

ASSESSING THE TOXICITY OF DODECYLBENZENE SULFONATE TO THE MIDGE
CHIRONOMUS RIPARIUS USING BODY RESIDUES AS THE DOSE METRICHAEJO HWANG,[†] SUSAN W. FISHER,*[†] KYEOK KIM,[†] PETER F. LANDRUM,[‡] ROBERT J. LARSON,[§] and
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(Received 5 March 2002; Accepted 12 July 2002)

Abstract—Dodecylbenzene sulfonate (DBS) is a component of linear alkylbenzene sulfonate (LAS), an anionic surfactant, mainly used in household detergents. Due to the large quantity of DBS in use, there is concern over adverse environmental effects. This work examined the toxicokinetics and toxicity of the 2-phenyl isomer of dodecylbenzene sulfonate in 4-d, 10-d, and partial life-cycle tests on the midge, *Chironomus riparius*, exposed to aqueous solutions. Toxicokinetics were determined in 10-d uptake and 5-d elimination tests. The toxicokinetics were based on parent compound concentration in water and yielded an uptake coefficient (k_u) of 17.5 (14.87–20.20) ml/g/h, an elimination rate constant (k_e) of 0.073 (0.062–0.085) per h, a bioconcentration factor (BCF) of 56 to 240, and a half-life ($t_{1/2}$) of 9.5 (8.0–11.0) h. Biotransformation measurements did not reveal evidence for DBS metabolism. Thus, body residues, determined in the toxicity study, represent parent compound. In toxicity tests, 4- and 10-d LR50s (the body residue required to cause 50% mortality) in live midges were 0.72 (0.65–0.79) and 0.18 (0.08–0.42) mmol/kg, respectively. Thirty-day LR50s were 0.18 (0.09–1.64) and 0.21 (0.15–0.39) mmol/kg in duplicate studies. Of the sublethal endpoints, only developmental time increase was significant, with the lowest-observed-effect residues of 0.085 (0.067–0.105) and 0.100 (0.087–0.114) mmol/kg for male and female midges, respectively. Deformities in surviving larvae were also observed as chronic responses for body residues exceeding the 30-d LR50. The body residues required for mortality suggest that DBS acts like a polar narcotic in the midge.

Keywords—Body residue Dodecylbenzene sulfonate Midges Toxicokinetics Chronic endpoints

INTRODUCTION

Dodecylbenzene sulfonate is widely used in industrial and household cleaning products. Its annual production in 1998 was 879 million kilograms worldwide [1], with most material discharged into wastewater [2]. During wastewater treatment, the majority of dodecylbenzene sulfonate is removed by biodegradation and sorption to biomass [3]. For example, dodecylbenzene sulfonate was largely removed by activated sludge (99.3 ± 0.61%), lagoon (98.5 ± 1.81%), oxidation ditch (98.0 ± 4.24%), rotating biological contact (96.2 ± 6.10), and trickling filter (77.4 ± 15.5%) methods [4].

Even though dodecylbenzene sulfonate is highly biodegradable, an average concentration of 95 µg/kg of sediment was found downstream from a wastewater treatment plant outfall [5]. Further, the average dodecylbenzene sulfonate concentration in effluent sewage from activated sludge plants was 6.0 µg/L, while its counterparts from trickling filter plants was 750 µg/L [5]. Although McAvoy et al. [5] predicted the 90th percentile dodecylbenzene sulfonate concentrations under low-flow conditions to be 185 µg/L or less, the detection of dodecylbenzene sulfonate in aquatic systems warrants further scrutiny into the potential effects of dodecylbenzene sulfonate in the environment.

To investigate the effects of an environmental contaminant such as dodecylbenzene sulfonate, the contaminant concentrations in the environmental exposure medium such as water or sediment have been commonly adopted as the dose metric.

However, the effects of the contaminant on the test organism are a function of the compound concentration and duration at the target site, not necessarily the environmental concentration, due to potential bioavailability limitations. For example, benthic organisms such as *Chironomus riparius*, *Lumbriculus variegatus*, and *Diporeia* spp. exposed to sediments spiked with pyrene, benzo[*a*]pyrene, or trans-chlordane all exhibited significantly different levels of bioaccumulation, with *C. riparius* having the least and *Diporeia* spp. the greatest [6]. This result was attributed to differences in feeding selectivity, lipid content, and physiological/metabolic variation among organisms. Similar results were obtained when invertebrates with different feeding habits were exposed to chlordane-contaminated sediments. In this case, the surface feeders or surface dwellers had higher bioaccumulation than suspension feeders [7]. Finally, pentachlorophenol concentrations in zebra mussels varied by a factor of 177 following exposure to pentachlorophenol under different combinations of pH and temperature [8]. Thus, the way in which organisms interact with the environment and the characteristics of the environment can result in significantly different levels of bioaccumulation.

Using body residues rather than environmental concentrations as the dose metric may circumvent problems such as differential bioavailability, varying feeding habitats, and physiological/metabolic variability, thereby providing a clearer connection between exposure and effects. Body residues are surrogates for the contaminant concentration at the target site and should reflect the toxic potential and genetic differences of the organisms [9]. Using body residues in interpreting toxicity of contaminants was proposed by McCarty [10–12]; their

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usage subsequently was expanded to ecological risk assessment by McCarty and Mackay [13]. Body residues have been used successfully to assess the environmental hazard of persistent contaminants [14–19]. However, the availability of body residue data is still too limited to be used routinely in ecological risk assessment.

Our study examines the body residues of dodecylbenzene sulfonate (an important component of linear alkylbenzene sulfonate [20]) in relation to mortality, reduction in growth and reproduction, and developmental time for the midge, *C. riparius*, for 4-, 10-, and 30-d exposure durations. The toxicokinetics of dodecylbenzene sulfonate were determined during a 10-d uptake phase, followed by a 5-d elimination period.

MATERIALS AND METHODS

Water

Hard standard reference water (HSRW), having a pH of 8.3 to 8.5, an alkalinity of 120 mg/L as CaCO₃, and a hardness of 160 mg/L as CaCO₃ was used in all experiments [21]. The salts used to prepare the water were predissolved in smaller vessels and added to deionized water (20°C) to produce HSRW. The water was mixed overnight, and water-quality parameters were confirmed according to standard laboratory protocols [21].

Organism

Chironomus riparius was reared in the Environmental Toxicology Laboratory at The Ohio State University (Columbus, OH, USA) according to the method of Estenik and Collins [22]. The midge larvae initially were collected in 1974 from the Jackson Pike Water Treatment Plant (Columbus, OH, USA) and have been maintained in culture with periodic outbreeding to wild midges. Midges were chosen as test organisms because they are sediment dwelling, are easy to culture, are ecologically important, and have a broad geographical distribution [23].

Test chemical

Procter and Gamble (Cincinnati, OH) donated ¹⁴C- and ¹²C-dodecylbenzene sulfonate. In addition to different chain lengths of linear alkylbenzene sulfonate, the phenyl position is variable in commercial linear alkylbenzene sulfonate. The dodecylbenzene sulfonate used to quantify water and tissue concentration was the 2-phenyl isomer. This isomer is bioconcentrated to a greater extent than isomers that have the phenyl group attached further along the alkyl chain [20]. Radioactive 2-phenyl dodecylbenzenesulfonate (dodecylbenzene sulfonate; specific activity, 50.77 Bq) was approximately 90% pure based on a combination of thin-layer chromatography and liquid scintillation counting (LSC). Thin-layer chromatography used silica gel plates (Silicagel; pore size 150 Å, thickness 250 μm; Whatman, Clifton, NJ, USA), and the plates were developed in CHCl₃:MeOH:H₂O:HCOOH (70:35:3:2). Radioactivity attributable to parent compound and metabolites was measured by removing and counting the silica gel with a Beckman 6000 liquid scintillation counter (Beckman, Fullerton, CA, USA) in 5 ml ScintiVerse® E scintillation cocktail (Fisher Scientific, Fair Lawn, NJ, USA). The LSC samples were corrected for quench using the external standards ratio method after subtracting background using the automatic quench correction. The ¹⁴C-dodecylbenzene sulfonate was purified using thin-layer chromatography preparative plates with the same solvent system. The purified ¹⁴C-dodecylbenzene sulfonate was then eluted from the silica gel with methanol. The purified

product was determined to be >98% pure. For toxicity tests, we used a mixture of ¹⁴C- and ¹²C-dodecylbenzene sulfonate. The specific activity of the mixture was recalculated to account for isotopic dilution. The ¹²C-dodecylbenzene sulfonate consisted of a mixture of C₁₂ isomers consisting of 5/6-phenyl (26.9%), 4-phenyl (13.2%), 3-phenyl (21.8%), and 2-phenyl (38.1%).

Toxicokinetics

The toxicokinetics were performed as two separate phases, an uptake phase (lasting 10 d) followed by an elimination phase (lasting 5 d). For the uptake test, 3 L of HSRW were placed in an 8-L aquarium and dodecylbenzene sulfonate was added at a concentration of 4.5 (±0.4) × 10⁻³ mg/L. The solution was stirred with a magnetic stirrer for 2 h before use. Each of three 1-ml water samples was combined with 15 ml ScintiVerse E scintillation cocktail and kept in the dark overnight before determining radioactivity by LSC.

The uptake test was started by placing approximately 500 third instar midges into the aquarium. Five midges were sampled at predetermined time intervals, initially four times per day for the first 3 d, then twice daily thereafter. Exact times were recorded and used for the toxicokinetics calculations. At every other sampling time, 15 additional midges were removed and stored at -80°C to determine biotransformation. At the same time that the midges were removed for biotransformation analysis, a 20-ml water sample was also taken and stored at -80°C to determine biodegradation in the water. The midges were individually weighed, transferred into a scintillation vial, and digested in 100 μl nitric acid and 50 μl hydrogen peroxide at 50°C until the tissue had dissolved (~2 min). Upon cooling to room temperature, 15 ml of scintillation cocktail were added, and the samples were stored in the dark overnight before radioactivity was measured. Three 1-ml water samples were also taken along with midges to follow the dodecylbenzene sulfonate concentrations in water.

During the uptake test, water was changed daily to minimize linear alkylbenzene sulfonate biodegradation. Before and after the water change, three 1-ml water samples were taken and analyzed using LSC. The water was prepared as described previously, and the midges were transferred to the fresh exposure water using a fish net. Midges were fed a mixture of *Chlorella vulgaris* and commercial trout chow every other day. The feeding occurred just before transfer to fresh exposure media. Algae and trout chow, in an amount sufficient to insure that the food supply was not depleted during the feeding period, were placed in 300 ml of water in a glass dish and stored in a growth chamber at 20°C overnight to facilitate settling. Midges were transferred to the feeding beaker and allowed to feed for 1 h before being transferred to the freshly prepared exposure water.

For the elimination test, the midges remaining from the uptake test were transferred to a mini-flow-through system (500 ml volume with a flow rate of ~600 ml/h) and allowed to eliminate the contaminant. Five midges were collected at predetermined time intervals (2, 4, 14.3, 25, 34, 53, 61, 72.5, 84, 97, 106.5, 126 h) and treated in the same manner as in the uptake test. As above, an additional 15 midges were collected at every other sampling time interval for biotransformation.

The midges collected for biotransformation were lyophilized, homogenized, and extracted with methanol. The methanol extraction was performed a total of three times (10 ml/each) and the extracts were combined. The methanol in the

extracts was then evaporated using a rotary evaporator (Büch Laboratories-Technik, Buchi, Switzerland). The residue was reconstituted with 1 ml of methanol, and 100 μ l of the reconstituted solution was spotted for thin-layer chromatography analysis as described above. After development, the plate was removed from the solvent, air dried in a hood, sectioned into 1-cm sections, and the silica gel for each section removed for LSC.

The relative amount of dodecylbenzene sulfonate degradation products in the water was determined by a modified solid-phase extraction technique [24]. Frozen water samples were thawed, and three 1-ml samples were taken for radioactivity determination. Fifteen milliliters of water were passed through a C_2 cartridge (Maxi-Clean C_2 Cartridge; Altech Associates, Deerfield, IL, USA) that had been preconditioned with 5 ml of methanol followed by 5 ml of water. During the sorption process, three 1-ml eluent samples, representing degradation products, were collected for LSC. The parent dodecylbenzene sulfonate that was retained on the column was extracted by passing three 5-ml methanol aliquots through the column. The amount of parent dodecylbenzene sulfonate was determined by taking three 1-ml methanol samples for LSC. The extent of degradation was determined based on the total in the methanol representing parent compound and the total passing through the C_2 column representing degradation products.

Biotransformation of dodecylbenzene sulfonate by midges was not detected, but dodecylbenzene sulfonate was lost rapidly from the water due to degradation. The fractions of parent compound in the water were used to correct the total measured radioactivity in the water for kinetic modeling. The following one-compartment first-order kinetics model (Eqn. 1) was applied to estimate toxicokinetic parameters:

$$\frac{dC_a}{dt} = k_u \times C_w - k_e \times C_a \quad (1)$$

where C_a is contaminant concentration in whole organism (μ g/g), k_u is uptake rate constant (ml/g/h), C_w is contaminant concentration in water (μ g/ml), k_e is elimination rate constant (h^{-1}), and t is time (h).

The body residues from the uptake and elimination tests and concentrations of parent dodecylbenzene sulfonate or total dodecylbenzene sulfonate in water were simultaneously fit to Equation 1 to estimate toxicokinetic parameters [25].

Four- and 10-d toxicity bioassays

Twenty-four hours before test initiation, 400 third instar midges were removed from culture and placed in HSRW in an environmental chamber at 20°C and 14:10-h light:dark cycle. The next day, 500 ml HSRW were placed into eighteen 1-L beakers (six concentrations including the controls with three replicates per concentration). A mixture of ^{14}C - and ^{12}C -dodecylbenzene sulfonate sufficient to create five concentrations ranging from 1 to 8 mg/L and from 0.4 to 8 mg/L for 4- and 10-d tests, respectively, were added to each beaker. In the control, only methanol carrier solvent was added. The amount of methanol added, in this case, was equivalent to that at the highest exposure concentration ($\sim 400 \mu$ l/L). The beakers were stored in an environmental chamber for 2 h to allow equilibration and acclimation to the test temperature. Before adding the organisms, a 1-ml water sample was taken from each beaker and the radioactivity was determined by LSC. Twenty midges were added to each beaker. The beakers were

loosely covered with aluminum foil and returned to the environmental chamber. The exposure medium was changed every other day by transferring the midges to fresh media. The midges were fed before transfer to fresh media in the same manner as described for the uptake studies.

The beakers were examined visually for mortality twice daily. Dead midges were collected, blotted dry, weighed, and prepared for LSC as above. At the end of the exposure period, all midges, dead or alive, were collected, blotted dry, weighed, and radioactivity determined. Concentrations in the midges were calculated from the amount of radioactivity and the new specific activity determined from the isotopic dilution.

Partial life-cycle test

One day before the test, 600 second instar midges were removed from culture and placed in HSRW in an environmental chamber. The next day, 480 ml HSRW were placed into each of eighteen 1-L beakers, followed by 20 ml (~ 1 billion cells) of *C. vulgaris* solution and 30 mg of defatted trout chow. Then a mixture of ^{14}C - and ^{12}C -dodecylbenzene sulfonate was added to the beakers to create nominal concentrations of 0.04, 0.1, 0.2, 0.4, and 0.8 mg/L. Additional methanol (total methanol concentration 200 μ l/L) was added to each beaker, including beakers in the control, to correct for differences in the solvent among different exposures. The contents of the beakers were swirled with a glass rod and the beakers were stored in an environmental chamber for 2 h to facilitate equilibration. After 2 h, 1-ml water samples were taken from each beaker to confirm the concentration by LSC. In addition, 20 ml from each exposure concentration (5 ml from each of four beakers chosen at random) were collected and stored at $-80^\circ C$ for biodegradation. Then 30 second instar midge larvae were added to each beaker. The beakers were loosely covered with aluminum foil and returned to the environmental chamber set at 20°C and 14:10-h light:dark photoperiod. The water was exchanged every other day, and samples for concentration and degradation were taken as above.

Midges that were dead or metamorphosed to adults were collected and weighed. Dead and male midges were individually prepared for LSC as previously described. Female midges were dissected under a microscope and the ovarioles removed. The ovariole was broken open, and the ova from each ovariole were visually scored under a microscope. The test endpoints were developmental time from second instar to adult, number of ova/female, and weight of adults. At the end of the exposure, 15 midges from each group, i.e., pupae, males, and females, were sampled for biotransformation. Biotransformation was determined as described previously.

Data analysis

The toxicokinetic parameters, k_u and k_e along with 95% confidence intervals (CI), were determined using Scientist® [25]. The bioconcentration factor (BCF) was calculated as the ratio of k_u/k_e , and the half-life ($t_{1/2}$) was calculated from $\ln 2/k_e$. The 95% CIs for both BCF and $t_{1/2}$ were estimated by propagation of errors [26]. The median lethal residue (LR50) and median lethal concentration (LC50) along with 95% CIs were calculated by a Probit analysis [27]. Mortality data were corrected using Abbott's equation [27]. Concentrations for LC50 analyses used the time-weighted average exposure concentrations.

For the chronic data, the values from the same exposure were pooled and subjected to the general linear model analysis,

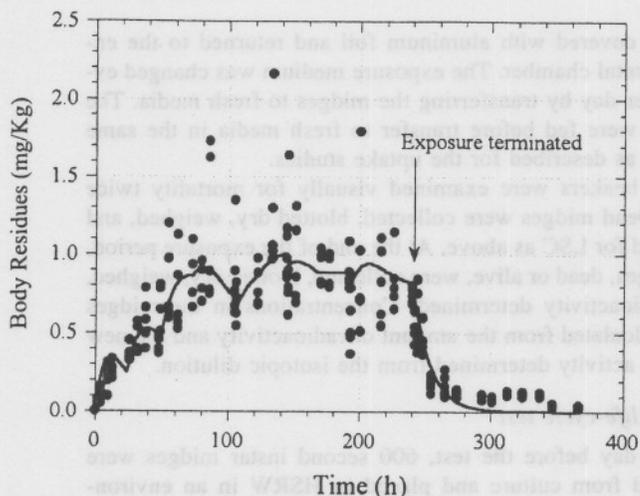


Fig. 1. Body residues and parent chemical concentrations in water were fit simultaneously to the one-compartment first-order kinetic model. The arrow indicates the start of the depuration phase.

followed by a Tukey test on SAS® [28] to determine the lowest-observed-effect residues for each endpoint. The null hypothesis of no difference was rejected at $p \leq 0.05$.

RESULTS

Toxicokinetics

The biotransformation potential measured in parallel with toxicokinetics revealed that dodecylbenzene sulfonate was not metabolized by the midges but rapidly degraded in water. In the exposure water, only $52.2 \pm 16.3\%$ (mean \pm standard deviation) of the dodecylbenzene sulfonate was parent compound after 24 h. However, the amount of the parent compound remaining in the water before successive water exchanges increased with time during the exposure period. Fitting the kinetics data using only parent dodecylbenzene sulfonate to the first-order toxicokinetic model results in a fit that did not show a smooth hyperbolic line due to the significant changes in water concentration (Fig. 1). The resulting toxicokinetic parameters (95% CI) estimated from the model were uptake rate constant, k_u , 17.5 (14.87–20.20) ml/g/h; elimination rate constant, k_e , 0.073 (0.062–0.085)/h; bio-concentration factor, BCF, 240 (164.1–316.2) L/kg; and biological half-life, $t_{1/2}$, 9.5 (8.0–11.0) h.

Four- and 10-d toxicity tests

The LC50s (95% CI) based on the time-weighted average dodecylbenzene sulfonate concentration in water for 4- and 10-d exposures were 2.2 (1.76–2.55) and 1.3 (0.49–3.05) mg/L, respectively (Tables 1 and 2). Median lethal residues (LR50s) were calculated based on body residues of dead and live midges separately. The 4-d LR50s were 1.17 (0.31–4.32)

and 0.72 (0.65–0.79) mmol/kg for dead and live midges, respectively, while the 10-d LR50s (95% CI) were 0.40 (0.14–1.10) and 0.18 (0.08–0.42) mmol/kg for dead and live midges, respectively (Tables 1 and 2). We found no statistically significant difference in the values for residues measured either in live or dead organisms. However, the lethal residue values based on dead organisms were more variable than those based on live organisms. The concentrations in dead midges were measured once the organism had succumbed to the toxicant, and the concentrations in live midges came only from the time point at the end of the experiment. We presumed that greater body residues were needed to produce toxicity when the exposure was of shorter duration, so organisms that died before the end of the exposure could be expected to have had greater body residues. Although the beakers were examined twice daily for mortality, dodecylbenzene sulfonate concentrations in dead organisms could have changed between the time of death and sampling due to absorption of water or loss of internal fluids as the body began to deteriorate. These factors could have contributed to the observed greater variability in LR50 values for dead organisms and may be responsible for the somewhat larger median values. Further, both LC50s and LR50s were lower in the 10-d toxicity test than in the 4-d test (Fig. 2).

Partial life-cycle test

Two partial life-cycle tests (tests 1 and 2) were performed. In both tests, significant mortality occurred (Table 3). The LR50s based on the body residues of live midges were 0.18 (0.09–1.64) and 0.21 (0.15–0.39) mmol/kg for tests 1 and 2, respectively. The LC50s based on the parent chemical in water were 0.8 (0.39–9.82) and 1.2 (0.84–2.11) mg/L for tests 1 and 2, respectively.

In test 2, in addition to mortality, deformities were also observed and were significantly increased at a body residue of 0.38 mmol/kg and above (Table 3, Fig. 3). The observed deformities included an abnormally long abdominal section with abnormal legs and small wings (Fig. 3d); underdeveloped ovarioles where ovarioles in the same female were different, with one short and thick and the other long and slim (Fig. 3e); and failure to emerge, with subsequent mortality (Fig. 3f). In addition to abnormalities pictured in Figure 3, midges with deformed wings (small or swollen with blood inside) were also frequently found. Some midges also contained very small pale ova, which were very difficult to recognize and count. The only abnormality observed in the controls was failure to emerge. The body residues required for significant deformities were larger than the 30-d LR50 by a factor of approximately two. We did not observe deformities in test 1.

Although female midge larvae are consistently heavier than male midge larvae, dodecylbenzene sulfonate at concentrations up to 0.75 mg/L (test 1) and 1.7 mg/L (test 2) did not adversely

Table 1. Four-day mortality data for dodecylbenzene sulfonate in the midge

Concn. in water (mg/L)	Dose in dead midges (mmol/kg)	Dose in live midges (mmol/kg)	Dead midges	Total	Observed mortality (%)	Corrected mortality (%)
Control	0	0	4	65	5.3	
0.8	0.29	0.56	9	42	21.4	16.9
2.1	1.07	0.59	20	46	43.5	40.2
4.7	1.68	1.11	55	64	85.9	85.1
7.9	1.98	1.34	56	59	94.9	94.6

Table 2. Ten-day mortality data for dodecylbenzene sulfonate in the midge

Concn. in water (mg/L)	Dose in dead midges (mmol/kg)	Dose in live midges (mmol/kg)	Dead midges	Total	Observed mortality (%)	Corrected mortality (%)
Control	0	0	7	75	9.33	—
0.37	0.13	0.06	28	74	37.8	30.4
1.07	0.44	0.25	39	74	52.7	47.0
2.17	0.63	0.26	45	75	60	55.2
3.33	1.02	0.32	48	75	64	59.7
7.09	1.38	0.62	71	75	94.7	94.0

affect body weight in either sex on day 28 (Table 4). At these concentrations, the average body residues ranged from 0.44 to 0.20 mmol/kg across sex and test.

Dodecylbenzene sulfonate-induced effects on development time were complex. Increased developmental time was observed in test 2 but not in test 1, and the changes in development time in test 2 did not follow a dose-response relationship (Table 5). In test 1, the developmental time of male midges from the second instar to adult ranged from 19.6 (SE 0.62) to 21.9 (0.54) d. Female developmental time ranged from 21.7 (0.34) to 22.7 (0.34) d, and no significant changes were found relative to controls. In test 2, male developmental time ranged from 18.2 (0.47) to 21.3 (0.42) d, while female developmental time ranged from 20.7 (0.30) to 23.2 (0.31) d. Significant increases in development time were found at 0.085 (SE 0.010) and 0.100 (0.007) mmol/kg for males and females, respectively. We intended to replicate exposures identically between the two tests, but the midges in test 2 accumulated higher concentrations, which is presumed to account for the observation of significant changes in development time in test 2 but not in test 1. The lack of a strong dose-response pattern suggests some type of interaction with other biological processes was occurring because the concentrations required to produce the changes in development time were of similar magnitude to the 30-d LR50 values.

No significant reductions in fecundity occurred in either test. In test 1, the number of ova (SE) ranged from 431.6 (21.0) to 473.6 (19.4), and in test 2, the number of ova ranged from 457.9 (24.1) to 493.5 (14.7) (Table 6). The controls values were 473.6 (19.4) and 491.3 (13.7) ova.

The 30-d partial life-cycle test was not designed to measure bioconcentration factors, but the fact that the midges appear to come to steady state within 60 h allowed the use of body-

burden data collected at 30 d to estimate bioconcentration. Across both tests and sexes, BCF values (i.e., concentration in the midge divided by the exposure concentration) ranged from 21 to 56. These values are substantially lower than those determined from the 10-d toxicokinetics assay.

DISCUSSION

Toxicokinetics and metabolism tests

Biodegradation significantly reduces toxicity of dodecylbenzene sulfonate. The LC50 of parent compound in a commercial linear alkylbenzene sulfonate preparation (C₁₂-C₁₄) to *Daphnia magna* was 3 mg/L [29]. However, the LC50 of commercial dodecylbenzene sulfonate in which the parent product was 80 to 90% metabolized was 20 to 35 mg/L [29]. A similar reduction in toxicity and eventual elimination of toxicity with increasing biodegradation was found for bluegills and snails [30]. Thus, the degree of linear alkylbenzene sulfonate biodegradation is important to correctly estimate its environmental hazard.

We were surprised to find that the midges did not biotransform linear alkylbenzene sulfonate because midges can biotransform other xenobiotics [31,32] and linear alkylbenzene sulfonate is readily biodegraded by microorganisms [33,34]. However, *Hyalella azteca* and bluegill sunfish also failed to biotransform linear alkylbenzene sulfonate (D. Versteeg, Procter and Gamble, Cincinnati, OH, USA, personal communication). The inability of *H. azteca* to biotransform dodecylbenzene sulfonate was confirmed in separate studies in our laboratory (Procter and Gamble, unpublished data). Therefore, the lack of biotransformation capability for dodecylbenzene sulfonate in midges was not unique to midges.

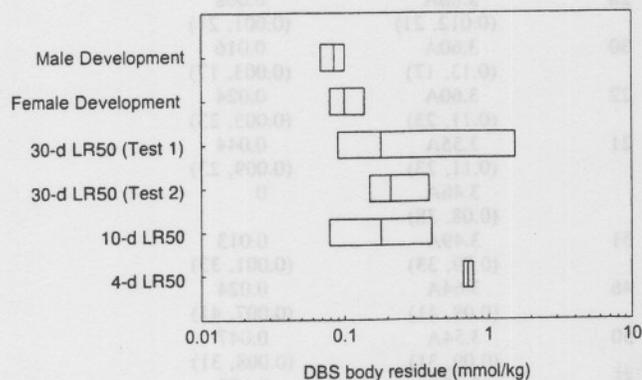


Fig. 2. A spectrum of effects of dodecylbenzene sulfonate (DBS) on the midges *Chironomus riparius*. The bars represent the 95% confidence intervals, with the median represented by the solid line in each bar.

Table 3. Mortality results in tests 1 and 2 and the number of deformities observed in each test. The values in a column with the same letters are not statistically different. SE represents standard error; *n* is number

Test	Water concn. (mg/L)	Mortality	Total	Body residue in mmol/kg (SE, <i>n</i>)	Deformed
1	0	14	57	0	—
	0.037	24	76	0.007 (0.0006, 24)	0
	0.10	39	84	0.024 (0.0018, 39)	0
	0.19	50	85	0.058 (0.0047, 50)	0
	0.38	43	79	0.072 (0.0078, 43)	0
	0.75	51	89	0.189 (0.0162, 51)	0
2	0	15	90	0	3A
	0.09	17	90	0.015 (0.003, 17)	2A
	0.19	26	90	0.031 (0.005, 26)	6AB
	0.33	32	96	0.005 (0.018, 32)	4A
	0.63	39	98	0.097 (0.020, 39)	7AB
	1.72	61	97	0.379 (0.108, 61)	13B

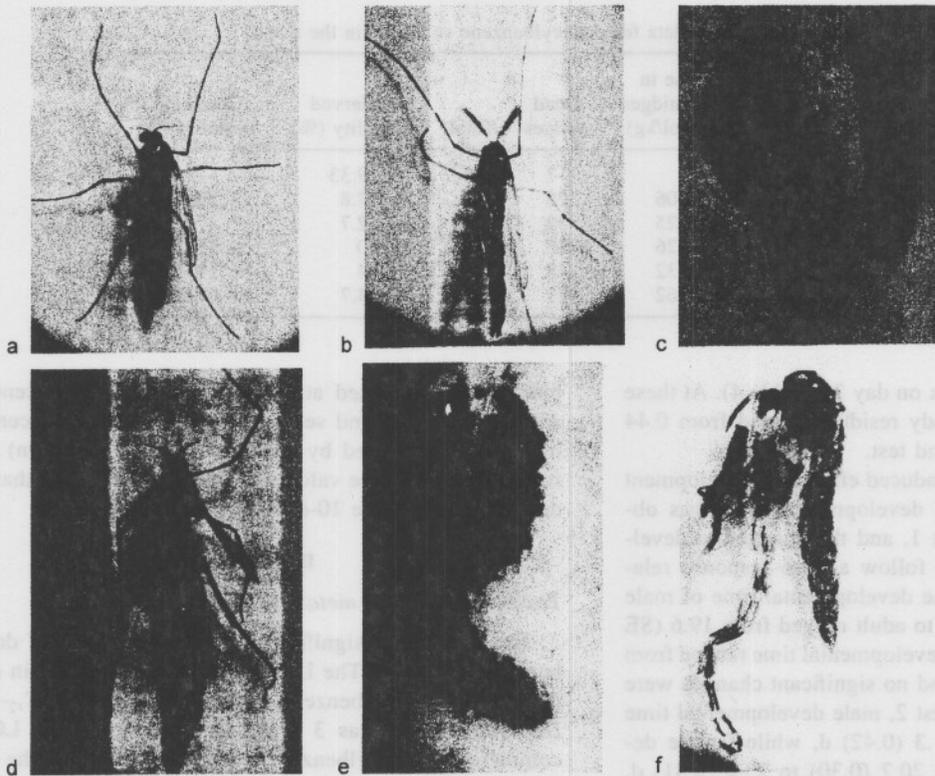


Fig. 3. Pictures of the midges: (a) a normal female midge, (b) a normal male midge, (c) a normal ovary at $\times 100$ magnification, (d) an abnormal female with long abdominal portion, (e) abnormal egg masses with unmatched sizes, and (f) a midge that failed to emerge.

The relatively rapid loss of dodecylbenzene sulfonate from water was suspected to be due to microbial biodegradation and was accounted for in determining toxicokinetic and LC50 values. Because of the experimental design with frequent water

exchange, the extent of dodecylbenzene sulfonate loss between exchanges declined as the experiment progressed. This probably was due to continual dilution of the microbial community as the water was exchanged.

Table 4. Body weights and bioconcentration factors (BCF) along with corresponding body residues for midges in tests 1 and 2. The numbers with the same letters in the same column of each test are not statistically different. SE represents standard error; n is number

Test	Concn. (mg/L)	Male midges			Female midges		
		BCF	Weight in mg (SE, n)	Body residue in mmol/kg (SE, n)	BCF	Weight in mg (SE, n)	Body residue in mmol/kg (SE, n)
1	Control		2.04A (0.094, 13)	0		3.57A (0.14, 4)	0
	0.037	48	2.12A (0.102, 11)	0.005 (0.001, 11)	29	3.52A (0.12, 18)	0.003 (0.0004, 18)
	0.010	64	2.21A (0.084, 16)	0.018 (0.007, 16)	28	3.60A (0.012, 21)	0.008 (0.001, 21)
	0.19	47	2.21A (0.082, 17)	0.025 (0.003, 17)	30	3.60A (0.13, 17)	0.016 (0.003, 17)
	0.38	34	2.08A (0.098, 12)	0.036 (0.012, 12)	22	3.60A (0.11, 23)	0.024 (0.005, 23)
	0.75	22	2.25A (0.098, 12)	0.046 (0.016, 12)	21	3.55A (0.11, 23)	0.044 (0.009, 23)
	2	Control		1.89A (0.065, 29)	0		3.46A (0.08, 38)
0.09		35	1.90A (0.058, 36)	0.009 (0.007, 36)	51	3.49A (0.09, 33)	0.013 (0.001, 33)
0.19		48	2.00A (0.082, 18)	0.025 (0.010, 18)	46	3.64A (0.08, 41)	0.024 (0.007, 41)
0.33		36	1.96A (0.061, 33)	0.034 (0.007, 33)	50	3.54A (0.09, 31)	0.047 (0.008, 31)
0.63		48	2.16A (0.087, 16)	0.085 (0.010, 16)	56	3.45A (0.08, 42)	0.100 (0.007, 42)
1.72		29	1.93A (0.097, 13)	0.142 (0.11, 13)	41	3.59A (0.13, 15)	0.200 (0.015, 15)

Table 5. Developmental time along with corresponding body residues of midges (*Chironomus riparius*) in tests 1 and 2. The numbers with the same letters in the same column of each test are not statistically different. SE is standard error; n is number

Test	Concn.	Male midges		Female midges	
		Days (SE, n)	Body residue in mmol/kg (SE, n)	Days (SE)	Body residue in mmol/kg (SE, n)
1	Control	20.4A (0.52, 17)	0	22.6A (0.44, 14)	0
	0.037	19.8A (0.65, 11)	0.005 (0.001, 11)	22.4A (0.39, 18)	0.003 (0.001, 21)
	0.01	21.9A (0.54, 16)	0.018 (0.007, 16)	22.6A (0.35, 22)	0.008 (0.001, 21)
	0.19	19.5A (0.52, 17)	0.025 (0.003, 17)	22.7A (0.39, 18)	0.016 (0.003, 17)
	0.38	20.2A (0.62, 12)	0.036 (0.012, 12)	22.7A (0.34, 23)	0.024 (0.005, 23)
	0.75	19.6A (0.62, 12)	0.046 (0.016, 12)	21.7A (0.34, 24)	0.044 (0.009, 23)
	2	Control	19.6ABC (0.3, 30)	0	21.4AB (0.3, 40)
0.09	18.6BE (0.3, 36)	0.009 (0.007, 36)	20.7B (0.3, 33)	0.013 (0.001, 33)	
0.19	19.4ABCDE (0.4, 18)	0.025 (0.010, 18)	22.1AC (0.3, 42)	0.024 (0.007, 41)	
0.33	20.4ACD (0.3, 33)	0.034 (0.007, 33)	22.3A (0.3, 33)	0.047 (0.008, 31)	
0.63	21.3D (0.4, 16)	0.08 (0.010, 16)	23.2C (0.3, 42)	0.100 (0.007, 42)	
1.72	18.2BE (0.5, 13)	0.142 (0.011, 13)	22.6AC (0.5, 15)	0.200 (0.015, 15)	

Typical application of a first-order toxicokinetics model involves a constant infusion model with three assumptions, which are constant contaminant concentration in water, no biotransformation, and constant toxicokinetic parameters. In the present study, the dodecylbenzene sulfonate concentrations were not constant because of rapid microbial degradation in water. The variability in water concentrations for this study included removal of the midges from exposure after 1 h of feeding. The second assumption (no dodecylbenzene sulfonate biotransformation) was confirmed through measurement. The third assumption (constancy of the toxicokinetics parameters, k_u and k_e) could not be confirmed or rejected based on our experimental design. To fit the data, however, these parameters

must be assumed constant over the course of the experiment. The fluctuation in water concentration due to the loss of dodecylbenzene sulfonate through biodegradation and the exchange into uncontaminated water during the 1-h daily feeding periods eliminates the ability to exactly integrate the differential equation describing the uptake and elimination. Thus, the model must be fit by numerical integration. We fit the data using a fourth-order Runge-Kutta approach [25]. Fitting the data with the variation in water concentration including the interruption in exposure during the feeding period results in a zigzagging pattern of accumulation (Fig. 1). This fit shows the influence of elimination on the overall progress toward steady state as the exposure fluctuates. Despite the fluctuation in exposure, the model demonstrates that the midges approached steady state within about 60 h. The time to reach steady state may be estimated by modifying a half-life equation, i.e., $t_{1/2} = \ln 2/k_e$, and the time to reach 99% of steady state was similarly estimated as 61.4 h. Thus, the 4- and 10-d mortality studies represent midges that are at steady state with their exposure.

Literature values for the toxicokinetics of dodecylbenzene sulfonate in the midges were not available for comparison. However, linear alkylbenzene sulfonate toxicokinetic values in different aquatic organisms were similar to that of the midge. For instance, the k_u , k_e , and BCF of C₁₂, 2-phenyl linear alkylbenzene sulfonate (dodecylbenzene sulfonate) in *Hyalella azteca* were 5.0 ml/g/h, 0.015/h, and 332 L/kg, respectively [35]. Similarly, in fathead minnows, the k_u (% error), k_e (% error), and steady-state BCF (SD), estimated by dividing the linear alkylbenzene sulfonate concentration in fish by the water concentration at steady state, were 4.8 (0.25) ml/g/h, 0.046 (0.83)/h, and 47.6 (26) L/kg, respectively [20]. The kinetics from these two studies compare favorably with the midge, while the overall kinetics suggest a BCF of 240 compared with

Table 6. Fecundity changes along with corresponding body residues of midges in tests 1 and 2. The numbers with the same letters in the same column of each test are not statistically different. SE is standard error; n is number

Concn.	Test 1		Test 2	
	Ova (SE, n)	Body residue in mmol/kg (SE, n)	Ova (SE, n)	Body residue in mmol/kg (SE, n)
Control	473.6A (19.4, 13)	0	491.3A (13.7, 3.7)	NA
1	431.6A (21.0, 11)	0.003 (0.0004, 18)	477.2A (22.7, 14)	0.013 (0.001, 33)
2	467.4A (15.6, 20)	0.008 (0.001, 21)	491.3A (16.3, 26)	0.024 (0.007, 41)
3	448.1A (18.0, 15)	0.016 (0.003, 17)	473.9A (16.3, 26)	0.047 (0.008, 31)
4	460.3A (15.2, 21)	0.024 (0.005, 23)	493.5A (14.7, 32)	0.100 (0.007, 42)
5	447.3A (17.5, 16)	0.044 (0.009, 23)	457.8A (457.8, 12)	0.200 (0.015, 15)

the two organisms above, with BCF values ranging from 48 to 332. This range of BCF values for linear alkylbenzene sulfonate is similar to that reported in daphnids when converted to a wet-weight basis (100–800) [36]. Thus, despite the fact that 2-phenyl dodecylbenzene sulfonate was used in our studies, the dodecylbenzene sulfonate toxicokinetics for the midge are similar to those reported for other small aquatic organisms, i.e., they are characterized by relatively slow accumulation from water compared with, e.g., the uptake of DDE by *C. riparius* [37].

Methanol was used to enhance the solubility of dodecylbenzene sulfonate in toxicity and toxicokinetic studies. While it is possible for solvents to confound estimates of bioconcentration and affect bioavailability, we consider the probability of such interference to be negligible. Effects of solvents on partitioning behavior occur at mole fractions of approximately 10% [38]. Further, Landrum [39] has shown that cosolvents were not effective in reducing uptake of polycyclic aromatic hydrocarbons until solvent concentrations exceeded 100 ml/L. Our methanol levels were substantially lower, making it unlikely that the presence of methanol affected our results.

Mortality

Four toxicity tests with different durations (i.e., 4-, 10-, and two 30-d) were used to study the relationship between LR50 and exposure duration. Currently, in aquatic toxicology, it is recommended to allow sufficient time for steady state between water and body residues so that toxicity is not limited by toxicokinetics. This allows one to determine the incipient LC50 [40]. After steady state is established, a toxicity index such as LC50 should remain constant, even though exposure duration increases, if the mechanism of toxic action does not change. Combining the incipient LC50 with the steady-state BCF leads to an estimated critical body residue or the body residue to kill 50% of test population. The latter is reported to be a constant value, i.e., 2 to 8 mmol/kg, for nonpolar narcotics (anesthetics) for the fish with 5% lipid [14]. Recently, however, inconsistency of contaminant toxicity with increasing exposure duration has been observed both for compounds with specific modes of toxic action and nonpolar narcotics (anesthetics) [41–44]. For example, body residues of 2,3,4,5-tetrachloroaniline in dead guppies decreased within the first 48 h, after which the body residues reached a constant value of approximately $0.7 \pm 0.5 \mu\text{mol/kg}$ [41]. A decreasing trend in the internal lethal body residue of halobenzenes was found for *Gambusia affinis* [42]. Recently, the toxicity of polycyclic aromatic hydrocarbon to *H. azteca* was measured at lower body residues with increasing duration of exposure [45]. For toxic chemicals, toxicity is defined not only by exposure dynamics but by the toxicodynamics of the compound as well. This understanding led to the following formulation of a damage-assessment model for evaluating the hazard represented by exposure to aquatic contaminants [45]:

$$\text{LR50}(t) = \frac{D_L}{k_a} \frac{1}{(1 - e^{-k_e t})} \times \left(\frac{e^{-k_r t} - e^{-k_e t}}{k_r - k_e} + \frac{1 - e^{-k_e t}}{k_r} \right) \quad (2)$$

where D_L is the critical level for damage required to produce 50% mortality, k_a is the rate of damage, k_r is the rate of damage repair, k_e is the elimination rate constant, and t is time. If the

time-dependent LR50 data are fit to the above equation, $D_L/k_a = 50.6 \pm 9.1 \text{ mmol/kg/h}$ and $k_r = 0.0026 \pm 0.002 \text{ per h}$. The coefficient of determination for the model was 0.91. Thus, with this model, the time-dependent toxicity of dodecylbenzene sulfonate in the midge can be estimated for various exposure durations.

Comparing the LR50 values of dodecylbenzene sulfonate with the critical body residue values reported for differing modes of action suggests that our 4-d LR50 of 0.7 (0.65–0.79) mmol/kg is within the range proposed for the acute critical body residue of polar narcotic chemicals (0.6–1.9 mmol/kg) [13]. Further, the 10-d LR50 0.18 (0.08–0.42) mmol/kg is lower than the range for acute toxicity as a result of longer exposure and is within the range for chronic toxicity of polar narcotics [13]. The slight reduction in LR50 for midges in this work may be due to lower lipid content of midges, which varied from 1.53 to 3.53% of wet weight in larval and adult midges, respectively [46]. In contrast, 4-d LR50s of 0.6–1.9 mmol/kg were determined in fish with 5% lipid.

The lethal body residues for polar narcotics (0.6–1.9 mmol/kg) in this study and others fell below the lethal body residues identified for nonpolar narcotics (2–8 mmol/kg) for fish with 5% lipid, prompting the suggestion that the two classes of compounds may have distinct modes of action. However, van Wezel et al. [17] have shown that the amount of chemical needed at the target site to produce narcosis is similar for polar and nonpolar narcotics. The distribution of the two classes of contaminants in different types of lipids does vary, with polar narcotics preferentially partitioning into polar lipids such as phospholipids [47]. In contrast, nonpolar narcotics predominate in nonpolar lipids, such as triglycerols and cholesterol. Because the target site for narcosis is believed to be the phospholipid fraction [17] and because polar narcotics have a greater affinity for polar lipids than nonpolar narcotics, it may appear that the dose of polar narcotic needed to cause lethality is lower. However, the amount of nonpolar narcotic in the phospholipid target site needed to cause death is the same as for polar narcotics. A significant amount of nonpolar narcotic will partition into the nonpolar lipid and thus contribute little to toxicity while raising the apparent lethal body burden that is calculated on a whole-body basis.

The LR50 values of dodecylbenzene sulfonate in midges from this work also are similar to reported values in other species. The measured body residue of dead *H. azteca*, e.g., was 2.8 mmol/kg after a 4-d exposure [35], which is similar to the 4-d LR50 in the midge. Further, the 10-d LR50 for dodecylbenzene sulfonate was statistically the same as the 10-d LR50 for 2,4,5-trichlorophenol (0.113 [0.097–0.136] mmol/kg) in *C. riparius* [48]. Trichlorophenol is another compound that is often thought to act by polar narcosis. Thus, while the toxicity of linear alkylbenzene sulfonate to some species seems to manifest as a nonpolar narcotic (anesthetic) in the midge, the toxicity of dodecylbenzene sulfonate seems to be more in the range of toxicity of polar narcotics.

In 4- and 10-d tests, LR50 values calculated from the body residues of dead organisms were larger but not statistically different from those calculated from body residues of live midges. Further, the 95% confidence intervals for the LR50s based on dead midges are consistently wider than those based on live midges. Larger body residues among dead organisms have also been found for *H. azteca* and *Corbicula fluminea* [35]. The larger LR50 values based on dead midges may be attributable to three factors, i.e., the variation in toxicokinetics

between dead and live midges, the sampling time represented, and the accuracy of the measurement of concentrations within dead organisms. The effects of these factors are explained below.

The first factor that may increase LR50s in dead midges is that, within a population, some individuals may accumulate the contaminant more rapidly, either through more rapid uptake or slower elimination. Either of these conditions would allow the compound to accumulate to lethal levels, thereby resulting in differential effect levels for live versus dead organisms. This hypothesis cannot be tested within the current experimental framework. The second factor that could have contributed both to higher concentrations and greater variability is the sampling time for dead organisms. The concentrations for the live organisms were data from a single sampling point at the end of the experiment, while concentrations for the dead organisms were determined throughout the duration of the experiment. Organisms apparently require greater concentrations to succumb at short exposure times, which may increase the body-residue estimate and the variability in the body residues for organisms succumbing early in the exposure. Finally, the third factor that may have increased body residues in dead midges was that, although dead organisms were removed twice daily, invertebrates degrade rapidly after death. Partial degradation could have resulted in either greater or lower measured concentrations, depending on when the organisms died and their state of decomposition.

The toxicity of dodecylbenzene sulfonate can further be compared with other studies through the LC50. The LC50 value in our study, 2.16 mg/L for the 4-d exposure, was similar to literature values. In a 72-h exposure, 88.5% of newly hatched *C. riparius* larvae survived exposure to a linear alkylbenzene sulfonate concentration of 1.0 mg/L, although 100% mortality occurred at 4.7 mg/L [49]. Thus, the 72-h LC50 would lie in the same range as our 4-d LC50, even considering differences in exposure time (72 vs 96 h) and life stages (newly hatched vs third instar).

Sublethal endpoints

The partial life-cycle tests were performed in duplicate and produced generally comparable data when evaluated on a body residue basis. The LR50 values were statistically the same. The sublethal endpoints also were consistent based on body residue concentrations between the two tests, with no significant changes in growth or reproduction in either test and no changes in developmental time for male or female midges at dodecylbenzene sulfonate concentrations lower than 0.085 mmol/kg (Table 3). Only in test 2 were significant changes in development time found and only at high dodecylbenzene sulfonate body residues (Table 1). Where chronic sublethal effects were observed, the dodecylbenzene sulfonate concentrations in the midges were at or above the range of concentrations causing 50% chronic mortality. It is not surprising that highly stressed organisms would show responses other than mortality when the concentrations were in the lethal range.

As shown by this study and others (D. Versteeg, personal communication), survival was often more sensitive than other endpoints for dodecylbenzene sulfonate intoxication in midges. However, fecundity and development time were sensitive for describing the effects of hexachlorobiphenyl on the midge [46]. While exposure to hexachlorobiphenyl produced significant sublethal effects at concentrations lower than those required to cause mortality, no statistically significant effects

were observed for body weight with hexachlorobiphenyl [46], which was similar to our results for dodecylbenzene sulfonate. The failure to produce significant changes in body weight may be due to insect-specific physiology. Insects must attain a specific body weight to pupate [50]. Therefore, pupal midges should be similar in weight, and following emergence, adults should be similar because pupal midges do not feed; they survive on the energy stored during the larval stage.

The ecological significance of LR50s and the lowest-observed-effect residues for development-time increase is not yet known. Assuming that dodecylbenzene sulfonate is representative of linear alkylbenzene sulfonate, then body residues could be estimated from the BCF and compared with the toxicity data generated in this study. Two estimates of BCF values were determined from this work, one from the toxicokinetics exposures with no food and one estimated at the end of the chronic test conducted with feeding. The values differed by a factor of about six (240 L/kg for water only vs 39 L/kg in chronic exposures), with the water-only exposures generating the higher estimate. This is not surprising. A recent investigation has shown that, when organisms eliminate contaminants in the presence of an organic medium, the medium will adsorb eliminated contaminants and thereby enhance the elimination rate of the organism [51]. The midges in the chronic study had a consistent supply of fresh food that could sorb the eliminated dodecylbenzene sulfonate, possibly leading to an increase in the elimination rate and reduction in the BCF. This possibility is consistent with other studies that have demonstrated that, when organisms are allowed to feed during testing, they have more rapid elimination rates (e.g., [52]). Thus, the BCF value measured for *Chironomus* in the absence of food probably represents a maximum value. Because the midges under the chronic test conditions likely better represent midges in the environment, we used the maximum BCF of 56 L/kg and an environmental concentration of 0.75 mg/L, which yield an estimated body residue of 42 mg/kg or 0.12 mmol/kg. The latter is well below the 10-d LR50 but within the range of the 30-d LR50. This estimate is a worst-case scenario, particularly given that dodecylbenzene sulfonate is one of the most strongly bioaccumulated congeners in the linear alkylbenzene sulfonate mixture [20]. Other investigators have reported linear alkylbenzene sulfonate concentrations of 0.05 to 0.2 mg/L in outfalls from wastewater treatment plants [53], 0.0001 to 0.0028 mg/L in Mississippi (USA) river water [54], 0.002 to 0.081 mg/L in effluent of a wastewater treatment plant with an activated sludge system, and 0.004 to 0.094 mg/L in effluent of a wastewater treatment plant with a trickling filter system [55]. All of these concentrations would allow bioaccumulation to a level below any of the effects levels observed in this study.

Based on a U.S. monitoring survey and river dilution models, McAvoy et al. [5] predicted that 90% of river waters would contain linear alkylbenzene sulfonate concentrations <0.185 mg/L at critical low flow. Use of a BCF of 240, which was observed during the BCF portion of this study, results in a body residue of approximately 0.1 mmol/kg, which is in the range at which effects were observed. However, use of the maximum 30-d BCF value of 56 results in a body residue of 10.3 mg/kg, or approximately 0.03 mmol/kg. This is well below the lowest effect level of dodecylbenzene sulfonate from this study, suggesting that chronic effects on midges should not be observed in most river waters of the United States even at low flow (Fig. 2). This is supported by measured total linear alkylbenzene sulfonate concentrations of 0.005 to 0.0039

mmol/kg in caged *Corbicula* and caged and feral fish exposed to effluent from an activated sludge wastewater treatment plant (D.J. Versteeg, personal communication).

Acknowledgement—This work was funded in part by the Ohio Sea Grant College Program, Project E/IF-1 under National Oceanic and Atmospheric Administration grant NA86RG0453, and by Procter and Gamble (Cincinnati, OH, USA). We thank Procter and Gamble for the donation of the ^{14}C and ^{12}C linear alkylbenzene sulfonate. This is Great Lakes Environmental Research Laboratory Contribution 1240.

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