Susceptibility of Bluegill Sunfish (*Lepomis macrochirus*) to Nonionic Surfactants

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Although both published (GILLETTE et al., 1952; LAWRENCE, 1962; SWISHER et al., 1964; MARCHETTI, 1965; CONWAY and WAGGY, 1966) and unpublished data exist on the acute toxicity of various types of surfactants to fishes, little, if any, information providing direct comparisons of various types of materials is available. Therefore, the objective of this study was to determine the susceptibility of bluegill sunfish, (Lepomis macrochirus), under both static and dynamic conditions, to a wide range of nonionic surfactants. The data generated were intended to provide a basis for comparing the susceptibility of this species to various alkylphenol ethoxylates (APE) and alcohol ethoxylates (AE); and for evaluating the effect of ethylene oxide (EO) chain length on susceptibility.

METHODS AND MATERIALS

This investigation was performed at the aquatic toxicology laboratory of Bionomics, E G & G, Inc. in Wareham, Massachusetts. Bluegill were obtained from a commercial fish farmer in Nebraska and had a mean weight of 1.0 g. The fish were held in the laboratory for at least 30 days prior to use in bioassays, during this period mortality in the test population was less than 4% and the fish were judged to be in good physical condition. The surfactants, the source of the sample, and a general chemical description are given in Table 1. All samples were commercially available products obtained from the manufacturers. Each was supplied as 100% active except Triton X-305 which was 70% in water. All solutions tested were made up based on the active ingredient content to enable direct comparison of all results.

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Table 1 - Tradename, source and chemical description[a] of the surfactants tested in bioassays with bluegill sunfish, (Lepomis macrochirus)

TRADENAME	SOURCE	CHEMICAL DESCRIPTION	
		ETHOXYLATE	CHAIN LENGTH[b]
SURFONIC N-40	Jefferson	alkylphenol	EO_4, C_9
SURFONIC N-95	"	"	EO_9, C_9
IGEPAL CO-520	GAF Corp.	"	EO_5, C_9
IGEPAL CO-630	"	"	EO_9, C_9
IGEPAL CO-880	"	"	EO_{30}, C_9
TRITON X-45	Rohm and Haas	"	EO_{4-5}, C_8
TRITON X-100	"	"	EO_{10}, C_8
TRITON X-305	"	"	EO_{30}, C_8
NEODOL 25-3	Shell	primary alcohol	EO_3, C_{12-15}
NEODOL 25-9	"	"	EO_9, C_{12-15}
ALFONIC 1012-60	Continental Oil	"	EO_6, C_{10-12}
SURFONIC TD-90	Jefferson	"	EO_9, C_{13}
TERGITOL 15-S-9	Union Carbide	secondary alcohol	EO_9, C_{11-15}

[a]The surfactants tested are of one of the following basic structures:

1) alkylphenol ethoxylate, R -  $-O(CH_2CH_2O)_nH$;

2) primary alcohol ethoxylate, $R-O(CH_2CH_2O)_nH$;

3) secondary alcohol ethoxylate, $H-\underset{\underset{CH_3}{|}}{\overset{\overset{R'}{|}}{C}}-O(CH_2CH_2O)_nH$, where R'=R-2

[b]Refers to the number of ethylene oxide units (n), and to the number of C atoms in branched or straight chain moiety (R), exclusive of phenyl ring.

The susceptibility of the bluegill to the surfactants was measured in terms of the median lethal concentration (LC50), the concentration of the surfactant in water which causes 50% response (death) under the test conditions during a specific time interval. The estimation of a LC50 value, and its 95% confidence interval, was based on the conversion of the concentrations tested and the corresponding observed percent mortalities to logs and probits, respectively, and the subsequent mathematical calculation of a linear regression equation. All samples were coded and provided to Bionomics, Inc. through Rohm and Haas Co. The specific chemical identity of samples was unknown to the personnel at Bionomics until after the bioassays were completed.

Static bioassays were conducted in 19-liter uncovered glass jars held in constant temperature ($18 \pm 0.5^{\circ}C$) water baths. The test diluent consisted of 15 liters of deionized well water of at least 1 million ohms resistivity which was reconstituted by adding 2 mg KCl, 30 mg $CaSO_4$, 30 mg $MgSO_4$ and 48 mg $NaHCO_3$ per liter. The pH of the diluent was 7.1 and the methyl orange alkalinity was 35 mg/l as $CaCO_3$. Bioassays were conducted without artificial aeration, and with a single introduction of the surfactant dissolved in water. Fish were acclimated to the test conditions for 72 hours, and to the test system for at least 24 hours, prior to testing. Test solutions were prepared by adding the appropriate amount of surfactant to 15 liters of the test diluent. Ten fish were tested at each concentration, using a minimum of six concentrations per bioassay; the mass/volume ratio never exceeded 1.0 gram of fish per liter of diluent. Dissolved oxygen concentrations ranged from 9.0 mg/l initially to 5.1 mg/l at the end of the test.

Dynamic bioassays were conducted according to Fish Bioassay Procedures described in Standard Methods (APHA, 1971), utilizing an intermittent-flow proportional dilution apparatus described by MOUNT and BRUNGS (1967). The apparatus provides for intermittent introduction of seven different concentrations of the test compound into seven different test vessels, and diluent water (no surfactant) to a control vessel. Flow rate to each 30-liter glass aquarium was 5 liters/hour. The test diluent consisted of aerated well water of pH 7.1, total hardness 38 mg/l as $CaCO_3$, and constant temperature of $21 \pm 1.0^{\circ}C$. Dissolved oxygen concentrations ranged from 9.4 to 8.9 mg/l throughout the test for each surfactant tested.

At various intervals during the static bioassays, samples of 100 ml of test solution were removed from selected concentrations in each test and preserved by adding 1 ml of formaldehyde (37%). These samples were shipped to Rohm and Haas Company, Bristol, Pa. for analysis to determine the variability between nominal and experimental concentrations at the beginning of the test and to determine if significant biodegradation had occurred during the test. The method of analysis was that described by WICKBOLD (1972) which consists of isolating and concentrating the nonionic surfactant by solvent sublation into ethyl acetate. After evaporation of the ethyl acetate, the residue was picked up in methanol and water. The nonionic was then precipitated with modified Dragendorff reagent ($KBiI_4$ + $BaCl_2$ + glacial acetic acid). After isolation by filtration, the precipitate in solution was then titrated potentiometrically with a pyrrolidinedithiocarbamate solution of pH 4-5 using platinum-calomel electrodes. The precision of the method is probably no better than $\pm$ 5% and the recovery of known nonionics spiked to control water is greater than 90% for nonionics with EO chain length between 6 and 30.

RESULTS AND DISCUSSION

The recovery of nonionic surfactants from samples taken at the beginning (0 hour) of bioassays ranged from 96-106% for both APE and AE surfactants indicating the nominal concentrations varied minimally (within the precision of the method) from actual concentrations of surfactant. There were no significant differences in the concentration of APE surfactants between water samples taken at the beginning and end of the static bioassays (96 hours), indicating little, if any biodegradation of these materials. On the other hand, measured concentrations of AE surfactants in samples taken during the static bioassays indicated a rather linear disappearance of surfactant during the 96 hours to the extent of 10-15%. Since fish mortality occurred principally in the first 24 hours, the significance of this disappearance is further minimized. Since the flow rate in dynamic bioassays was equivalent to one complete turnover per six hours, no chemical analyses of water samples from dynamic tests were conducted. In these bioassays nominal concentrations were assumed to be accurate and relatively constant, and LC50 values generated appear to support this assumption.

TABLE 2

Susceptibility of bluegill (Lepomis macrochirus) to alkylphenol and alcohol ethoxylate surfactants as determined by static bioassays.

SURFACTANT	TYPE	CHAIN LENGTH[a]	LC50-mg/l 24 HOUR	LC50-mg/l 96 HOUR
SURFONIC N-40	APE	EO_4, C_9	1.5(1.3-1.8)[b]	1.3(1.0-1.8)
IGEPAL CO-520	"	EO_5, C_9	2.8(2.4-3.2)	>2.4<2.8
TRITON X-45	"	EO_{4-5}, C_8	3.5(3.1-4.0)	>2.8<3.2
SURFONIC N-95	"	EO_9, C_9	7.8(6.2-9.9)	7.6(6.0-9.7)
IGEPAL CO-630	"	EO_9, C_9	8.9(5.9-13.6)	7.9(6.4-9.8)
TRITON X-100	"	EO_{10}, C_8	16.2(13.3-19.8)	12.0(8.4-17.2)
IGEPAL CO-880	"	EO_{30}, C_9	>1000.0	>1000.0
TRITON X-305	"	EO_{30}, C_8	1080.0(663.0-1470.0)	531.0(385.0-730.0)
NEODOL 25-3	AE	EO_3, C_{12-15}	1.8(1.2-2.4)	1.5(1.2-1.8)
NEODOL 25-9	"	EO_9, C_{12-15}	2.1(1.6-2.9)	2.1(1.5-2.8)
ALFONIC 1012-60	"	EO_6, C_{10-12}	6.4(4.2-9.6)	6.4(4.2-9.6)
SURFONIC TD-90	"	EO_9, C_{13}	7.8(6.2-9.9)	7.5(5.9-9.7)
TERGITOL 15-S-9	"	EO_9, C_{11-15}	4.7(3.7-5.9)	4.6(3.6-5.8)

[a] Refers to the number of ethylene oxide $(EO)_n$ units, and to the number of carbon atoms (C_n) in the branched or straight chain moiety, exclusive of any phenyl ring.

[b] 95% confidence interval.

TABLE 3

Susceptibility of bluegill (Lepomis macrochirus) to alkylphenol and alcohol ethoxylate surfactants as determined by dynamic bioassays.

SURFACTANT	TYPE	CHAIN LENGTH[a]		LC50-mg/l[b]		
		EO_n	C_n	24 HOUR	96 HOUR	INCIPIENT
IGEPAL CO-630	APE	EO_9	C_9	>10.0	>10.0	192 hours 6.3(4.1-9.8)
TRITON X-100	APE	EO_{10}	C_8	>10.0	>10.0	168 hours 9.6(4.0-16.9)
NEODOL 25-9	AE	EO_9	C_{12-15}	2.7(2.2-3.3)	2.1(1.7-2.6)	96 hours 2.1(1.7-2.6)
TERGITOL 15-S-9	AE	EO_9	C_{11-15}	>4.0 <5.6	4.8(3.3-7.2)	4.8(3.2-7.2)

[a]Refers to the number of ethylene oxide $(EO)_n$ units, and to the number of carbon atoms (C_n) in the branched or straight chain moiety exclusive of any phenyl ring.

[b]Values in parenthesis are 95% confidence values.

The 24-hour, 96-hour, and incipient LC50 values for all compounds tested in both static and dynamic systems are summarized in Tables 2 and 3. The incipient LC50 is a time independent estimate of the acute toxicity of a compound and is made at the time when no additional significant mortality (<10%) is observed in any concentration tested during a 48 hour period. The most obvious conclusion based on these data is that the susceptibility of bluegill to nonionic surfactants increases with decreasing EO chain length. All of the surfactants (both APE and AE) having EO chain lengths less than 6 had 96-hour LC50 values ranging from 1.3-3.2 mg/l. Except for Neodol 25-9, the seven surfactants having EO chain lengths of 9 or 10 had 96-hour LC50 values ranging from 4.6-12.0 mg/l. Finally, both surfactants with EO chain lengths of 30 had 96-hour LC50 values greater than 500 mg/l.

A second conclusion is that bluegill are no more susceptible, and, in fact, are probably less susceptibile to acute exposure to alkylphenol ethoxylates than to alcohol ethoxylates. For surfactants with EO chain lengths of 9-10, the range of six 96-hour LC50 values, based on static and dynamic bioassays, for alcohol ethoxylates was 2.1-7.5 mg/l, while five 96-hour LC50 values, based on both static and dynamic bioassays, for alkylphenyl ethoxylates were greater than 6.3 mg/l. A third conclusion is that the toxic effects of the surfactants are most pronounced during the first 24 hours of exposure in both the static and dynamic bioassays.

Finally, a comparison of 96-hour LC50 values for compounds tested under both static and dynamic bioassay conditions suggests that results with AE surfactants are virtually identical from both systems. A similar comparison for APE surfactants suggests bluegill are less susceptible to APE materials in dynamic systems. However, a prediction of LC50 values based on 192 hours continuous exposure yields LC50 values comparable to those observed for 96-hour static bioassays and suggests that a slightly longer exposure time is required in a dynamic system to elicit the same response of bluegills to APE surfactants. For example, the 192-hour LC50 of Igepal CO-630 for bluegill is 6.3(4.1-9.8) mg/l compared to a 96-hour LC50 (static) of 7.9 (6.4-9.8) mg/l; comparable values for Triton X-100 are 168-hour LC50 of 9.6 (4.0-16.9) and a 96-hour LC50 (static) of 12.0 (8.4-17.2). No additional significant response of bluegill to any of the surfactants tested under dynamic bioassays was observed during the final 48 hours of exposure.

A program is presently in progress to measure the acute toxicity of the degradation products of the four surfactants studied in the above dynamic tests. Surfactants are degraded by the use of semi-continuous activated sludge units which are initially acclimated to the parent compounds. The efficiency of the units is such that > 95% degradation, determined by CTAS analysis of residual surfactant in the effluent, occurs within 24 hours residence time. Performing a static bioassay (96 hr) using effluent from such units, mortality to bluegill was not observed for any of the four surfactants even when 32-40 ppm were charged to the sludge unit. It thus appears that the degradation products of the tested APE's and AE's are significantly less toxic to fish than are the surfactants themselves (Unpublished data, GAF Corp.).

SUMMARY

The present report provides a basis for direct comparison of the susceptibility of fish to alkylphenol and alcohol ethoxylates of various EO chain lengths. It is obvious from the data provided that susceptibility of bluegill to nonionic surfactants (both types) increases with decreasing EO chain length. Also it appears that bluegill are no more susceptible, and, in fact, are probably less susceptible to the acute effects of alkylphenol ethoxylates than of alcohol ethoxylates.

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