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ARTICLE

TOXICITY OF SODIUM DODECYL SULFATE TO FEDERALLY THREATENED AND PETITIONED FRESHWATER MOLLUSK SPECIES

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ABSTRACT

Anthropogenically caused physical and chemical habitat degradation, including water pollution, have caused dramatic declines in freshwater mollusk populations. Sodium dodecyl sulfate (SDS), a surfactant with no USEPA Water Quality Criteria (WQC), is commonly used in industrial applications, household cleaners, personal hygiene products, and herbicides. In aquatic habitats, previous SDS studies have associated deformities and death to mollusks found in these systems. The objective of this study was to determine EC₅₀ values for two freshwater juvenile unionids (*Villosa nebulosa* and *Hamiota perovalis*) and two freshwater caenogastropods (*Leptoxis ampla* and *Somatogyrus* sp.) endemic to the Mobile River Basin, USA, to SDS. Using the Trimmed Spearman-Kärber method, EC₅₀ values were calculated. Results found that EC₅₀ values were: *V. nebulosa* = 14,469 µg/L (95% CI: 13,436 – 15,581 µg/L), *H. perovalis* = 6,102 µg/L (95% CI: 4,727 – 7,876 µg/L), *Somatogyrus* sp. = 1,986 µg/L (95% CI: 1,453 – 2,715 µg/L), and *L. ampla* = 26 µg/L (95% CI: 6 – 112 µg/L). Freshwater gastropods were more sensitive to SDS than freshwater unionids. *Leptoxis ampla* was the most sensitive species tested and had such a low EC₅₀ value that more protective regional criteria may be required. Therefore, future research should include additional testing on mollusk species, particularly regionally isolated species that may display increased sensitivity.

KEY WORDS - SDS, threatened, mollusk, Mobile River Basin, Water Quality Criteria

INTRODUCTION

In North America, freshwater mollusks are the most imperiled aquatic fauna with 74% of 703 identified gastropods imperiled, followed by unionids with 72% of 298 identified species imperiled (Wilcove and Master 2008; Johnson et al. 2013). Many freshwater mollusk species are highly endemic, particularly in the Mobile River Basin, USA, which includes 139 endemic freshwater mollusk taxa (34 unionids and 105 gastropods) (Neves et al. 1997; Williams et al. 2008; Ó Foighil et al. 2011). Stenotypic species are often underrepresented in traditional toxicity testing that normally utilize broad ranging

species, usually distributed across multiple drainage basins (Ó Foighil et al. 2011; Wang et al. 2010; Wang et al. 2011).

Declines in freshwater gastropod and unionid populations are attributed to increases in the human population, leading to alteration or destruction of habitat both physically and chemically (Villemela et al. 2004; Johnson et al. 2013). Pollution is ranked as the second leading cause of stream impairment (USEPA 2009), following physical habitat alteration (Neves et al. 1997). Toxicity testing is important in protecting organisms by providing information on specific pollutant effects, such as reduced survival and growth or inhibited biological processes on a particular life stage (American Society for Testing and Materials (ASTM) 2013). Using data from these tests, criteria inclusive of imperiled organisms can be established to help protect remaining populations.

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Sodium dodecyl sulfate (SDS), a surfactant, is the most widely used synthetic organic chemical found in detergents, shampoos, cosmetics, household cleaners, herbicides, and dispersants used in oil-spill cleanups (Cowan-Ellsberry et al. 2014). Sodium dodecyl sulfate is an alkylsulfate with sodium as the counter ion with a chain length of 12 carbons (Cowan-Ellsberry et al. 2014). Sodium lauryl sulfate (SLS) and SDS are often used synonymously in reporting of product ingredients (Singer and Tjeerdema 1993; NIH, 2014). Concentrations >67% SLS (active ingredients) can be found in household products, dispersants, and herbicides (Lewis 1991; Singer and Tjeerdema 1993; Kegley et al. 2014). Sodium dodecyl sulfate is also used in the cleanup of polycyclic aromatic hydrocarbons (e.g., oil and gas products). The major exposure route for SDS to aquatic environments is through contaminated waters, sediments, or soils, which threatens drinking water supplies or organisms living in these environments (Singer and Tjeerdema 1993). Contamination of groundwater by surfactants is caused primarily by leaching from industrial and municipal sewage systems, but can also be introduced to the environment by domestic and industrial effluents from discarded cleaning products (Singer and Tjeerdema 1993; Chaturvedi and Kumar 2010). In the United States, per capita detergent consumption is about 10 kg/year (Rebello et al. 2014), but consumption declined 3.9% per year during 2008 – 2013 (Cowan-Ellsberry et al. 2014). However, in 2008, 76% of alkylsulfate consumed in North America were found in household laundry detergents (59%) and personal care products (17%) (Cowan-Ellsberry et al. 2014).

Sodium dodecyl sulfate is not currently monitored in water systems or listed as a ground water contaminant (Kegley et al. 2014). Other surfactants with similar uses are monitored (reviewed by Rebello et al. 2014 and Singer and Tjeerdema 1993). In the United Kingdom, surfactant concentrations in surface waters have been recorded as high as 416 $\mu\text{g/L}$ (Fox et al. 2000), while sewage effluents have had concentrations documented up to 1,090 $\mu\text{g/L}$ (Holt et al. 1989). Treated sludge has been found to have concentrations of linear alkylbenzene sulfonate as high as 30,200,000 $\mu\text{g/kg}$ (dry weight) (Berna et al. 1989). All monitored concentrations for sulfates exceed the predicted no-effect concentration value (250 $\mu\text{g/L}$) for surfactants by van de Plassche et al. (1999). In Massachusetts, the Town River had reported concentrations between 40 $\mu\text{g/L}$ and 590 $\mu\text{g/L}$ (Lewis and Wee 1983), while other major rivers in the United States had reported surfactant concentrations that ranged from 10 $\mu\text{g/L}$ to 3,300 $\mu\text{g/L}$ (A.D. Little Co. 1981) or 10 $\mu\text{g/L}$ to 40 $\mu\text{g/L}$ (Hennes and Rapaport 1989).

Sodium dodecyl sulfate was formally classified as ‘environmental friendly’ based on its readily biodegradable and low bioaccumulation properties, meaning it does not persist long in the environment (Belanger et al. 2004). However, some studies have suggested that SDS can be lethal in acute exposures (e.g., 19,040 $\mu\text{g/L}$ for *Utterbackia imbecilis*, Keller 1993; summarized in Singer and Tjeerdema 1993; summarized in Kegley et al. 2014; Table 2). Because of

Table 1. Data from acute toxicity trials using SDS on four mollusk species, including toxicant concentrations, number of dead organisms, and number of organisms exposed.

Species	Concentration ($\mu\text{g/L}$)	Dead	Number exposed
<i>Villosa nebulosa</i>	Control	3	10
	5,000	1	30
	10,000	1	30
	15,000	16	30
	20,000	28	30
	30,000	30	30
<i>Hamiota perovalis</i>	Control	0	10
	5,000	11	30
	10,000	16	30
	15,000	17	30
	20,000	28	30
	30,000	30	30
<i>Leptoxis ampla</i>	Control	0	10
	1	3	30
	10	11	30
	100	21	30
	1,000	23	30
	10,000	18	30
<i>Somatogyrus</i> sp.	Control	0	10
	1,000	7	30
	3,000	20	30
	10,000	30	30
	30,000	30	30
	100,000	30	30

its fast acting, nonselective, and consistent toxicity, SDS is commonly used as a reference toxicant in toxicity tests (USEPA 2002). Developmental abnormalities in *Illyanassa obsoleta* embryos, such as incomplete or inhibited formation of lobe-dependent structures (e.g., foot, operculum, and eyes) of gastropods have been attributed to SDS exposure (treatments ranging from 10,000 – 30,000 $\mu\text{g/L}$) (Render 1990). Tarazona and Nuñez (1987) reported that SDS exposure significantly decreased shell weights in lymnaeid gastropods and impeded normal shell deposition ($\text{EC}_{50} = 540 \mu\text{g/L}$ for *Lymnaea vulgaris* and 610 $\mu\text{g/L}$ for *Physa heterostropha*). When exposed to SDS ($\text{EC}_{50} = 31,400 \mu\text{g/L}$), *Corbicula fluminea* displayed avoidance behaviors and gill damage which decreased oxygen consumption and reduced siphoning activity (Graney and Giesy 1988).

Previous studies suggest early life stages of unionids are more sensitive than later life stages or other commonly used aquatic test organisms (Keller et al. 2007; Augspurger 2013). Until recently, freshwater unionid toxicity tests were not included in establishing Water Quality Criteria (WQC) due to limited information available (e.g., life cycle, host fish, sensitivity, populations), and the inability to culture them in sufficient numbers to support testing needs (Keller et al. 2007).

Table 2. Median effective concentrations (EC₅₀) for 96 h acute toxicity tests of sulfate surfactants on mollusk species.

Mollusk species	Common name	Age	Hardness (mg CaCO ₃ /L)	96 h EC ₅₀ (µg/L)	References
Bivalves					
<i>Corbicula fluminea</i>	Asiatic Clam	NR	NR	31,400	Graney and Gisey 1988
<i>Utterbackia imbecillis</i>	Paper Pondshell	Juveniles	NR	19,040 ^a	Keller 1993
<i>Hamiota perovalis</i>	Orangenacre Mucket	Juveniles	43	6,102	Current Study
<i>Villosa nebulosa</i>	Alabama Rainbow	Juveniles	43	14,469	Current Study
Gastropods					
<i>Physa heterostropha</i> *	Pewter Physa	NR	NR	34,161	Patrick et al. 1968
<i>Lymnaea peregra</i> *	Wandering Pond Snail	NR	NR	15	Misra et al. 1984
<i>Lymnaea vulgaris</i> *	Great Pond Snail	Juveniles	35.6	540	Tarazona and Nuñez 1987
<i>Lymnaea vulgaris</i> *	Great Pond Snail	Juveniles	35.6	610	Tarazona and Nuñez 1987
<i>Leptoxis ampla</i>	Round Rocksnail	Juveniles	43	26	Current Study
<i>Somatogyrus</i> sp.	Cahaba Pebblesnail	Adults	43	1,986	Current Study

NR = not reported

^a = 48 h LC₅₀ value

* = pulmonate gastropod

Similarly, caenogastropods (respire using a gill or ctenidia) are considered among the most sensitive aquatic organisms to contaminants (Besser et al. 2009), but are rarely used for toxicity testing due to slow growth and low reproductive rates, which make them difficult to culture or test in the laboratory (Besser et al. 2009). New propagation and rearing techniques have been recently developed that allow sufficient numbers of organisms to support formal toxicity testing (Barnhart 2006). The goal of the current study was to evaluate acute SDS exposure to four Mobile River Basin endemic mollusks, two freshwater juvenile unionids and gastropods. These data may eventually contribute to the development of a specific WQC for SDS for freshwater mollusks.

METHODS

Two lotic freshwater unionids (*Hamiota perovalis* and *Villosa nebulosa*) and two lotic freshwater caenogastropods (*Leptoxis ampla* and *Somatogyrus* sp.) endemic to the Mobile River Basin were used. *Hamiota perovalis* (Orangenacre Mucket) and *Leptoxis ampla* (Round Rocksnail) were federally listed as threatened under the Endangered Species Act (ESA) (USFWS 1993, 1998). *Villosa nebulosa* (Alabama Rainbow) has been formally petitioned for federal protection under the ESA (Center for Biological Diversity 2010). The specific taxonomic position of *Somatogyrus* sp., Cahaba Pebblesnail, is unclear (E.E. Strong and P.D. Johnson, Alabama Aquatic Biodiversity Center (AABC), personal observation).

Unionids were propagated by the AABC, Marion, Alabama, using host-fish infections and standard culturing methods (Barnhart 2006). *Villosa nebulosa* adults (n=6) were collected from South Fork Terrapin Creek in Celburne County, Alabama while *H. perovalis* adults (n=4) were collected from Rush Creek in Winston County, Alabama. Both species used

Largemouth Bass (*Micropterus salmoides*) for transformation. Unionids were fed a diet of Nanochloropsis, Shellfish diet (Reed Mariculture) at a concentration of ~50K cells/mL.

For gastropods, *Leptoxis ampla* was propagated by AABC, while *Somatogyrus* sp. was collected from the Cahaba River (Latitude: 32° 57.577' N, Longitude: 87° 08.441' W). The AABC commonly uses this location for reintroductions, restocking, and translocations of threatened and endangered mollusk species. Juvenile unionids were 30 – 60 days post-transformation, and gastropods were 5 – 8 months post hatch. *Somatogyrus* sp. is considered to be an annual species with juveniles hatching in spring. Adults die soon after the reproductive season is concluded (Johnson et al. 2013). Test organisms were kept in an aquarium with dilution water prepared following ASTM (2007) guidelines and used in testing within 14 days of arrival, so feeding was not necessary (ASTM 2013). Since it can absorb contaminants (Newton and Bartsch 2007), sediment was not used during toxicity testing, which reduced the likelihood of organisms being exposed to contaminants that may be present in sediment or uncontrolled chemical reactions occurring within the sediments.

Experimental Conditions

Static renewal toxicity tests for both classes of organisms were completed following ASTM Standard Guide for Conducting Laboratory Toxicity Tests with Freshwater Mussels (E2455-06) (ASTM 2013). Dilution water recipe additions included sodium bicarbonate (NaHCO₃), calcium sulfate (CaSO₄ ·H₂O), magnesium sulfate (MgSO₄), and potassium chloride (KCl) following ASTM Standard Guide for Conducting Acute Toxicity Tests on Test Materials with Fishes, Macroinvertebrates, and Amphibians (E729-96) (ASTM 2007). The mean physiochemical variables of the dilution water were as follows: pH - 7.3 (7.1 – 7.4), hardness -

41 (40 – 42) mg CaCO₃/L, alkalinity - 33.5 (33 – 34) mg CaCO₃/L, and conductivity - 168 (130 – 189) μΩ/cm. Dissolved oxygen saturation measured ≥90%, temperature was kept at 25 ± 1 °C, and ambient light from overhead fluorescent laboratory lights was used with a photoperiod of 16L: 8D. Test organisms were acclimated in the dilution water for at least 24 hours before trials by adjusting the temperature no more than 3 °C/h until reaching 25 °C (Wang et al. 2007; ASTM 2013). Ten individuals were used in triplicate per concentration (n = 30). Chambers (600 mL Pyrex® beakers) were filled with 300 mL of either dilution water (controls) or toxicant solution and were changed at 48 h (Table 1). Stock SDS toxicant solutions (Laboratory grade, Lot # AD-14008-56, Carolina Biological Supply) analyses were not conducted to quantify concentrations in toxicant exposure; therefore, EC₅₀ values are based on nominal concentrations.

Endpoint determinations were completed after the 96 h tests, unionids were placed under a microscope to view heartbeat or foot movement. If no movement was observed after five-minutes, the unionid was classified as dead. Gastropods were classified as dead if no movement was detected within a five-minute observation period (Archambault et al. 2015) or after the “tickle” test, performed by touching the organisms with a soft pick to provoke a stimulus response. An eyelash stick was used to prevent any excess pressure being placed on the foot and observing a false reaction. Non-decaying individuals were placed in fresh dilution water for thirty minutes and rechecked for survival. Control survivorship had to exceed 90% at the end of each trial for results to be acceptable (ASTM 2013) (Table 1).

Data Analysis

The 96 hour EC₅₀ values for SDS were determined using ToxStat® 3.5 from West, Inc. (downloaded from <https://www.msu.edu/course/zol/868/>) using the Trimmed Spearman-Kärber method (TSK). The EC₅₀ values of test organisms were then compared to EC₅₀ values of other species in the published literature.

RESULTS

In accordance with ASTM (2013) protocols, control survivability was ≥90% for each unionid trial and 100% for each gastropod trial. Gastropods were active and scaling the beaker walls within control test chambers. The EC₅₀ value for *Hamiota perovalis* was 6,102 μg/L (95% CI: 4,727 – 7,876 μg/L), and *Villosa nebulosa* was 14,469 μg/L (95% CI: 13,436 – 15,581 μg/L), more than double the EC₅₀ value of *H. perovalis* (Table 2). In all treatments containing SDS, juvenile unionids of both species purged a mucus-like substance that coated the entire organism. While little foot movement was observed, a heartbeat was always detected in live unionids. However, in high concentrations of SDS, no soft tissue was observed in the shells at the end of the 96 h acute toxicity tests.

Gastropods tested in the current study were more sensitive to SDS than unionid species evaluated. *Leptoxis ampla* had an EC₅₀ value of 26 μg/L (95% CI: 6 – 112 μg/L), which was the lowest EC₅₀ value calculated in this study. *Somatogyrus* sp. had an EC₅₀ value of 1,986 μg/L (95% CI: 1,453 – 2,715 μg/L) (Table 2). Similar to unionids, soft tissues dissolved or completely separated from the shell in the highest concentrations, and most dead gastropods had begun to decompose so death was easily determined. Living gastropods from lower concentrations appeared to begin normal activity once transferred to water lacking SDS. Movement was observed without “tickling” in most low concentrations.

DISCUSSION

Sodium dodecyl sulfate is commonly found in high concentrations in detergents and household cleaners and contaminates drinking water and aquatic ecosystems (Cowan-Ellsberry et al. 2014); however, it has no WQC. Keller (1993) reported a 48 h LC₅₀ value of 19,040 μg/L for juvenile *Utterbackia imbecillis*, a species classified as having a stable conservation status (Williams et al. 2008). Both juvenile unionid species exposed to SDS in the current study had 96 h EC₅₀ values (*V. nebulosa*: 14,469 μg/L (federally petitioned); *H. perovalis*: 6,102 μg/L (federally threatened)) below the value reported by Keller (1993). In a related study, Graney and Giesy (1988) reported a 96 h LC₅₀ value of 31,400 μg/L for SDS using *Corbicula fluminea* (Asiatic clam), which is 2x and 5x higher than the EC₅₀ values determined for *V. nebulosa* and *Hamiota perovalis*, respectively, in the current study.

The gastropod species used in the current study, *L. ampla* and *Somatogyrus* sp., were generally more sensitive to SDS as compared to other published studies, but Tarazona and Nuñez (1987) reported a 96 h LC₅₀ value of 540 μg/L for SLS using *Lymnaea vulgaris*, a pulmonate gastropod. This reported value was higher than the 96 h EC₅₀ value for *Leptoxis ampla* reported in the current study (26 μg/L), but *Somatogyrus* sp. had a higher 96 h EC₅₀ value at 1,986 μg/L, suggesting it may be more tolerant than *Lymnaea vulgaris*. Patrick et al. (1968) reported a LC₅₀ value of 34,161 μg/L for alkylbenzene sulfonate using the pulmonate gastropod *Physa heterostropha*, which was a greater concentration than any EC₅₀ value reported in this study. Misra et al. (1984) reported an EC₅₀ value of 15 μg/L (endpoint: calcium uptake) for alkylbenzene sulfonate using *Lymnaea peregra*, which was similar to the EC₅₀ value for *Leptoxis ampla*. No other published EC₅₀ or LC₅₀ values were close to the EC₅₀ value of *Leptoxis ampla*, suggesting that this highly stenotypic species (Cahaba River Basin endemic) could be one of the most sensitive aquatic species to SDS tested to date. These reported values suggest that caenogastropods display increased sensitivity over pulmonate gastropods to SDS contamination.

Fish have more frequently been subjected to SDS acute toxicity testing than freshwater mollusks and tend to have slightly higher LC₅₀ values than mollusks (Table 3). However,

Table 3. Median effective concentrations (EC₅₀) for 96 h acute toxicity tests of sulfate surfactants on macroinvertebrate and fish species.

Species name	Common Name	Hardness (mg CaCO ₃ /L)	96 h EC ₅₀ (µg/L)	References
Invertebrates				
<i>Daphnia magna</i>	Cladoceran	NR	10,300	Keller 1993
<i>Daphnia magna</i>	Cladoceran	NR	5,400 – 15,000 ^a	Lewis and Weber 1985
<i>Daphnia pulex</i>	Cladoceran	NR	1,400 – 15,200 ^a	Lewis and Weber 1985
Fish				
<i>Danio rerio</i>	Zebrafish	NR	7970	Fogels and Sprague 1977
<i>Danio rerio</i>	Zebrafish	NR	8810 ^a	Fogels and Sprague 1977
<i>Danio rerio</i>	Zebrafish	NR	9,900 – 20,100	Newsome 1982
<i>Cichlasoma nigrofasciatum</i>	Convict Cichlid	NR	16,100 – 30,000	Newsome 1982
<i>Ctenopharyngodon idella</i>	Grass Carp	NR	7,700	Susmi et al. 2010
<i>Cynopoecilus melanotaenia</i>	Killfish	NR	14,900	Arenzon et al. 2003
<i>Cyprinus carpio</i>	Common Carp	NR	1,310	Verma et al. 1981
<i>Jordanella floridae</i>	Flagfish	NR	8,100	Fogels and Sprague 1977
<i>Macrones vittatus</i>	Asian Striped Catfish	NR	1,390	Verma et al. 1978
<i>Menidia beryllina</i>	Inland silverside	NR	9,500	Hemmer et al. 2010
<i>Oncorhynchus mykiss</i>	Rainbow Trout	NR	4,620	Fogels and Sprague 1977
<i>Piaractus brachipomus</i>	Red Pacu	24	11,290	Reátegui-Zirena et al. 2013
<i>Pimephales promelas</i>	Fathead Minnow	NR	6,600	Conway et al. 1983
<i>Pimephales promelas</i>	Fathead Minnow	NR	10,000 – 22,500	Newsome 1982
<i>Pimephales promelas</i>	Fathead Minnow	NR	8,600	USEPA 2002
<i>Poecilia reticulatus</i>	Guppy	NR	13,500 – 18,300	Newsome 1982

NR = not reported

^a = 48 h LC₅₀ value

invertebrates tend to be more sensitive than fish to SDS, and few toxicity studies have focused on freshwater unionids using SDS. The cladoceran *Daphnia magna* had 48 h LC₅₀ value of 10,300 µg/L (Keller 1993), while *Daphnia pulex* had 48 h LC₅₀ values ranging between 1,400 µg/L and 15,200 µg/L (Lewis and Weber 1985; Singer and Tjeerdema 1993) (Table 3). These lower values compared to the EC₅₀ values of *Somatogyrus* sp. reported in the current study suggest that *D. pulex* and *Somatogyrus* sp. may have similar SDS sensitivity. Few toxicity studies have been conducted on caenogastropods (Besser et al. 2009; Johnson et al. 2013), and previous investigations with surfactants have used pulmonate gastropods (e.g., Patrick et al. 1968; Misra et al. 1984; Tarazona and Nuñez 1987).

Other surfactants have been studied more extensively on freshwater mollusks. Ostroumov and Widdows (2006) examined surfactants hindering filter feeding for unionids by reporting a drop in clearance rates after a 10 minute exposure. Bringolf et al. (2007b) examined the components of Roundup® using *Lampsilis siliquoidea* and found that MON 0818, the polyethoxylated tallow amine (POEA) surfactant blend that helps the active ingredients penetrate the waxy coverings of plant leaves, was the most toxic component of that herbicide. In a similar study, Bringolf et al. (2007a) further examined MON 0818 and reported that unionids during their earlier life stages (i.e., glochidia and juvenile stages) are among the most sensitive organisms tested.

The EC₅₀ values of the current study were lower than most

reported in the literature (Tables 2 – 3) for SDS, suggesting that some freshwater mollusks may be among the most sensitive aquatic species tested to date. The majority of mollusk species previously tested have broad geographical ranges and occur across multiple basins and are, therefore, probably adapted to a broad range of physical and chemical water quality. In contrast, many federally listed freshwater mollusks have distributions limited to a distinct, regional drainage, such as the Mobile River Basin species utilized in the current study. These regionalized species would be adapted to specific regional physical and chemical parameters. Freshwater unionids and caenogastropods in the current study were found to be sensitive to SDS, particularly the Cahaba River Basin endemic, *Leptoxis ampla* had the lowest LC₅₀ value recorded to date. Threatened species, such as *L. ampla* or *H. perovalis*, often demonstrate increased sensitivity to environmental changes than other broad ranging species. The stenotypic biology of small range endemic mollusks with unique generic placement (e.g., *Hamiota*) may represent increased sensitivity to a variety of toxicants (Gibson 2015) than wider ranging species adapted to a broad range of normal water quality variables (e.g., *Lampsilis siliquoidea*).

Toxicity tests are important tools that provide information for risk assessment of chemicals and are used when determining USEPA WQC. This study is one of the few testing toxicity of SDS using Mobile River Basin freshwater mollusks, specifically using federally threatened or petitioned species. Many mollusks have a narrow endemic range which

may increase sensitivity to water quality as compared to the broader ranging species. Further testing is urged for regional mollusk species, and especially determination of WQC for SDS, which currently does not exist. Criteria may need to be established using a suite of organisms from various river basins to include stenotypic species (e.g., Cahaba River Basin endemics) that may display increased sensitivity to SDS.

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