether (DEGEE) (D), and Fenmedefo (E) in Lake Davis following CFT Legumine treatment. Points represent the mean of sites 6, 8, and 10 ($n = 3$). Standard deviations not shown for clarity (see Supplemental Data).

solubility (0.296 mg/L) [5], low vapor pressure (6×10^{-6} Pa) [5], and large organic carbon partitioning coefficient value ($\log K_{OC}$ 3.60) [6] of rotenone suggest that it would partition from water to sediments more than other formula constituents.

Rotenone's large octanol–water partition coefficient ($\log K_{OW} = 4.10$) [7] suggests that it would bioconcentrate in fish if water concentrations remained elevated for an extended period, which they did not. However, rotenone is metabolized and eliminated

Table 2. Physical and chemical properties of the CFT Legumine constituents subject to monitoring

Chemical	MW (g/mol)	Boiling point (°C)	Water solubility (mg/L at 25°C)	Vapor pressure (torr at 25°C)	Henry's Law constant (atm \times m ³ /mol)	Log K_{OW}	Log K_{OC}
Rotenone	394.4	157–175 at 0.5 mmHg [13]	0.296 [5]	6×10^{-6} Pa (at 25°C) [5]	1.1×10^{-13} [14]	4.10 [7]	3.60 [15]
Rotenolone	412.42	NA	NA	NA	NA	NA	NA
Methyl pyrrolidone	99.13	202 [16]	Miscible [17]	0.345 mmHg at 25°C [18]	4.46×10^{-8} [15]	-0.54 [7]	1.23 [19]
Diethylene glycol monethyl ether	134.2	196 [20]	Miscible [21]	0.13 [18]	2.2×10^{-8} [6]	-0.54 [7]	1.08 [6]
Fenmedefo 99							
Triethylene glycol	150.2	285 [20]	Miscible [22]	1.32×10^{-3} mmHg at 25°C (est) [18]	3.2×10^{-11} [14]	1.98 [23]	1.00 [19]
Tetraethylene glycol	194.2	327 [21]	Miscible [24]	4.65×10^{-5} mmHg at 26°C [25] ^a	5.5×10^{-19} [14]	-2.02	1.00 [19]
Pentaethylene glycol	238.3	338–340 at 2 mmHg [26]	NA	NA	NA	NA	NA
Hexaethylene glycol	282.3	217 at 4 mmHg [27]	NA	NA	NA	NA	NA
Heptaethylene glycol	326.5	NA	NA	NA	NA	NA	NA
Octaethylene glycol	370.5	NA	NA	NA	NA	NA	NA
Nonaethylene glycol	370.5, 414.5	NA	NA	NA	NA	NA	NA
Decaethylene glycol	458.5	NA	NA	NA	NA	NA	NA

^a See also <http://www.epa.gov/oppt/exposure/pubs/episuitedl.htm>.

by fish, leading to relatively low bioconcentration factors of 10.8 for vicera to 27.9 for the head during the equilibrium phase [8]. Retained residues were probably also transformed to rotenolone and then eliminated. There was no evidence of prolonged bioaccumulation of either rotenone or rotenolone in fish tissues as a result of the treatment; water concentrations rapidly declined, limiting the opportunity for uptake.

Although rotenolone is more water soluble than rotenone (Table 2), it was still predisposed to sediment sorption (Fig. 6), because average rotenolone concentrations were observed to be greater in the sediments compared with tissues and water from sites 6, 8, and 10. Rotenone behaved as would be predicted, showing the lowest concentrations in water and tissues compared with sediment at sites 6, 8, and 10 (Fig. 6). Sediment residues are subject to biotic (anaerobic microbial) and abiotic degradation processes over time, which ultimately contribute to complete degradation [4]; rotenone has been previously shown to degrade in bottom sediments [9]. The $t_{1/2}$ of rotenone in water during the 1997 treatment was 7.7 d [10], similar to but slightly longer than the 2007 treatment of 5.6 d. For California impounded waters, the estimated $t_{1/2}$ for rotenone varies from 0.65 to 7.7 d and generally is inversely related to temperature [11]. Photolytic degradation of rotenone in the light-penetration zone of surface waters might also have contributed to the overall dissipation of rotenone after treatment, because rotenone is known to photodegrade [12]. Sediment residues persisted for approximately six months in both the 1997 [10] and the 2007 treatments, despite the use of different rotenone formulations.

Methyl pyrrolidone, DEGEE, and Fenmedefo 99

Both DEGEE and Fenmedefo 99 showed greater average water concentrations at sites 6, 8, and 10 compared with sediments and tissues posttreatment. However, MP possessed a higher sediment concentration 3 d posttreatment compared with water despite its miscibility (Table 2 and Fig. 6). Based on the relatively high water solubility values of these three compounds, compared with rotenone (Table 2), they would be expected to remain more readily in aqueous solution. Estimated $\log K_{OC}$ values for all three constituents range from 1.00 to 1.23 (Table 2), suggesting very low potential for sediment sorption. Although Fenmedefo 99 (at 48.5 d) possessed a longer $t_{1/2}$ in sediments than either rotenone (31.1 d) or rotenolone (31.8 d), the poor correlation for the rate constant, and the fact that it dropped below reporting limits before either rotenone or rotenolone, suggests that it was less persistent in sediments. Also, because of their relatively high water solubility values, MP, DEGEE, and Fenmedefo 99 accumulation by fish was not expected; any residues absorbed would be quickly depurated. This was indeed observed in this study. The relatively small estimated $\log K_{OW}$ values (Table 2) also suggest low bioconcentration potential compared with rotenone. Likewise, low Henry's law constants (Table 2) for MP, DEGEE, and the polyethylene glycols of the Fenmedefo 99 formulation also suggest that they would not readily volatilize from the lake, because all the values are less than $3 \times 10^{-7} \text{ atm} \times \text{m}^3/\text{mol}$ [4].

CONCLUSIONS

Concentrations of all five CFT Legumine constituents rapidly declined in Lake Davis water following the 2 d treatment period, with both sediment and fish tissue concentrations following suit in similar fashion. The more water-soluble agents, MP, DEGEE, and Fenmedefo 99, most likely degraded in place via microbes or sunlight because their physicochemical proper-

ties would have limited volatilization, sorption, and bioaccumulation. The less water-soluble constituents, rotenone and rotenolone, though also not particularly volatile, would have been more prone to sorption and bioaccumulation. However, with their water concentrations in rapid decline following the 2-d treatment, also most likely because of degradation, sorbed and accumulated residues again followed suit. Both rotenone and rotenolone are known to be readily degraded by both microbes and fish. Ultimately, the result was that no trace of the formulation remained by the final sampling event, 212 d posttreatment. Thus, the chemical constituents behaved as would be predicted from their physicochemical properties, and, most likely as a result of various degradation processes, all formulation constituents ultimately dissipated from the lake in a timely fashion.

SUPPLEMENTAL DATA

Tables S1–S3.

Supplemental Data References. (275 KB DOC).

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