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Bioconcentration and Toxicity of Dodecylbenzene Sulfonate (C_{12}LAS) to Aquatic Organisms Exposed in Experimental Streams

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Abstract. Fish, mollusks, and crustaceans were caged in the tail pool of streams during a C_{12}LAS (dodecyl benzene sulfonate) model ecosystem experimental program. Bioconcentration of total C_{12}LAS and individual isomers and acute and chronic toxicity were investigated during this study. Toxicity endpoints were based on water and tissue (i.e., body burden) concentrations at which adverse effects were observed. At 32 days, total C_{12}LAS bioconcentration factors (BCFs) for the fathead minnow and three invertebrate species ranged from 9 to 116. In general, bioconcentration was affected by isomer position, exposure concentration, and species. BCF values tended to decrease as isomer position moved from external (e.g., 2-phenyl) to internal (e.g., 5,6-phenyl). BCFs also decreased as exposure concentration increased. Mean acute 4-d LC_{50} values ranged from 1.5 to >3.0 mg/L for the six species tested. Lethal body burdens associated with 50% mortality (LBB_{50}) varied from 0.21 to 0.60 mmole/kg (wet weight). During the 32-day chronic exposures, the EC_{20} values were 0.27 (0.204–0.352), 0.95 (0.597–1.29), and approximately 1.0 mg/L for *Corbicula* (length), *Hyalella* (survival), and fathead minnow (survival), respectively. At these EC_{20} values, C_{12}LAS body burdens were 0.035, 0.23, and 0.19 mmoles/kg wet weight in *Corbicula, Hyalella,* and fathead minnow, respectively. Fish exposed to wastewater treatment plant effluent had total C_{12}LAS tissue concentrations ranging from 0.0005 to 0.0039 mmoles/kg wet weight. These concentrations are approximately 45–360 times below the tissue concentration associated with subtle effects in the model ecosystem stream exposures. Total C_{12}LAS body burdens in feral and caged *Corbicula* exposed to WWTP effluents were approximately 0.0013 mmoles/kg; approximately 25-fold below concentrations associated with effects in stream exposures.

Linear alkylbenzene sulfonate (LAS) is an anionic surfactant used in a variety of detergent applications due to its cleaning efficiency, solubility, foaming properties, relative hardness insensitivity, and low cost (Rosen 1989). The LAS used in detergents typically contains a mixture of alkyl chain lengths ranging from decyl to tetradecyl with the phenyl ring attached at any non-terminal carbon. Due to its facile biodegradation and sorption characteristics, it is well removed during wastewater treatment. Effluent monitoring combined with dilution modeling predicts ninetieth percentile river water concentrations of 3–10 μg/L in Dutch surface waters, 1000 m below municipal effluents (Feijtel *et al.* 1999) and 3.7–185 μg/L in the United States, under mean and low flow conditions, respectively (McAvoy *et al.* 1998). LAS has been measured at 2–10 μg/L in an impacted estuarine system (Scheldt estuary, The Netherlands), and below the detection limit of 0.5 μg/L, 15 km offshore (Stalmans *et al.* 1991). LAS's environmental fate and effects have been studied and the risk posed by these compounds assessed in the freshwater, marine, and terrestrial compartments (Fendinger *et al.* 1994; van de Plassche *et al.* 1999; Versteeg *et al.* 1999; Jensen *et al.* 2001; Temara *et al.* 2001). The continued reevaluation of this mixture of compounds is appropriate for several reasons including the volume of LAS used and disposed and the continued progress in the fields of environmental fate, effects, and risk assessment.

Risk assessment schemes world-wide assume a connectivity between laboratory studies and field effects despite the general lack of information directly comparing effects in these two systems. The use of controlled model ecosystems provide an opportunity to link effects observed in the laboratory with the field. In this study, we have linked data from laboratory toxicity tests, in situ (i.e., cages in artificial streams) exposures, a model ecosystem study (Belanger *et al.* 2002), and biological monitoring of caged and feral organisms in the environment in an attempt to evaluate the importance of each piece of information. Single species toxicity data are evaluated using cumulative single species approach allowing probabilistic statements about the percentage of species potentially affected at any exposure concentration (Versteeg *et al.* 1999; Aldenberg and Jaworska 2000). Naturally exposed organisms are linked into the risk assessment via body burdens, a relatively new toxicological paradigm which asserts that tissue concentrations at which effects occur are constant for a given mode of action (McCarty and Mackay 1993; Fisher *et al.* 1999). While this approach can be criticized due to high variability in effective

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body burdens (Barron *et al.* 2001), it offers the potential to base risk on tissue burdens of contaminants, allowing advances in mixture assessments and in validating risk assessment predictions in the environment. This linkage of laboratory, model ecosystem, and field data provides information on the relative value of data from these sources in compiling an effects assessment. Our purpose was to better understand the bioconcentration, toxicity, and body burden levels at which effects occur for C_{12}LAS in traditional laboratory species. Water column concentrations and body burdens associated with adverse effects were compared with impacts on model ecosystem communities (Belanger *et al.* 2002) to better understand the utility of single species toxicity tests and body burden measurements in ecological risk assessments.

Methods and Materials

Test Material

The C_{12}LAS mixed isomer sample (CAS Number 25155-30-0, Condea Vista, Austin TX, USA) used for the Experimental Stream (ESF) and analytical method validation contained five positional isomers: 2-phenyl = 35%, 3-phenyl = 19%, 4-phenyl = 15%, 5-phenyl = 15%, and 6-phenyl = 14%. The homolog distribution was C_{10} 0.39%, C_{11} 1.6%, C_{12} 97%, C_{13} 0.32%, C_{14} 0.59%, other homologs 0.31%.

Model Streams

All exposures occurred at the Procter & Gamble Experimental Stream Facility (ESF) in Milford, Ohio. This facility houses a series of 12-m-long streams. The streams consist of a head tank, a gentle sloping (1°) periphyton region which is 30 cm wide and 4.3 m long, a macroinvertebrate region (5° slope, 50 cm × 4.3 m), and a tail pool. Single-species exposure occurred in cages suspended in the tail pools. The tail pool is a 460-L tank (93 cm long, 61 cm wide, 82 cm deep) and receives approximately 167 L/min of test material and dilution water. During this experiment, the periphyton region contained unglazed terra cotta tiles to support a periphyton community. The macroinvertebrate region contained cobble packed into trays for sampling of invertebrate communities. River water (Lower East Fork of the Little Miami River, approximately six miles upstream of Milford, OH) was used to colonize and conduct tests. Macroinvertebrate and periphyton communities colonized the streams; their response to LAS exposures is discussed in Belanger *et al.* (2002). Test chemical was metered into river water and allowed to flow through the streams on a continuous flow, once-through basis. Five streams, each at a unique C_{12}LAS concentration, were used in this study. The nominal test concentrations were 0 (control), 0.15, 0.30, 1.0, and 3.0 mg/L of C_{12}LAS.

Single Species Studies

The current study presents data on single species exposed to C_{12}LAS in cages in the tail pool of the model streams. C_{12}LAS accumulation and toxicity were investigated in separate studies. Organisms used and their initial size (mean (standard deviation)) were: Asiatic clam (*Corbicula fluminea,* length 10.9 mm (0.44), weight 0.70 g (0.09)), snails (*Elimia* species, weight 0.36 g (0.092)), fathead minnows (*Pimephales promelas,* length 29.2 mm (3.0), weight 0.19 g (0.053)), channel catfish (*Ictalurus punctatus,* length 88.6 mm, weight 4.79 g (1.29)), and the amphipod (*Hyalella azteca,* weight 0.0027 g (0.0006)). Clams and snails were obtained from the Lower East Fork of the Little Miami River in the vicinity of Milford, Ohio prior to the start of the exposure. Fish were obtained from Charles River–Aquatic Research Organisms (ARO) (Hampton, NH). *Hyalella azteca* came from an in-house culture with original organisms obtained from ARO. Organisms were acclimated to water quality conditions for a minimum of four days and to flow conditions for a minimum of three days.

Bioconcentration was investigated in an eight-day exposure to a single concentration of C_{12}LAS and in a 32-day exposure to all concentrations. During the eight-day exposure, bluegill sunfish, fathead minnow, channel catfish, *Corbicula, Elimia,* and *Hyalella* were exposed to a nominal concentration of 0.3 mg/L which is approximately 10–20% of the C_{12}LAS LC_{50} values (this study). This portion of the study was conducted between days 7 and 15 (days 49 and 53 channel catfish) of the ESF exposure, when 30 organisms per species (60 for *Hyalella*) were transferred from the control stream, where they were acclimating to stream flow, into the appropriate exposure stream or control. On exposure days 0, 1, 2, 4, and 8, five individuals of each species (10 for *Hyalella*) were removed, measured for length (except *Hyalella* and *Elimia*) and wet weight, and frozen for LAS and dry weight analyses. Organisms were also removed from the test on day 32, the end of the toxicity phase of the study. These organisms were analyzed for LAS to understand bioconcentration and body burdens after a longer exposure to a wider range of C_{12}LAS concentrations.

Acute and chronic toxicity and total body burdens of LAS were determined in the toxicity phase. The toxicity phase was initiated on day −6 (day −5 for *Hyalella*) when 20 organisms of each species (fathead minnow, *Corbicula, Elimia, Hyalella*) were placed in cages suspended in the tail pool of each stream. The first six days (day −6–0) allowed acclimation to stream flow conditions. Exposure and effects data for days 0–4 were used to evaluate acute toxicity. Exposure and effects data for days 0–32 were used to evaluate chronic toxicity. However, growth of *Elimia* and *Hyalella* were insufficient to draw robust conclusions about chronic toxicity. On days −4, 0, 1, 2, and 4, approximately, organisms were examined to determine survival. On days 0, 8, 16, and 32, test organisms were examined for lesions and survival. Growth was also measured on these days in *Corbicula* (weight and length) and in fish (length via photographs with ruler and standards in photograph). Any dead or moribund organisms were removed from the test, weighed, measured for length and frozen for analysis. Moribund organisms were killed by rapid chilling and were stored at −80°C until analyzed for lipid, dry weight, and/or LAS analyses. On day 32, all remaining organisms were sacrificed, measured for length and wet weight, and lyophilized for dry weight and LAS analyses. Channel catfish acute toxicity testing was conducted by transferring fish that had been acclimated in a control stream into treated streams at the end of the ESF study (days 49–53). During this period, the average stream temperature was 15°C.

Field Exposures

In October 1997, fathead minnow and *Corbicula* were moved from tanks receiving river water in the ESF to cages in the effluent channel which carries effluent from the Lower East Fork Wastewater Treatment Plant (Milford, OH) to the Lower East Fork of the Little Miami River (LEFLMR). The wastewater treatment plant treats predominantly domestic sewage. The treatment plant consists of primary solids removal, rotating biological contactors, clarification, and rapid sand filtration. Effluent from the plant travels through a stream for approximately 250 m before emptying into the LEFLMR. The stream was approximately 100% effluent due to little to no upstream flow. After seven days of exposure, caged organisms were sacrificed and frozen for LAS analysis. On the first day of exposure, seines were used to collect feral fish and hand collection was performed on *Corbicula* from

the effluent stream. The feral fish were small (less than 10 cm), were caught in the upper 200 m of the stream and, due to the short home range of these organisms, were believed to have been in the stream for a number of days to weeks prior to sampling. Feral organisms were immediately sacrificed and frozen for LAS analyses.

Analytical Methods

Water samples were preserved with 1–3.5% formalin and a deuterated C_{12}LAS internal standard added. Sample volumes ranged from 10–200 mL, depending on the expected LAS concentration. Water samples were extracted and analyzed as described by Morrall *et al.* (2000). Briefly, LAS was isolated from water on a C2 bonded phase cartridge (Analytichem International, Harbor City, CA), then eluted with methanol. LAS was isolated from the methanol on a strong anion exchange (SAX) solid phase extraction column (Analytichem International, Harbor City, CA), and recovered in 10 mL of 50% 2N HCl / 50% methanol. The acidified methanol LAS eluent was evaporated to dryness under a nitrogen stream, and redissolved in 1 mL 50/50 methanol water, filtered, and analyzed by HPLC/MS. Biological samples were frozen at −80°C and lyophilized. Dried *Hyalella* were extracted by crushing to a fine powder and sonicated in 5 mL of methanol in a sonicating bath (Branson Ultrasonics Corporation, Danbury, CT, USA) twice for a total of 20 min. Extracts were filtered through a glass microfibre filter (Whatman Paper Ltd., Maidstone, England, UK) and then analyzed by HPLC/MS. *Corbicula, Elimia,* fathead minnows, and channel catfish were ground for 30 s (Micro-Mill; Bel-Art Products, Pequannock, NJ, USA) with enough hydromatrix (diatomaceous earth/crystalline silica; Varian, Harbor City, CA, USA) to cover the blades. The ground samples and additional hydromatrix were extracted on an accelerated solvent extractor (Dionex ASE 200; Dionex Corporation, Sunnyvale, CA, USA) with hexane to remove interfering substances and methanol to extract LAS. The hexane extraction used the following parameters: temperature 40°C, 1500 psi, heating time 5 min, static time 5 min, flush volume 60%, purge 60 s. The hexane extraction was repeated twice and the extracts discarded. Cells were then extracted with methanol using the following parameters: temperature 150°C, 1500 psi, heating time 7 min, static time 5 min, flush volume 60%, purge 60 s. The ASE methanol extracts were taken to dryness, then reconstituted in 2 mL of methanol with deuterated internal standard for LAS analysis by Midwest Research Institute (Kansas City, MO) using HPLC/MS by methods described in Morrall *et al.* (2000). The major ions monitored in the selected ion recording (SIR) mode corresponded to the [M-Na] negative ions of C_{12}-LAS (m/z = 325) and d_4 or $d_7$2-ϕC_{12}-LAS internal standard (m/z = 329 or 332). Method precision (triplicate samples) was determined for each biological matrix and each isomer. Extraction efficiencies of spiked samples averaged 103% for tissue and 101% for water samples. The relative standard deviations ranged from 1.2–9.9% across all isomers for extracts from tissue samples. Limits of quantitation, at a signal to noise ratio of 10:1, were approximately 1.3 μg C_{12}LAS/g tissue (dry weight) or 0.004 μmol C_{12}LAS/mg tissue (dry weight) for a 100-mg tissue sample and 3.0 μg C_{12}LAS/L for a 50-ml water sample. Internal standards were used to account for changes in MS response. Variations in background signal occurred due to variable amounts of endogenous matrix components. The potential for interferences was evaluated by analysis of matrix blanks using tissue and water samples not exposed to the test compound and subsamples of these matrix blanks spiked with test material. Variable amounts of matrix interference precluded quantitation at trace levels. Matrix interference was minimal for all samples reported.

Water Quality

Water quality parameters were monitored throughout the model ecosystem study and changed based on the meteorological and hydrological conditions in the area. Water quality parameters (mean (standard deviation)) during the study were: hardness 140 (25) mg/L as $CaCO_3$, alkalinity 120 (17) mg/L as $CaCO_3$, temperature 18 (3.6)°C, pH 7.8–8.3 (range), dissolved oxygen 8.1 (0.96) mg/L, conductivity 320 (54) μS, and total organic carbon 7.2 (4.9) mg/L. The test was conducted from September to October, 1996. Temperatures decreased from approximately 24°C, during the first four days, to 17°C during the last four days of the 32-day exposure period. A 96-h exposure of channel catfish was conducted during late October and early November. The temperature decreased from 17 to 13°C during this exposure. Time course graphs of dissolved oxygen, pH, temperature, and conductivity are available in Belanger *et al.* (2002).

Statistics

Bioconcentration factors (BCF) were based on the ratio of measured body burdens to measured water concentrations. Effect of isomer distribution and exposure concentration on BCF values were determined using analysis of covariance (ANCOVA). Differences in BCF values among species were determined by ANCOVA with the Tukey-Kramer multiple comparison of least squares mean test. LC_{50} values were determined using the Binomial or Probit methods. LBB_{50} (body burden associated with 50% mortality) were estimated from the BCF times LC_{50} based on the exposure concentration closest to the LC_{50} value. Probit (Finney 1971) (mortality data) or the nonlinear iterative technique of Bruce and Versteeg (1992) (continuous data) were used to determine the EC_{20} and EBB_{20} values; where EC_{20} refers to the effective concentration in water that reduces the biological endpoint 20% relative to control levels and EBB_{20} refers to the body burden concentration at which the biological endpoint was reduced 20%. Single species distributional parameters were estimated as described in Versteeg *et al.* (1999) using a log-logistic distribution. All statistical analyses used SAS release 6.12 (SAS Institute, Cary, NC, USA).

Results and Discussion

Exposure Concentrations

Mean C_{12}LAS concentrations measured during the accumulation and toxicity portions of the study ranged from 87–100% of nominal concentrations. Trace levels of C_{12}LAS were detected in the control treatment. These levels were below the quantitation limit and were likely derived from a municipal WWTP, approximately 6.5 miles upstream of the ESF riverwater intake. At exposure concentrations above the control, isomer distributions were consistent across streams consisting of 33–35% 2-phenyl, 19–20% 3-phenyl, 15% 4 phenyl, and 30–32% 5 and 6-phenyl.

Bioconcentration

During the eight-day exposure, total C_{12}LAS concentrations in all species, except possibly *Elimia* and *Hyalella,* appeared to have achieved steady state. At the 0.3 mg/L nominal exposure concentration, mean body burdens ranged from 0.022 (*Cor-*

Table 1. Whole body concentrations of C_{12}LAS isomers and total C_{12}LAS during the 8-day exposure period—organisms exposed to a nominal concentration of 0.3 mg/L

	C_{12}LAS Tissue Concentrations[1] (mmoles/kg)				
		Isomer Position			
Organism	Total C_{12}LAS	2-phenyl	3-phenyl	4-phenyl	5,6-phenyl
Fathead minnow	0.0450	0.0282	0.0073	0.0042	0.0053
Channel catfish[2]	0.0610	0.0466	0.0094	0.0028	0.0022
Corbicula	0.0221	0.0148	0.0029	0.0015	0.0030
Elimia	0.0848	0.0375	0.0144	0.0125	0.0204
Hyalella	0.167	0.0776	0.0275	0.0208	0.0408

[1] C_{12}LAS tissue concentrations based on mean body burdens on days 4 and 8 of exposure except for catfish, which were exposed for four days.
[2] Channel catfish exposure initiated later in the study than the other species at an average stream temperature 15°C.

Table 2. Whole body concentrations of C_{12}LAS isomers and total C_{12}LAS during the 32-day toxicity phase of the study

			C_{12}LAS Tissue Concentrations (mmoles/kg)				
				Isomer Position			
Organism	C_{12}LAS Exposure Concentration (mg/L)	Total C_{12}LAS BCF	Total C_{12}LAS	2-phenyl	3-phenyl	4-phenyl	5,6-phenyl
Fathead minnow	0.126	96	0.0347	0.0251	0.0042	0.0017	0.0037
	0.293	79	0.0667	0.0467	0.0080	0.0037	0.0083
	0.927	65	0.174	0.129	0.0181	0.0086	0.0191
Channel catfish	0.126	104	0.0378	0.0274	0.0068	0.0021	0.0016
	0.293	72	0.0610	0.0466	0.0094	0.0028	0.0022
	0.927	42	0.111	0.0894	0.0153	0.0037	0.0026
Corbicula	0.126	33	0.0119	0.0075	0.0019	0.0009	0.0015
	0.293	22	0.0188	0.0125	0.0031	0.0011	0.0022
	0.927	21	0.0550	0.0406	0.0064	0.0026	0.0054
	2.98	9	0.0810	0.0604	0.0091	0.0044	0.0072
Elimia	0.126	42	0.0153	0.0088	0.0039	0.0011	0.0016
	0.293	37	0.0312	0.0175	0.0052	0.0023	0.0062
	0.927	18	0.0493	0.0302	0.0055	0.0040	0.0095
	2.98	11	0.0965	0.0599	0.0129	0.0083	0.0153
Hyalella	0.126	119	0.0429	0.0180	0.0090	0.0070	0.0089
	0.293	36	0.0307	0.0174	0.0057	0.0038	0.0038
	0.927	66	0.176	0.0872	0.0299	0.0241	0.0346

Fathead minnow, *Corbicula*, *Elimia*, and *Hyalella* were exposed to C_{12}LAS for 32 days; channel catfish were exposed for 96 h.

bicula) to 0.167 (*Hyalella*) mmole/kg of total C_{12}LAS across species (Table 1). During the 32-day exposure to 0.3 mg/L, mean body burdens ranged from 0.019 (*Corbicula*) to 0.067 (fathead minnow) mmole/kg of total C_{12}LAS across species (Table 2). For *Corbicula* and fathead minnows exposed to 0.3 mg/L, LAS tissue concentrations were similar between the eight- and 32-day exposures, but for *Hyalella* and *Elimia*, tissue concentrations decreased 2.5- to 5-fold between the eight- and 32-day exposures. Whole body concentrations of individual isomers decreased as the isomer position moved from external (2- and 3-phenyl) to internal in the eight- and 32-day exposures to 0.3 mg/L (Tables 1 and 2). For example, the 2-phenyl isomer comprised 44–76% and 56–70% of the total C_{12}LAS body burdens on days 8 and 32, respectively, despite comprising only 35% of the total C_{12}LAS starting material.

Total C_{12}LAS BCF values ranged 9–119 L/kg, but were affected by exposure concentration, phenyl position, and species (Table 2, Figure 1). Total C_{12}LAS BCFs declined with exposure concentration in all species except *Hyalella*. Analysis of covariance was used to assess the contribution of each factor to the overall BCF across species. Collapsing across exposure concentrations, mean total C_{12}LAS BCF values in fathead minnow and channel catfish were 73 and 80 L/kg, respectively, and were not significantly different from each other. Mean total BCF values in *Elimia* and *Corbicula* were 24 and 27 L/kg, respectively, and were not significantly different from each other, but were significantly less than BCF values for all fish species.

For channel catfish, *Corbicula* and *Elimia*, joint effects of exposure concentration and isomer distribution on BCF values occurred (Figure 1). In other words, the effect of exposure concentration on BCF changed with isomer position (i.e., slopes of the BCF versus exposure concentration regressions are different between isomers). For fathead minnow and *Hyalella*, the effect of exposure concentration on BCFs was similar for all isomers (slopes are the same among isomers across exposure concentrations). The generally decreasing BCF with exposure concentration agrees with the bluegill sunfish data of Bishop and Maki (1980) who reported C_{12}LAS BCF values

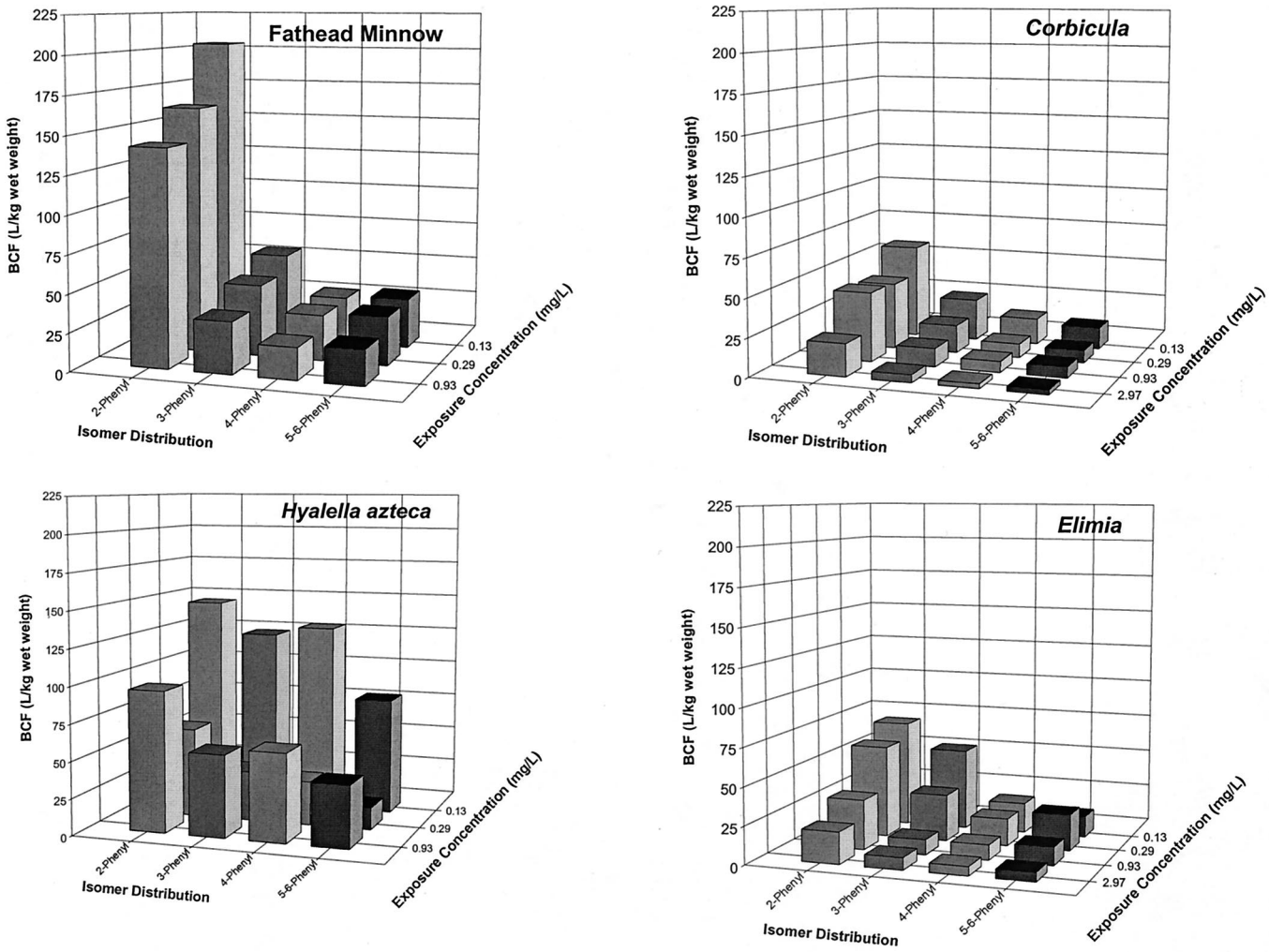

Fig. 1. Effect of isomer position and exposure concentration of C_{12}LAS on the bioconcentration factor (BCF) in experimental streams during the toxicity phase of the experiment. Fathead minnow, *Corbicula*, *Hyalella*, and *Elimia* were exposed for 32 days

decreased from 280 to 130 L/kg as exposure concentrations increased from 0.064 to 0.68 mg/L. Tolls *et al.* (2000a) showed a trend towards decreasing BCF values with exposure concentration (0.007 to 0.23 mg/L). This trend was not statistically significant but changes in conditions among the exposures may have contributed to uncertainty in the BCF values.

Our C_{12}LAS BCF values are generally in line with reported values. Tolls *et al.* (1997) reported BCF values ranging from 22 to 91 L/kg in fathead minnows, depending on the isomer distribution assumed for the starting material. This study used specific analytical, taking into account the potential for LAS metabolism which can account for up to 40% of the loss of LAS in fathead minnows (Tolls *et al.* 2000b). BCF values range from 108 to 280 L/kg in bluegill sunfish (Kimerle *et al.* 1975; Bishop and Maki 1980), 8 to 103 L/kg in daphnids (Comotto *et al.* 1979), and 56 to 240 L/kg in *Chironomus* (Hwang *et al.* in press). The range of *Chironomus* BCF values reported by Hwang *et al.* (in press) appears to be due to feeding. In a conventional 10-day uptake depuration study without feeding, a BCF value of 240 L/kg was measured. In chronic toxicity tests with feeding, the BCF value of 56 L/kg was observed. The bluegill sunfish, daphnid, and *Chironomus* studies cited here used radiolabeled LAS to quantify BCF values possibly leading to overestimation of the BCF since metabolites would be quantified as parent. However, bluegill sunfish, *Daphnia magna*, and *Chironomus* do not appear to metabolize LAS to a significant extent (Comotto *et al.* 1979; Hwang *et al.* in press; Rawlings and Versteeg submitted).

Phenyl position affected BCF values for fathead minnow, channel catfish, *Corbicula*, and *Elimia* with BCF values increasing in the more external isomers (Figure 1). The increased BCFs we observed for the more external isomers (i.e., 2-, and 3-phenyl) are consistent with the observed direct relationship between LAS BCF values and hydrophobicity (Kimerle *et al.* 1975; Comotto *et al.* 1979; Bishop and Maki 1980; Tolls *et al.* 1997). Tolls *et al.* (1997) observed a 5- to 10-fold decrease in fathead minnow BCFs in moving from the 2-phenyl to the 5- and 6-phenyl C_{12}LAS. The increased hydrophobicity for the external isomers is reflected in the greater $\Delta G°_{abs}/A_{min}$ values of Rosen *et al.* (2001), where the $\Delta G°_{abs}$ is the standard free energy of adsorption of the surfactant at the air water interface

Table 3. Toxicity of C_{12}LAS to single species caged in model ecosystem streams

	Acute		Chronic	
Organism	LC_{50} mg/L	LBB_{50} mmoles/kg	EC_{20} mg/L	EBB_{20} mmoles/kg
Fathead minnow	1.7 (0.93–2.98)	0.32 (0.174–0.557)	1.0[1] (survival)	0.19[1]
Corbicula	>3.0	>0.078	0.27 (length) (0.204–0.352)	0.035 (0.0310–0.0405)
Hyalella[2]	3.1 (2.53–4.24)	0.59 (0.480–0.804)	0.95 (survival) (0.597–1.29)	0.23[3]
Elimia	>3.0	>0.096	>2.9 (survival)	>0.096
Channel catfish	1.7 (0.93–2.98)	0.21 (0.114–0.364)	NA[4]	

Results and 95% confidence interval in parentheses of acute (4 days) and chronic (32 days) toxicity tests with associated body burden concentrations. LC_{50} and EC_{20} values refer to water concentrations; LBB_{50} and EBB_{20} values refer to tissue concentrations (i.e., body burdens in whole organisms) associated with the LC_{50} and EC_{20} effects.
[1] Estimated graphically.
[2] *Hyalella* chronic test ended on day 24 as control mortality increased on day 32.
[3] Calculated using probit; confidence intervals could not be calculated.
[4] Chronic endpoint not available for this species.

and A_{min} is the minimum cross-sectional area of the surfactant at the interface. Mechanistically, the reduced hydrophobicity as the phenyl position moves internally is due to carbon–carbon interaction, resulting in a reduced number of water molecules needed to solvate the hydrocarbon chain (Roberts 1988).

Toxicity

Acute 4-d LC_{50} values ranged from 1.7 to >3.0 mg/L for the five species tested (Table 3). Lethal body burdens associated with 50% mortality (LBB_{50}) varied from 0.21 to 0.59 mmole/kg (wet weight). In laboratory acute toxicity studies with C_{12}LAS, LBB_{50} values of 0.26, 0.54, 0.93, 1.4, and 1.2 mmol/kg for bluegill sunfish, fathead minnow, *Corbicula, Hyalella,* and *Chironomus riparius,* respectively, have been reported (Rawlings and Versteeg submitted, Hwang *et al.* in press).

Chronic toxicity tests in the tail pools were successfully completed with fathead minnows, *Corbicula, Elimia,* and *Hyalella* (Table 3, Figure 2). For *Corbicula* and fathead minnows, weight gains were 33% and 17%, respectively, during the course of the study. For *Elimia* and *Hyalella,* interpretation of growth data was difficult due to poor growth in the controls (i.e., less than 3% of initial body weight during the 32-day exposure). For *Hyalella,* survival data is reported on day 24 due to poor control survival on day 32.

C_{12}LAS at concentrations up to 2.9 mg/L did not adversely affect survival of *Corbicula* or *Elimia* (Figure 2). For *Corbicula,* the EC_{20} values were 0.27 (0.204–0.352) (Table 3) and 0.36 (0.278–0.470) mg/L for length and weight, respectively. The *Hyalella* EC_{20} value was 0.95 (0.597–1.29) mg/L based on survival. For fathead minnow, survival after 32 days of exposure was 85% at 0.93 mg/L and 0% at 2.9 mg/L. Growth (length gain) was increased above the control at all exposure concentrations below 2.9 mg/L. Based on the most sensitive endpoint, survival, the fathead minnow 32-day EC_{20} value was 1 mg/L (estimated graphically). The fathead minnow chronic toxicity value agrees with data in the literature (van de Plassche *et al.* 1999). Chronic toxicity values for *Elimia* and *Hyalella* could not be obtained from the literature, but these water column chronic values are within the range of values for freshwater invertebrates reported for other species which generally range from 2.0 to 10 mg/L (van de Plassche *et al.* 1999, data normalized to dodecyl chain length; Figure 3). The chronic EC_{20} value for *Corbicula* is the lowest available toxicity value for an invertebrate species and is among the lowest chronic toxicity values available for C_{12}LAS, suggesting a potentially different mode of action of this compound in this species. This may be due to the ability of bivalves to detect the presence of surfactants at low concentrations and reduce their siphoning. This behavioral response reduces feeding resulting in slower growth. Reduced siphoning in the presence of xenobiotics has been reported for a variety of compounds (Salanki and Varanka 1978; Doherty 1990; Kontreczky *et al.* 1997).

At the EC_{20} or NOEC value from the chronic exposures, body burdens for the most sensitive endpoint were 0.035 (length), >0.096 (survival), 0.23 (survival), and 0.19 (survival) mmoles/kg wet weight in *Corbicula, Elimia, Hyalella,* and fathead minnow, respectively (Table 3). These values are similar to the C_{12}LAS LOER (lowest observed effective residue) values for male and female development of 0.085 and 0.100 mmoles/kg wet weight, respectively, reported by Hwang *et al.* (in press). For *Hyalella* and fathead minnows, body burdens at the EC_{20} (approximately 0.2 mmoles/kg) are similar to those reported to cause chronic effects for polar narcotics (McCarty and Mackay 1993).

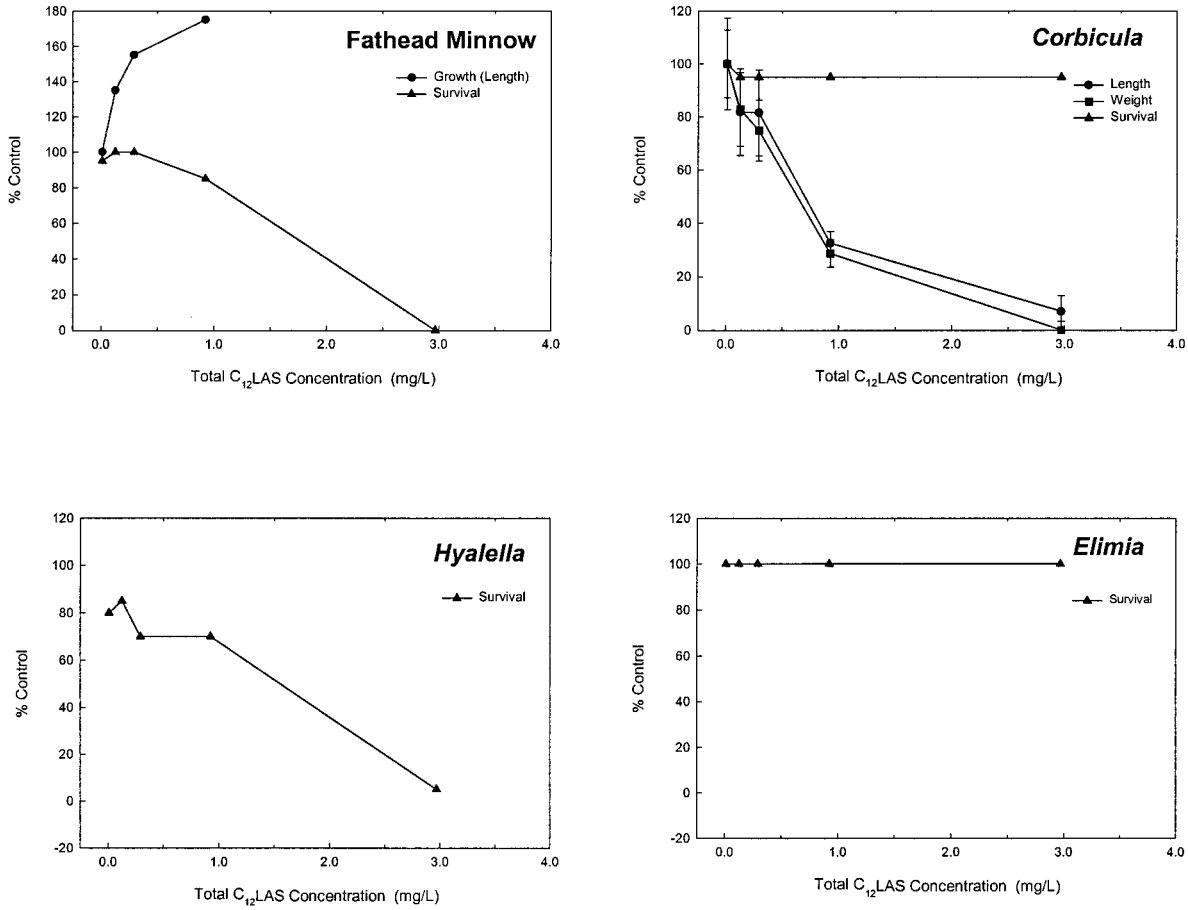

Fig. 2. Survival (percent) and growth (change in weight or length as a percent of control) during the 32-day exposure of fathead minnow, *Corbicula*, *Elimia*, and *Hyalella* to C_{12}LAS in streams. All organisms were caged in the tail pools. For *Hyalella*, survival at 24 days is shown due to an increase in control mortality during the last 8 days of exposure. Change in weight based on wet weight

Field Exposure

For the effluent exposures, fish and *Corbicula* were transferred from the laboratory, where they were exposed to river water containing trace levels of C_{12}LAS, to the effluent where they were naturally exposed to C_{12}LAS for seven days. Five species of fish representing a variety of feeding strategies were captured in the effluent. A greater percent of the total C_{12}LAS in fish was in the more internal 5,6-phenyl isomer relative to *Corbicula* (Table 4). The observed isomer distribution may be due to a complex combination of multiple factors including the effluent isomer concentrations, isomer specific metabolism, isomer specific uptake and depuration kinetics, and differences in the feeding strategies, food sources, and ability to process sediment (i.e., *Corbicula*). Despite the complexity involved in understanding tissue concentrations, these levels can be used to assess potential effects in caged and feral organisms. Total C_{12}LAS body burdens in feral and caged *Corbicula* were approximately 0.0013 mmoles/kg. This is approximately 25-fold below concentrations associated with effects in in-stream exposures. Fish tissue concentrations ranged from 0.0005 to 0.0039 mmoles/kg wet weight, approximately 45–380 times below the tissue concentration associated with subtle effects in the in-stream exposures. Based on tissue concentrations, ad-

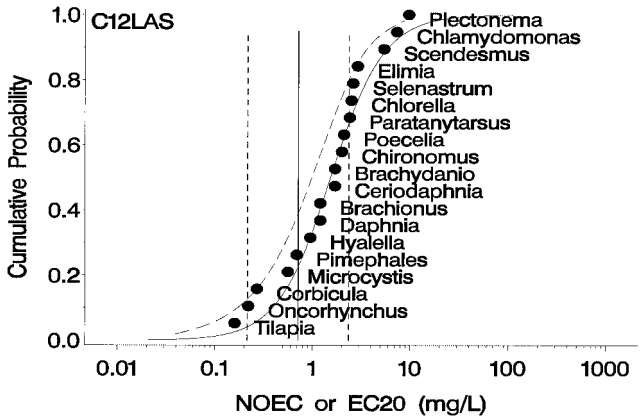

Fig. 3. Cumulative single species toxicity plot with model ecosystem data. Single species toxicity data from Van de Plassche *et al.* (1999) and Versteeg *et al.* (1999) with *Elimia*, *Hyalella*, and *Corbicula* values from this study plotted and fit to a cumulative log-logistic function. Lower 95% confidence limits of the distribution percentiles are shown (dashed). Model ecosystem data from Belanger *et al.* (2002) presented as geometric mean (solid vertical line) and 95% confidence interval (dashed vertical line)

Table 4. Total C_{12}LAS body burdens (mmole/kg wet weight) and relative isomer distribution in fathead minnows and *Corbicula* caged in the effluent for 7 days and feral fish and *Corbicula* sampled immediately downstream of the effluent

		Mean Tissue Isomer Distribution (%)								Total C_{12}LAS Body Burden (mmoles/kg)	
		2-Phenyl		3-Phenyl		4-Phenyl		5,6-Phenyl			
	N	Mean	SD	Mean	SD	Mean	SD	Mean	SD	Mean	SD
Caged											
Pimephales promelas (fathead minnow)	11	8.4	4.4	7.3	2.5	9.7	0.8	74.6	7.0	0.0039	0.0023
Corbicula fluminea	6	30.2	5.0	22.7	3.1	12.8	1.3	34.3	3.7	0.0013	0.0006
Feral organisms											
Catostomus commersoni (common white sucker)	2	29.4		21.2		10.6		38.7		0.0017	
Semolitus atromaculatus (Northern creek chub)	10	19.5	4.0	7.5	3.4	10.1	5.2	62.9	9.6	0.0012	0.0005
Ambloplites species[1] (sunfish)	1	21.4		14.3		14.3		50.0		0.0005	
Rhinichthys cataractae (long nose dace)	7	26.3	5.9	13.8	4.5	17.0	7.3	42.8	13.3	0.0006	0.0001
Hybognathus regius silvery minnow	6	25.7	4.6	12.8	4.3	9.9	4.2	51.7	9.0	0.0006	0.0002
Corbicula fluminea (indigenous)	12	30.3	11.4	17.2	4.1	19.0	8.0	33.5	7.2	0.0012	0.0008

[1] Species identification tentative.

verse effects on these species should not be caused by the C_{12}LAS concentrations achieved in this effluent. Since LAS usually occurs as a mixture of alkyl chain lengths with an average of approximately dodecyl, the concentrations of other homologs would have to be measured in tissues to fully assess total LAS effects.

Comparison of Single Species Data with Model Ecosystem Data

The single species sensitivity data is summarized in a cumulative species sensitivity plot (Figure 3). This plot combines literature chronic toxicity values primarily from Versteeg *et al.* (1999) with the *Elimia, Hyalella,* and *Corbicula* EC_{20} values from the current study. Due to biological issues with *Elimia* and *Hyalella* toxicity tests, the analysis was conducted with these toxicity values included and excluded. The two datasets produced similar distributions; the single species distribution, with the *Elimia* and *Hyalella* values included, is discussed. Single species data are plotted as a log-logistic distribution with model ecosystem data represented as a mean with upper and lower 95% confidence intervals (Figure 3). All C_{12}LAS model ecosystem NOEC values from Belanger *et al.* (2002), normalized if needed, with a duration greater than four days were used. The single species log-logistic distribution (intercept, 0.4268; scale, 0.6217) was fit with the 19 available chronic single species toxicity values and has a mean value of 1.4 mg/L. The single species distribution overlaps with model ecosystem values (mean 0.71 mg/L (n = 11) with a lower 95% confidence interval of 0.21 mg/L, Belanger *et al.* 2002). Since single species toxicity data are often used to establish the "safe" or low effect concentration in the environment, it is informative to compare the mean and lower 95% confidence interval on the model ecosystem data with the C_{12}LAS concentration expected to be less than most single species NOEC or EC_{20} values. C_{12}LAS concentrations of 0.39, 0.25, and 0.088 mg/L would be protective of 90, 95, and 99% of species, respectively. Approximately 25% of species' chronic toxicity values are lower than the mean model ecosystem NOEC. Approximately 5% of the single species NOEC values are less than the lower 95% confidence interval on the model ecosystem NOEC values.

The model ecosystem study (Belanger *et al.* 2002), during which we exposed single species to C_{12}LAS in cages, had a NOEC of 0.29 mg/L based on benthic abundance of immature stream macroinvertebrates. At this NOEC, the cumulative single species effect distribution predicts 7% of the single species NOEC values would be exceeded. Van de Plassche *et al.* (1999) reported that a $C_{11.6}$LAS concentration of 0.32 mg/L was protective of 95% of species. Based on this single species value and the model ecosystem data available at the time, 0.25 mg/L was selected as the predicted no-effect concentration in the environment. Taken together, the similarity between model ecosystem NOEC values and single species NOEC values suggest single species values, when appropriately evaluated, can be used to understand effects in model ecosystems and, perhaps, the ecosystem.

Comparisons of laboratory and model ecosystem data and extrapolation to the ecosystem can be criticized due to differences between systems in test compound bioavailability, low replication in model ecosystem studies, lack of a particular type or level of effect, short duration of model ecosystem studies, and the fact that not all ecosystems are evaluated in model ecosystem studies (Suter *et al.* 2001). For LAS, the bioavailability issue is a minor concern (Traina *et al.* 1996) and the number and variety of model ecosystem

studies performed help address the replication, duration, and ecosystem-diversity issues. At a more fundamental level, however, is the intended use of both single species and model ecosystem data. Both are intended to establish an understanding of the low or minimal effect concentration in the environment and concurrence between the two different types of studies lends credence to both. The observation for C_{12}LAS that a sufficient number of single species toxicity data can be used to establish a concentration protective of model ecosystems and likely whole ecosystems is consistent with the findings of others concerning a wider variety of compounds (Emans *et al.* 1993; Okkerman *et al.* 1993; Versteeg *et al.* 1999). However, effects assessments which combine single species, model ecosystem, and field data in a weight-of-evidence approach will likely provide the most rigorous approach to establishing environmental effects criteria.

Conclusions

For C_{12}LAS, bioconcentration factors were affected by exposure concentration, species, and chemical structure. For the species tested, lethal body burdens ranged from 0.21 to 0.59 mmole/kg while body burdens associated with low levels of chronic effects (i.e., EC_{20}) ranged from 0.035 to 0.23 mmole/kg for total C_{12}LAS. C_{12}LAS body burdens in caged and feral organisms exposed to 100% effluent were well below these tissue concentrations suggesting C_{12}LAS does not pose an issue in this effluent. Single species sensitivity distributions and model ecosystem data suggest C_{12}LAS concentrations of approximately 0.25 to 0.30 mg/L should pose a low risk to the environment.

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