Bull. Environ. Contam. Toxicol. (1995) 55:751–758

Environmental Contamination and Toxicology

Responses of Invertebrates and Fish to Alkyl Sulfate and Alkyl Ethoxylate Sulfate Anionic Surfactants During Chronic Exposure

S. E. Belanger, K. L. Rupe, R. G. Bausch

Environmental Science Department, Ivorydale Technical Center, The Procter & Gamble Company, 5299 Spring Grove Avenue, Cincinnati, Ohio 45217, USA

Received: 7 September 1994/Accepted: 15 March 1995

Anionic surfactants are used in high volumes by the consumer products industry in laundry, dishwashing, shampoo, toothpaste, and cosmetic products (Fendinger *et al.* 1994). A desirable facet of these ingredients are rapid biodegradation which reduces exposure in the environment following waste treatment and prevents accumulation in aquatic and terrestrial compartments (Larson 1991). While this aspect of surfactant chemistry is an environmental benefit it leads to difficulties in executing ecotoxicity tests. Microbially mediated reductions in test concentrations argue for flow-through testing with analytical verification of exposure concentration.

This manuscript describes results from flow through chronic toxicity tests using non-standard species. Choice of organisms were based on availability and regional ecological importance. Results from these and other traditional single species tests were used to assess the applicability of statistical and uncertainty factor extrapolation techniques to predict effects at the ecosystem level (Aldenberg and Slob 1991; OECD 1992) determined during comprehensive stream mesocosm exposures to the same surfactants (Belanger *et al.* In Press; Belanger et al. 1994).

MATERIALS AND METHODS

The anionic surfactants dodecyl alkyl sulfate (AS or sodium dodecyl sulfate, CAS NO. 151-21-3) and alkyl ethoxylate sulfate (AES, CAS NO. 68585-34-2) were used in these studies. AS was obtained as 99% pure from Sigma Chemical Company (St. Louis, Missouri). Purity and structure were confirmed by fast atom bombardment-mass spectrometry (FAB-MS) and infra-red spectrometry (IR). Concentrations of AS in toxicity test waters were determined by the gas chromatographic method of Fendinger *et al.* (1994). AES was specially synthesized at Procter & Gamble by sulfating a 50:50 mixture of C_{14} and C_{15} linear alcohol ethoxylates. The structure of the AES was confirmed by nuclear magnetic resonance (NMR) and liquid chromatography/mass spectrometry (LC/MS) and had an average 2.17 ethoxylates per mole. The final test material and LC/MS methods for determining AES concentrations in toxicity

Correspondence to: S. E. Belanger

tests are described in Popenoe *et al.* (1994).

Four species were used in these experiments: *Corbicula fluminea* (the Asian clam), *Goniobasis* sp. (a snail), *Limnephilus* sp. (a case-building caddisfly) and *Pimephales promelas* (the fathead minnow). Tests were conducted at the Procter & Gamble Experimental Stream Facility (ESF) (Belanger 1992) located approximately 25 km from Cincinnati, Ohio, on the Lower East Fork of the Little Miami River. Background water quality is described in Belanger (1992) and Walker *et al.* (Procter and Gamble, unpublished manuscript). Tests were conducted in either flow through aquaria drawing water from experimental stream channels (AS: snail, caddisfly, fish) or from cages held in the terminal tail pool of ESF streams (AS: clams; AES: clams and snails) that received unfiltered Lower East Fork water. Aquaria and cages held 10 and 5 individuals of each species tested, respectively. Triplicate aquaria, 75 L in volume, drew water from each of 6 streams by peristaltic pumps (exposure concentrations are given in Table 1). Average in-flow and resulting hydraulic residence times were 400 mL/min with 160 min in each system. Test species were separated by subdivisions in each aquaria. In-flow to each aquarium was on the opposite end of the out-flow standpipe thereby ensuring mixing. Cages held in stream channel tail pools were stainless steel wire mesh (2 mm^2) and their use has been previously described in detail (Belanger *et al.* 1993). Hydraulic residence time in the tail pool (568 L volume) was approximately 3 min.

Invertebrates were collected from natural systems prior to testing as described below. *Corbicula* (10-15 mm shell length) were obtained from the New River, Virginia by standard techniques and shipped to the test site the same day by air courier to Ohio for the AS bioassay. Mortality was less than 0.5% (n = 1000 clams). For AES, clams were obtained from the a stream channel receiving unamended Lower East Fork River water in the ESF. *Goniobasis* were collected by hand from a riffle in the Lower East Fork River. Caddisflies were hand collected underneath large stones at 0.1-0.5 m depth from the Little Miami River north of Xenia, Ohio. Fish (7 d post hatch) were obtained from stock cultures at Virginia Tech (Dr. John Lauth, Department of Biology,

Table 1. Surfactant concentrations in water in single species tests during the 8-wk exposure period. Nominal AS and AES concentrations are given before and after the slash in the first column, respectively. Means ± 1 SD are given for combined samples over the 8 wk period (n = 7 per stream).

Nominal AS (μg/L)	Measured AS (μg/L)	Nominal AES (μg/L)	Measured AES (μg/L)
Control (0)	2 ± 5	Control (0)	< 0.1
26	23 ± 30	12	14 ± 7
78	51 ± 17	37	27 ± 7
233	151 ± 44	111	75 ± 49
700	418 ± 103	333	234 ± 72
2100	1357 ± 408	1000	730 ± 151

Table 2. Dates and durations of exposure for experiments with caddisflies, snails, clams and fish during exposure to AS (1991) and AES (1992) at the ESF.

Species	Dates of Exposure	Duration	Dates of Exposure	Duration
	Alkyl Sulfate		Alkyl Ethoxylate Sulfate	
Limnephilus	8/20-10/14	56 d	---	---
Goniobasis	8/20-10/14	56d	8/24-10/19	56d
Corbicula	9/3-10/14	42d	8/24-10/19	56d
Fathead minnow	9/3-10/14	42d	---	---

Blacksburg, Virginia) and were 4 wk post hatch at the beginning of the test. Clams, snails, and caddisflies were allowed to acclimate in a Living Stream (Frigid Units, Toledo, Ohio) for 1 to 2 wk to ensure testability. Dietary needs for all test organisms were met by food carried by stream waters. Sensitivity of all four species to AS was assessed. Only molluscs were evaluated in AES tests.

Endpoints measured included mortality (all species), growth (snails, clams), biomass at test termination (all species), and emergence (caddisflies) over 6 to 8-wk time intervals depending on the species and surfactant (Table 2). Growth for clams and snails were based on repeated measures of known individuals by the technique of Belanger *et al.* (1993). Measurements of wet and dry weights were made using a Mettler AE 100 (to $\pm$ 0.1 mg). Exposures for all tests bracketed environmentally realistic to unrealistically high concentrations (Table 1) which were maintained by amending river water at the head of each stream with surfactant stocks in a dilution series. Stocks were prepared in de-ionized carbon filtered water (Walker *et al.*, Procter & Gamble unpublished manuscript; Fendinger et al. 1992; Popenoe et al. 1994).

Statistical analysis of collected data consisted of parametric and non-parametric (Kruskal-Wallis) analysis of variance as appropriate using SAS (Statistical Analysis System, 1990). Multiple range tests used were a Least Significant Differences Procedure and Wilcoxon Rank Sum, respectively, with statistical significance inferred at $\alpha = 0.05$. No-observed-effect-concentrations (NOECs) are defined as the highest exposure concentration which did not significantly impair the tested population relative to the control.

RESULTS AND DISCUSSION

Limnephilus survived well at all exposure concentrations except 1357 μg AS/L (control mortality was 3.3% over 8 wk) (Table 3). All caddisflies that survived the exposure successfully entered a pupal stage during the third to fourth wk of exposure. However, emergence was impaired at the highest exposure. The NOEC for caddisfly survival and emergence was 418 μg AS/L. This compares well with observations of other insects in the ESF stream mesocosms during the study. The NOEC for the macroinvertebrate community was 224 μg AS/L as a result of a reduction in the abundance of sensitive mayfly populations and reduction in Ephmeroptera, Plecoptera, and Trichoptera taxa (Belanger *et al.*, In Press). At the highest AS exposure levels the heterotrophic community was enhanced as microbes actively degraded AS. This led to an increase in bacterial biomass. We observed zones of anoxia underneath larger heterotrophic mats. Even in the bioassays, microbial growth may have played a role as caddisflies sealed off the end of their fine-grain sand cases before entering the pupal stage.

Occlusion of water exchange may have been partly responsible for reduced emergence as caddisflies became stressed. Overall, *Limnephilus* was a useful insect model in these studies and will be pursued in future investigations.

Fathead minnows in aquaria were not adversely affected at any AS concentration. In fact, fish in aquaria drawing water from AS amended streams reached a final dry weight of 44.5 $\pm$ 0.6 mg/individual at 1357 μg AS/L versus 16.5 $\pm$ 0.7 mg/individual in the controls. Increases were exposure concentration dependent ($p < 0.001$).

Table 3. NOEC responses of invertebrates and fish exposed to alkyl sulfate (AS) and alkyl ethoxylate sulfate (AES).

Species	Endpoint	AS NOEC (μg /L)	AES NOEC (μg/L)
Limnephilus	Mortality	418	---
	Emergence	418	---
Goniobasis	Mortality	>1357	>730
	Final weight	>1357	>730
	Growth	---	>730
Corbicula	Mortality	>1357	>730
	Growth	418	75
Fathead minnow	Mortality	>1357	---
	Final weight	>1357	---
	Final length	>1357	---

Survival was not impaired at any concentration. As for caddisflies, a potential link with the microbial community may be present with more food resources available in AS exposed aquaria. Clearly AS was not toxic to fish at mg/L levels as has been previously demonstrated in shorter term experiments (Fendinger *et al.* 1994). *Goniobasis* was relatively tolerant of surfactant exposure. AS and AES NOECs were at the highest exposure concentrations tested of 1357 and 774 μg/L, respectively (Table 3). Survival in all treatments was >90%. Final wet and dry weights of snails at the conclusion of chronic exposure were approximately 5-15% higher in AS treatments than in the control, but these differences were not significant. Interestingly, these findings are similar to observations of gastropod populations in ESF stream channels where abundances were significantly greater in AS amended streams, but biomass on an individual basis was not affected (Belanger *et al.*, In Press). Snail mortality, growth, and final biomass were not significantly affected by AES (Table 3, Fig. 1). Snail growth was modified by colder stream water temperatures late in the test. Figure 2 shows average daily stream water temperature over the 56 d exposure period. Snail growth ceased as temperatures reached 15-17°C. By the conclusion of the test, snails began a period of "degrowth" as defined by Russel-Hunter *et al.* (1984).

Corbicula was the most sensitive organism tested to anionic surfactants. NOEC values were 418 and 75 μg/L for AS and AES, respectively (Table 3, Fig. 3). *Corbicula* responses in ESF streams mirrored single species responses, although the effect level for AES was one concentration interval higher in the stream mesocosm (NOEC = 251 μg/L). This species is an important component of ESF streams and has demonstrated utility in other surfactant investigations (Belanger *et al.* 1993). Growth of Asiatic clams has proven useful in monitoring effluents under natural and stream mesocosm conditions as well as in single species bioassays under controlled conditions (Belanger 1990). The species is widely dispersed geographically and is now present on several continents including North and South America, the Middle East and native ranges in the Asian Pacific region. It has often been postulated that this distribution is due to the clam's tolerance to pollution and a wide range of environmental conditions, but it is perhaps more related to a highly successful reproductive strategy (maturation of larvae to the pediveliger stage in gill "marsupia"; relatively long periods of reproductive output; large numbers of larvae/adult female expelled during reproductive events).

Sensitivity of several species have been evaluated previously during exposure to SDS or sodium dodecyl sulfate, a common synonym for dodecyl alkyl sulfate. However, exposure concentrations in previous studies have never been confirmed. For example, Cowgill and Milazzo (1991) determined the 7-day chronic NOEC for the daphnid *Ceriodaphnia dubia* to be 30 mg/L. Unpublished P&G data confirmed that under the conditions used in the typical *Ceriodaphnia* studies exposure to AS declines rapidly to below detection limits within 8-12 hours. Toxicity data becomes difficult or impossible to interpret under such conditions. AES is not as rapidly mineralized as AS, but the same issues remain. For rapidly degradable materials confirmation of exposure by toxicant-specific analytical techniques is absolutely necessary.

The NOEC values for *Limnephilus* and *Corbicula* exposed to AS and for *Corbicula* exposed to AES are among the lowest reported for these compounds (Fendinger *et al.*

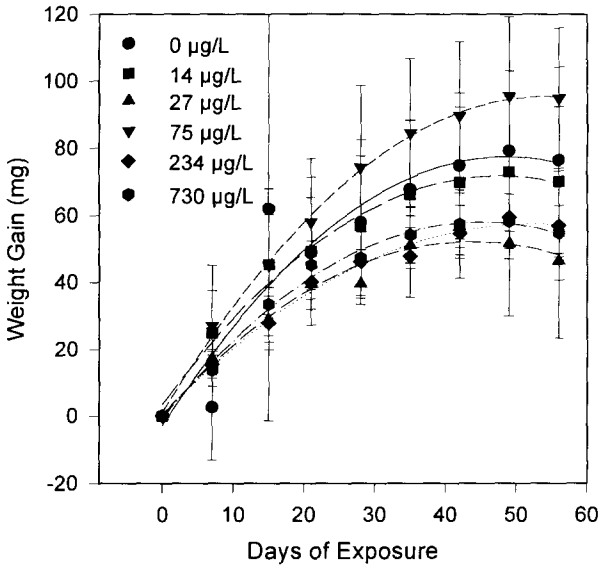

Figure 1. Growth of *Goniobasis* during exposure to AES. Error bars indicate ± 1 SD. Treatments were not significantly different from the control ($\alpha = 0.05$).

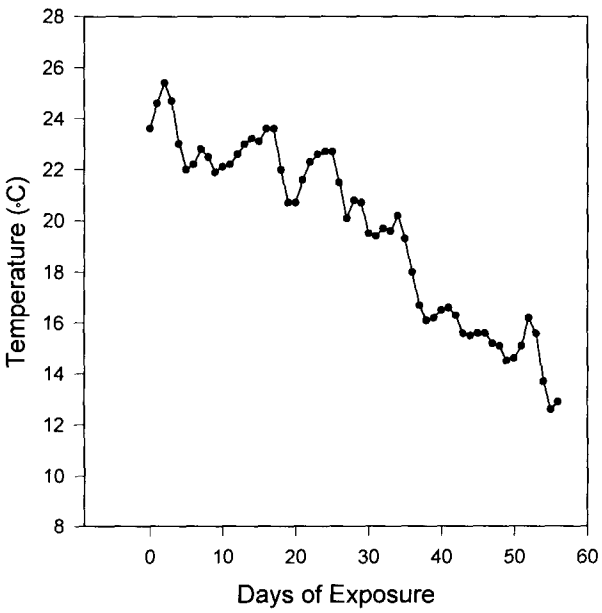

Figure 2. Mean daily water temperature in ESF stream channels during the AES experiment.

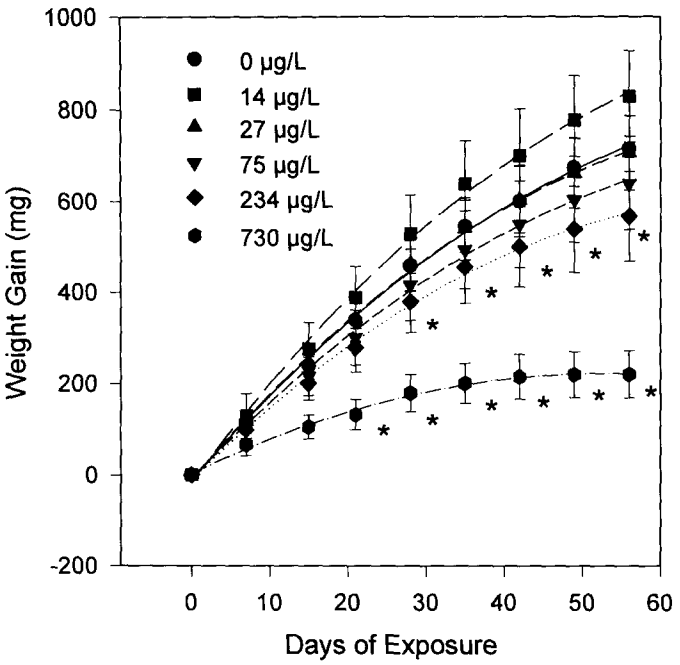

Figure 3. Growth of *Corbicula* during exposure to AES. Error bars are ± 1 SD of the mean. Groups that are significantly different from the control are indicated by an asterisk (*, $\alpha = 0.05$).

1994). This is likely due to the combined effects of employing sensitive taxa and confirming analytical exposure. Fendinger *et al.* (1992) and McAvoy *et al.* (Procter & Gamble, unpublished manuscript) showed that AS and AES levels in river water immediately below wastewater treatment plants were both < 40 µg/L (most often < 10 µg/L) under worst-case dilutions (dilution factors < 3). Therefore, a margin of environmental safety for both compounds exists based on data in this study.

REFERENCES

Aldenberg T, Slob W (1993) Confidence limits for hazardous concentrations based on logistically distributed NOEC toxicity data. Ecotoxicol Environ Saf 25: 48-63

Belanger SE (1990) The effect of dissolved oxygen, sediment, and sewage treatment plant discharges upon growth, survival and density of Asiatic clams. Hydrobiologia 218:113-126

Belanger SE (1992) Use of experimental stream mesocosms to assess ecosystem risk: A case study with a cationic surfactant. In: Cairns J, Niederlehner BR, and Orvos DR (eds), Predicting Ecosystem Risk. Princeton Scientific Publishing Company, Princeton, New Jersey, p 262

Belanger SE, Davidson DH, Farris JL, Reed D, Cherry DS (1993) Effects of cationic surfactant exposure to a bivalve mollusc in stream mesocosms. Environ Toxicol Chem 12:1789-1802

Belanger SE (1994) Review of experimental microcosm, mesocosm, and field tests used to evaluate the potential hazard of surfactants to aquatic life and the relation to single species data. In: Hill IR, Heimbach F, Leeuwangh P, and Matthiessen P. (eds), Freshwater Field Tests for Hazard Assessment of Chemicals. Lewis Publishers, Chelsea, Michigan, p 326

Belanger SE, Meiers EM, Bausch RG, Sweeney BN, Hart DD, Jackson JK, and Wolbach KC. Direct and indirect ecotoxicological effects of alkyl sulfate and alkyl ethoxysulfate on macroinvertebrates in stream mesocosms. Aquat Toxicol (In Press)

Cowgill UM, Milazzo DP (1991) The response of the three brood *Ceriodaphnia* test to fifteen formulations and pure compounds in common use. Arch Environ Contam Toxicol 21:35-40

Fendinger NJ, Begley WM, McAvoy DC, Eckhoff WS (1992) Determination of alkyl sulfate surfactants in natural waters. Environ Sci Technol 26:2493-2498

Fendinger, NJ, Versteeg DJ, Weeg E, Dyer SD, Rapaport RA (1994) Environmental behavior and Fate of anionic surfactants. In: Baker LA (ed), Environmental Chemistry of lakes and Reservoirs, ACS Advances in Chemistry Series No. 237. American Chemical Society, Washington, DC, p 528

Larson RJ (1991) Biodegradation in regulating the environmental exposure of detergent chemicals. Chemical Trends Times (July 1991):47-56

OECD (1992). Report of the OECD workshop on the extrapolation of laboratory aquatic toxicity data to the real environment. Organization for Economic Co-operation and Development Paris, France

Popenoe, DD, Morris, SJ, Horn, PS, and Norwood, KT (1994) Determination of alkyl sulfates and alkyl ethoxysulfates in in wastewater treatment plant influents and effluents and river water using liquid chromatography/ion spray mass spectrometry. Analyt Chem 66:1620-1629

Russell-Hunter WD, Browne RA, Aldridge DW (1984) Overwinter tissue degrowth in natural populations of freshwater pulmonate snails (*Halisoma trivolvis* and *Lymnaea palustris*). Ecology 65:223-229

Statistical Analysis System (1990) SAS® User's Guide: Statistics. Version 5. Cary, NC