

Acute Toxicity of Industrial Surfactants to *Mysidopsis bahia*

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Abstract. Three- to eight-day old *Mysidopsis bahia* were used to evaluate the acute toxicities of 17 industrial surfactants having a wide range of physicochemical characteristics. LC50s based on nominal concentrations covered approximately three orders of magnitude (<1 to >4,000 mg/L). The base structure of the surfactants (*i.e.*, aromatic or aliphatic, branched or linear) was not a factor controlling toxicity. Low solubility surfactants with low ethylene oxide (EO) molar ratios were the most toxic surfactants tested. Ethylene oxide chain length was the best predictor of toxicity, and would be a good parameter to use to "screen" for surfactant toxicity in hazard assessment. Substitution of terminal OH with SO₃ or PO₄ reduced toxicity of a selected group of surfactants. Sensitivity of *M. bahia* up to 26 days old was not significantly different from that of 3- to 8-day old animals. Use of one surfactant as a reference toxicant demonstrated that sensitivity of *M. bahia* was consistent throughout the various tests.

Many laws governing the production and distribution of chemicals require physicochemical and toxicological data on these chemicals prior to production and marketing (Bro-rasmussen and Christiansen 1984). Specifically, the Toxic Substances Control Act (15USC 1976) establishes a premanufacture testing policy which requires producers to generate toxicity data for use in hazard assessments. One of the first steps commonly taken in generating ecotoxicity data is to conduct acute, single-species toxicity tests. Since it is not economically feasible to assess the toxicity of all chemicals individually, increasing attention has been given to the use of quantitative structure activity relation-

ships (QSAR) to "screen" for likely chemical toxicity.

Industrial chemicals enter aquatic environments due to their presence in wash water, as wastes or by-products of production processes, or simply as a result of normal use and disposal. These chemicals often enter aquatic environments following wastewater treatment. However, the toxicity of many industrial chemicals is relatively unknown, particularly to marine and estuarine organisms. Industrial surfactants are an important group of chemicals for which saltwater toxicity data are scarce. In 1986, consumption of industrial surfactants was 2.6 billion pounds (Sherman *et al.* 1987).

The estuarine crustacean *Mysidopsis bahia* has been increasingly used to evaluate the toxicity of pure chemicals, effluents, and drilling muds in estuarine and marine environments (Nimmo and Hamaker 1982; USEPA 1987). *M. bahia* is as sensitive or more sensitive to toxic substances than other marine species (Nimmo and Hamaker 1982). It is also ecologically important due to its utilization as a major food source for bottom feeding fishes (Gentile *et al.* 1982). Although relatively new as a "standard" toxicity test organism, test methods for *M. bahia* have recently been adopted (USEPA 1985, 1987). Some states (New Jersey, Florida) have recently introduced *M. bahia* toxicity limitations in permits issued to industries and municipalities discharging wastewater to marine or estuarine environments. *M. bahia* was chosen as the test organism due to its ecological importance, sensitivity to a wide range of toxicants, the increased use of this organism in monitoring effluent toxicity, and to contribute to the developing data base on chemical toxicity to this saltwater species.

The purpose of this study was to determine the acute toxicities of a variety of industrial surfactants

to *M. bahia* and to gain some insight as to the importance of different chemical characteristics in influencing the toxicity of each surfactant. Ethoxylated surfactants were chosen as the test chemicals due to the lack of saltwater toxicity data on this group of chemicals and because of the wide range of physiochemical characteristics within this chemical group.

Materials and Methods

Test Species and Holding Conditions

Mysidopsis bahia were obtained from two commercial sources. All organisms had been cultured in natural saltwater of 27 to 29‰ salinity. Organisms were held in aged (>1 week) natural saltwater of 25 to 26‰ salinity for 24 to 48-hr prior to testing. All holding water had been filtered through 25 micron mesh filters prior to introduction to the 120-L holding aquaria. Holding water characteristics were adjusted to attain the following conditions: temperature $24 \pm 2^\circ\text{C}$, pH 7.70 to 8.33, dissolved oxygen >90% saturation, alkalinity 92 to 120 mg/L CaCO_3 ; NO_2 was non-detectable 0.27 mg/L. Mysids were maintained under a photoperiod of 16-hr light:8-hr dark. Holding conditions were generally maintained by the addition of new saltwater and NaOH for pH control. Temperatures were determined with hand-held thermometers, and pH with an Orion model SA250 pH meter. Dissolved oxygen was measured with a YSI model 54A dissolved oxygen meter and salinity determined with a YSI salinity/conductivity meter. Total alkalinity and NO_2 were measured with Hach test kits.

Prior to testing, mysids were held under the above conditions in round, clear plastic holding vessels (14 cm diameter by 20 cm high) having 63 or 202 micron mesh Nitex® net windows to allow a continuous supply of water. All mysids were fed live *Artemia* nauplii (<24-hr old) and small amounts of Zeigler Brothers larval fish food during holding periods. The holding vessels contained *Artemia* for easy capture by mysids and an airstone in each holding vessel ensured high levels of dissolved oxygen while providing a current to which mysids could orient to feed.

Test Procedure

Procedures used in all toxicity tests essentially followed those of USEPA (1985). All tests were 48-hr static renewals (renewals at 24 hr) at $25 \pm 1^\circ\text{C}$ under a light:dark photoperiod of 16-hr:8-hr. Tests were conducted in Blue-M Stabil-Therm® incubators. All mysids were 3 to 8 days old at the start of tests except when effect of age on mysid sensitivity was assessed. Aged, natural saltwater of 25 to 28‰ salinity (25 micron filtered) was the control and dilution water in all experiments. Test vessels were disposable 255 ml plastic cups (USEPA 1985, 1987) containing 100 ml of test solution. All toxicant exposures contained two replicates of four organisms per concentration and controls contained four replicates of four organisms. With the exception of one chemical (tp-NPEO_{1,5}), concentrations were nominal values obtained by adding pure chemical to the saltwater. A modification of the procedure described by Ahel and Giger (1985) was

used to measure tp-NPEO_{1,5} (gas chromatography following continuous distillation and extraction with octanol). *M. bahia* were fed live *Artemia* (<24-hr old) at the start of tests and after renewing solutions. Dissolved oxygen, pH, and salinity were monitored at the start, after 24-hr, at renewal, and at termination of experiments. Dissolved oxygen was measured in all solutions at the start, at 24-hr and 48-hr of all tests. Salinity and pH were monitored only in the controls and the two highest toxicant concentrations, since changes in these parameters were not observed as a result of addition of chemicals. With the exception of one test, only experiments with <20 percent control mortality were used in comparing the toxicity of different surfactants. Control mortality above the recommended 10% was deemed acceptable because this occurred on only a few occasions and lower levels of mortality occurred for mysids exposed to low levels of surfactant. A toxicant dose-response was otherwise observed in these tests. Reference toxicant data generated during the study demonstrated that sensitivity of mysids in tests with 11 to 19% control mortality was the same as that for mysids used in experiments with $\leq 10\%$ control mortality. Computer programs (USEPA 1985) calculated LC50 concentrations and 95% confidence limits, using the binomial and moving average procedures. To ensure that mysids obtained from the two suppliers were of similar and consistent sensitivity, one of the surfactants (tp-NPEO₉) was used as an internal reference toxicant throughout the tests, because it is readily soluble and of high acute toxicity.

Test Compounds

Surfactants having a wide range of physiochemical characteristics were obtained from a single commercial supplier (Table 1). All test compounds were anionic or nonionic surfactants, and, with one exception (methyl oleoyl taurate), were polyethoxylated. Purity of test chemicals was 99% except for one compound. Compensation for percent inactive ingredients was made when calculating this LC50. Chemical base structures of surfactants were aromatic or aliphatic, with molecular weights ranging from 272 to 2,420 g/mole. In aromatic based surfactants, chemicals containing both linear and highly branched alkyl groups were tested. Some surfactants have an active group substitution at the end of the ethoxylate chain.

Results and Discussion

Test Comparability

Parameters monitored during toxicity tests indicated that test conditions were acceptable and consistent. Dissolved oxygen concentrations were at saturation in all freshly prepared test solutions and never fell below the required 40% saturation level (USEPA, 1985). As a rule, dissolved oxygen concentrations were above 5 mg/L, with the lower values observed in tests with the more soluble surfactants. Test pH ranged from 7.40 to 8.08 but was generally 7.70 to 8.00. Changes in pH over a 24-hr period were never >0.5 units in a given exposure. Salinity and alkalinity ranged from 24 to 29‰ and 90 to 130 mg/L CaCO_3 , respectively. Nitrite con-

Table 1. Physiochemical characteristics of test surfactants

Surfactant	EO molar ratio	Chemical base structure	Molecular structure	Active group substitution	Molecular weight (g/mole)	Water soluble (Yes/No)
Linear APEO _{1.5} ^a	1.5	Aromatic	(C ₉ H ₁₉) [*] (OC ₂ H ₄) _{1.5} -OH	OH	286	?
Linear APEO ₉ ^a	9	Aromatic	(C ₉ H ₁₉) [*] (OC ₂ H ₄) ₉ -OH	OH	616	Yes
Linear APEO ₅₀ ^a	50	Aromatic	(C ₉ H ₁₉) [*] (OC ₂ H ₄) ₅₀ -OH	OH	2,420	Yes
tp-NPEO _{1.5}	1.5	Aromatic	(C ₉ H ₁₉) [*] (OC ₂ H ₄) _{1.5} -OH	OH	286	No
tp-NPEO ₉	9	Aromatic	(C ₉ H ₁₉) [*] (OC ₂ H ₄) ₉ -OH	OH	616	Yes
tp-NPEO ₁₅	15	Aromatic	(C ₉ H ₁₉) [*] (OC ₂ H ₄) ₁₅ -OH	OH	880	Yes
tp-NPEO ₄₀	40	Aromatic	(C ₉ H ₁₉) [*] (OC ₂ H ₄) ₄₀ -OH	OH	1,980	Yes
tp-NPEO ₅₀	50	Aromatic	(C ₉ H ₁₉) [*] (OC ₂ H ₄) ₅₀ -OH	OH	2,420	Yes
tp-NPEO ₉ Sulfonated	9	Aromatic	(C ₉ H ₁₉) [*] (OC ₂ H ₄) ₉ -OSO ₃ NH ₄	OSO ₃ ⁻	713	Yes
tp-NPEO ₉ Phosphated	9	Aromatic	(C ₉ H ₁₉) [*] (OC ₂ H ₄) ₉ ² -OPO ₃ H ₂	OPO ₃ ⁻²	695	Yes
OPEO _{1.5}	1.5	Aromatic	(C ₈ H ₁₇) [*] (OC ₂ H ₄) _{1.5} -OH	OH	272	No
OPEO ₅	5	Aromatic	(C ₈ H ₁₇) [*] (OC ₂ H ₄) ₅ -OH	OH	426	Yes
DAEO ₄	4	Aliphatic	R-(OC ₂ H ₄) ₄ -OH	OH	193 + R	Yes
TDAEO _{9.75}	9.75	Aliphatic	R-(OC ₂ H ₄) _{9.75} -OH	OH	446 + R	Yes
TDAE ₁₀ Cl-capped	10	Aliphatic	R-(OC ₂ H ₄) ₁₀ -OCl	Cl	491 + R	Yes
Castor Oil ₃₀	30	Aliphatic	—	OH		
Methyl oleoyl taurate ₁ , Sodium Salt	1	Aliphatic	C ₁₇ H ₃₃ -C ^O -N ^{CH₃} -CH ₂ -CH ₂ -SO ₃ Na	SO ₃	425	Yes

* = Benzene Ring, EO = Ethylene Oxide, APEO = Alkyl Phenol Ethoxylate, NPEO = Nonyl Phenol Ethoxylate, tp = Tripropylene (Highly Branched), OPEO = Octyl Phenol Ethoxylate (Highly Branched), DAEO = Decyl Alcohol Ethoxylate, TDAEO = Tridecyl Alcohol Ethoxylate

^a The linear APEO series surfactants consisted of 50/50 mixture of ethoxylated octyl- and decylphenols, with average detergency equivalent to that of nonylphenol ethoxylate

concentrations ranged from 0.06 to 0.21 mg/L. Differences in water chemistry parameters between duplicate toxicant exposures were negligible. The consistency in test conditions indicated that toxicity data were comparable in terms of physiochemical test conditions.

Results of ten reference toxicity tests with tp-NPEO₉ (for list of abbreviations refer to Table 1) conducted throughout the study indicated that the sensitivity of *M. bahia* was also consistent (Figure 1). Reference toxicant LC50s ranged from 0.71 to 2.0 mg/L and were not significantly different as determined by overlapping 95% confidence limits (ALPHA *et al.* 1980). Thus, the consistency of the water chemistry data and test organism sensitivity validates that direct comparison of the toxicity data for different chemicals is appropriate. Additionally, these data indicate that a soluble, highly toxic surfactant such as tp-NPEO₉ may prove useful as a routinely-used reference toxicant.

Toxicity of Test Compounds

Results of toxicity tests with the 17 surfactants are presented in Table 2. The 48-hr LC50s for all surfactants cover approximately three orders of magnitude (<1 mg/L to >4,000 mg/L). Unless other-

wise stated, comparisons for significant differences between LC50s (non-overlapping 95% confidence intervals) for 3 to 8 day old mysids were made using the moving average procedure due to the narrower confidence intervals around these LC50s. Comparisons using the binomial procedure were made when the moving average procedure would not calculate an LC50. Designations of statistically different LC50s were made only if confidence intervals did not overlap for all tests conducted. The five most toxic surfactants tested (all LC50s ≤ 2 mg/L) were tp-NPEO_{1.5}, the reference toxicant tp-NPEO₉, chlorine-capped TDAE₁₀, linear APEO₉, and OPEO₅. Chlorine-capped TDAE₁₀ was significantly more toxic than phosphated tp-NPEO₉, sulfonated tp-NPEO₉, OPEO_{1.5}, and sodium-methyl oleoyl taurate. Linear APEO₉ was significantly more toxic than phosphated and sulfonated tp-NPEO₉ and sodium-methyl oleoyl taurate. The toxicity of tp-NPEO₉ was also significantly more than that of sulfonated tp-NPEO₉ and sodium-methyl oleoyl taurate. Comparison of confidence limits generated by binomial procedure found the five most toxic surfactants were significantly more toxic than linear APEO₅₀. Lack of toxicity at even the highest concentrations of some chemicals precluded finding statistically significant differences in

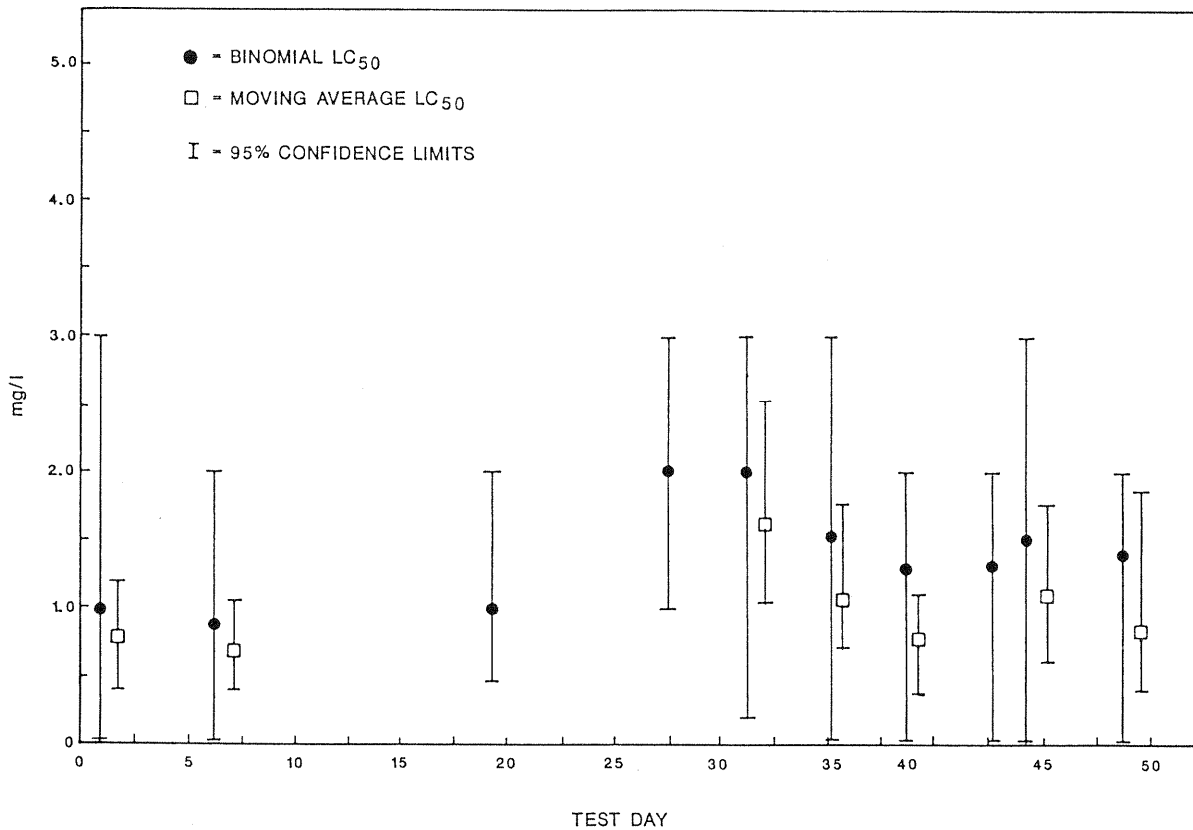


Fig. 1. Sensitivity of *M. bahia* to the surfactant tp-NPEO₉, used as a reference toxicant during the study.

toxicity between these and other chemicals. However, the five most toxic surfactants were markedly more toxic than tp-NPEO₄₀, tp-NPEO₅₀, and linear APEO₅₀.

Other relatively toxic surfactants (binomial and moving average LC₅₀s ~2 to 7 mg/L) include linear APEO_{1.5}, tp-NPEO₁₅, phosphated tp-NPEO₉, OPEO_{1.5}, DAEO₄, and TDAEO_{9.75}. Phosphated tp-NPEO₉ was significantly more toxic than sulfonated tp-NPEO₉. Sulfonated tp-NPEO₉ and sodium-methyl oleoyl taurate were of intermediate toxicity (LC₅₀s ~13 to 30 mg/L) and were significantly more toxic than linear APEO₅₀ as determined by binomial procedure. The least toxic surfactants were linear APEO₅₀, tp-NPEO₅₀, tp-NPEO₄₀, and castor oil ethoxylate. Linear APEO₅₀ was significantly less toxic than all other chemicals except tp-NPEO₄₀ and tp-NPEO₅₀ as determined by binomial procedure.

Toxicity data for surfactants similar to the ones used in this study are rare, especially for marine organisms. However, an extensive review of surfactant toxicity data (open literature and unpublished reports) has been published by the AD Little

Company (1977). This report found that acute LC₅₀s of alcohol ethoxylates to freshwater fish were usually within the range of 1 to 6 mg/L. Acute LC₅₀s of this same group of chemicals to invertebrates were approximately 1 to 100 mg/L (AD Little 1977). Swisher (1987) states the toxicity of surfactants to fish lies in the range of 1 to 100 mg/L. Maki *et al.* (1979) reported 96-hr *Pimephales promelas* LC₅₀s of 1.20 to 1.38 mg/L for a polyethoxylated, lauryl-derived surfactant. Lewis and Perry (1981) reported acute LC₅₀s of three different surfactants to *Daphnia magna* and *Lepomis macrochirus* to range from 0.08 to 5.63 mg/L. Some saltwater toxicity data are also available. From data presented by Wildish (1972), a 48-hr *Gammarus oceanicus* LC₅₀ of 11 mg/L can be derived for a polyoxyethylene lauryl ether. Swedmark *et al.* (1971) reported 96-hr LC₅₀s to *Mytilus edulis*, *Cardium edule*, and *Mya arenaria* of 50, <5, and 100 mg/L, respectively. The toxicities of the majority of surfactants evaluated in this study were within the ranges reported by the above investigators. This indicates that with the exception of some highly soluble surfactants, acute LC₅₀s to a variety of species lie

Table 2. Acute toxicity of test surfactants to *Mysidopsis bahia*

Surfactant	48 hr LC50 (95 percent Conf. Limits) mg/L	
	Binomial	Moving average
Linear APEO _{1.5}	2.00 (0–6) 3.34 (0.5–6)	1.66 (0– α) —
Linear APEO ₉	1.41 (0–2) 1.89 (1–2.5) 1.38 (0.5– α)	— 1.59 (1.4–1.87) 1.23 (0.76–1.89)
Linear APEO ₅₀	4,148 (325– α)	4,148 (0– α)
tp-NPEO _{1.5}	0.11 (1–3) ^a	—
tp-NPEO ₉	0.9–2 ^b (0–3) 2.28 (0.1– α) ^c 1.41 (0– α) ^d	0.71–1.56 ^b (0.39–2.56) 2.20 (1.43–5.84) 0.90 (0.12–3.35)
tp-NPEO ₁₅	2.57 (0–10)	—
tp-NPEO ₄₀	>40 >100 >1,000 ^e >4,110 ^e	>40 >100 >1,000 >4,110
tp-NPEO ₅₀	>6 (0.5– α)	—
tp-NPEO ₉ Sulfonated	>15 (6– α) 29.6 (0–50)	— 24.3 (17–32.3)
tp-NPEO ₉ Phosphated	3.40 (0–10) 5.23 (0–12) 7.07 (0–50)	3.42 (2.14–4.86) 5.23 (2.85–7.37) 6.51 (1.56–18.3)
OPEO _{1.5}	1.83 (0–5)	—
OPEO ₅	5.57 (1–10)	—
DAEO ₄	2.24 (1–5)	—
TDAEO _{9.75}	0.71 (0–2)	0.71 (0.53–0.95)
TDAE ₁₀ Cl-capped	>50 (0– α)	—
Castor Oil Ethoxylate ₃₀	19.1 (1.34–67)	13.8 (8.2–23.2)

^a Measured concentration

^b Ranges of LC50s only, see Figure 1 for individual values

^c Mysids 9 to 12 days old

^d Mysids 23 to 26 days old

^e Control mortality 25%, but value used due to lack of mortality in chemical exposures

within the range of 1 to 100 mg/L, with the majority being <20 mg/L.

Age of mysids apparently did not alter sensitivity to the reference toxicant tp-NPEO₉. LC50 values for 3 to 8 day old, 9 to 12 day old, and 23 to 26 day animals were not significantly different. The more soluble tp-NPEO₄₀ was of low toxicity to 3 to 8 day old and 9 to 12 day old mysids, with LC50s >100 mg/L for each group. Goodman, *et al.* (1987) also found that age of mysids did not affect the sensitivity to malathion, tetrabromo-bis-phenol-a, and tributyltin chloride.

A comparison of relative toxicities of all surfactants is presented in Table 3. Surfactants of various structures are present in each of the three groups of relative toxicity (LC50s \leq 2, ~2 to 30, and >30 mg/L). Thus, the general structure of the surfactant (highly branched or linear, aromatic or aliphatic) is not a factor controlling toxicity to *M. bahia*. An ethylene oxide (EO) molar ratio of \leq 15 was

common to all surfactants in the two most toxic groups. Surfactants with EO molar ratios of 30 to 50 were consistently of very low toxicity. Reductions in toxicity within a chemical group were also seen with increased EO molar ratios as the toxicity of both linear and branched (tp) NPEOs were reduced with increased EO. Alcohol ethoxylate toxicity to freshwater species was also shown to generally decrease as EO molar ratio increased (AD Little Company 1977). This same trend was demonstrated by Wildish (1972) for the freshwater species *Salmo salar* and the saltwater species *Gammarus oceanicus*. This trend has now been confirmed for a marine invertebrate. Figure 2 presents the relationship between the length of the EO chain and toxicity of the surfactants from the tp-NPEO series. It is apparent for this class of products that acute toxicity is approximately an exponential function of EO molar ratio. The only "group" of chemicals consistently toxic were the decyl and tridecyl al-

Table 3. Relative toxicity of test surfactants and summary of physiochemical characteristics

Test surfactant	LC50 or range of LC50s, mg/L binomial and moving avg	EO molar ratio	Active group
tp-NPEO _{1.5}	0.11	1.5	OH
Cl capped TDAE ₁₀	0.71	10	Cl
tp-NPEO ₉	0.71–2.0	9	OH
Linear APEO ₉	1.23–1.89	9	OH
OPEO ₅	1.83	5	OH
Linear APEO _{1.5}	1.66–3.34	1.5	OH
TDAEO _{9.75}	2.24	9.75	OH
tp-NPEO ₁₅	2.57	15	OH
tp-NPEO ₉ Phosphated	3.40–3.42	9	OPO ₃ ⁻²
DAEO ₄	5.57	4	OH
OPEO _{1.5}	6.51–7.07	1.5	OH
Methyl Oleoyl Taurate ₁	13.8–19.1	1	SO ₃
tp-NPEO ₉ Sulfonated	24.3–29.6	9	OSO ₃ ⁻
Castor Oil Ethoxylate ₃₀	50	30	OH
tp-NPEO ₄₀	100	40	OH
Linear APEO ₅₀	4,148	50	OH
tp-NPEO ₅₀	4,110	50	OH

cohols, having LC₅₀s of 0.71 to 5.57 mg/L, most likely due to their low EO molar ratio. Active group substitution altered toxicity in the few cases available for comparison. Chlorine substitution of TDAEO increased toxicity of this molecule. Substitution of the terminal OH group with SO₃ or PO₄ on tp-NPEO₉ reduced toxicity of this molecule, with phosphated tp-NPEO₉ being significantly more toxic than sulfonated tp-NPEO₉. The SO₃ group on sodium methyl oleoyl taurate apparently offset toxicity which would have been expected from this low EO chemical.

Some general conclusions may be drawn from the results of this work. A wide range of LC50s were obtained for the various surfactants, and toxicity could be associated with some characteristics of the chemicals. Toxicity of the ethoxylated alkylphenols, both branched (tp-NPEO, OPEO) and linear APEO, increases with decreasing EO molar ratio which corresponds to decreasing solubility in water. This is an expected finding, since decreasing solubility in water reflects increasing biophilic character of a molecule which in turn indicates that the molecule is more likely to absorb on lipid membranes and cause disruption of the membrane functions. Both aromatic and aliphatic based products with EO molar ratios of 30 or higher are relatively nontoxic, with LC50 values greater than 100 mg/L. Toxicity of the linear APEO (with the average alkyl chain length equal to that of nonylphenol) is similar to the toxicity of the corresponding (in terms of EO molar ratio) branched nonylphenol ethoxylates (tp-NPEO), indicating that the structure of the alkyl

group is not an important factor in pure product toxicity. Substitution of an anionic active group (OSO₃⁻, OPO₃⁻²) in place of the hydroxyl group at the end of the polyethoxy chain apparently decreases surfactant toxicity, most likely due to the increased solubility in water caused by the dissociating active group. Surfactant base structure (aromatic or aliphatic) does not appear to be an important factor in altering the toxicity of the chemicals tested.

The ethylene oxide molar ratio is a good indicator of surfactant toxicity, and would be a good parameter for evaluating surfactant toxicity when conducting hazard assessments on these chemicals. However, biological degradation reduces surfactant toxicity (Swisher 1987; Maki *et al.* 1979; Kimerle and Swisher 1977) and would have to be considered in evaluating possible environmental impacts of these chemicals. Both industrial and domestic surfactants often enter aquatic environments following wastewater treatment. Thus, it is likely that the toxicity of many surfactants is reduced during routine wastewater treatment. Very little data are available on environmental levels of surfactants. A review of data by the AD Little Company (1977) found that levels of nonionic surfactants in European sewage treatment plant effluents ranged from ~0.1 to 2.7 mg/L, with most values ranging from 0.1 to 1 mg/L. These values are in the range of acute effects levels for some of the surfactants considered in this and other studies. However, due to dilution and subsequent biodegradation, in-stream acute effects of surfactants would likely be less

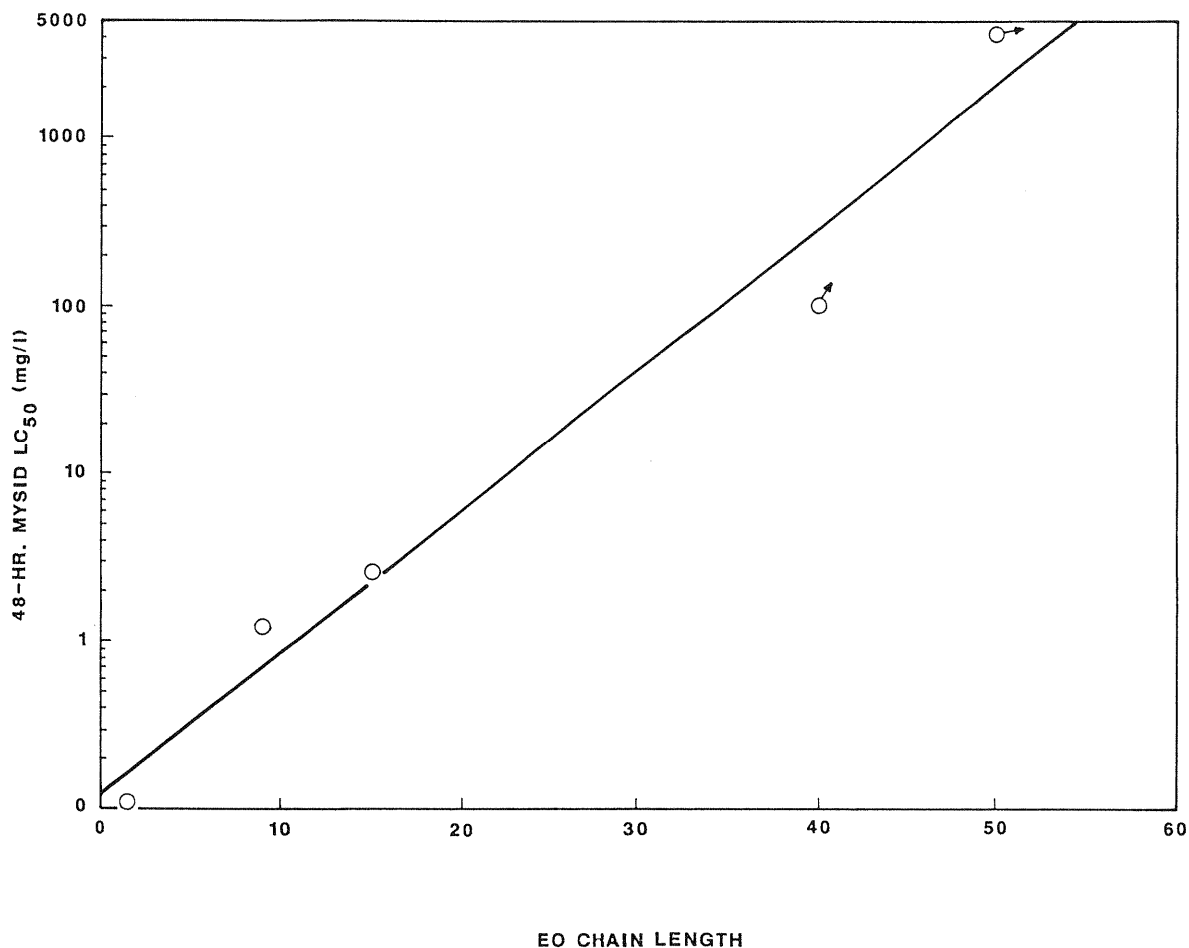


Fig. 2. Effect of EO molar ratio on toxicity of tp-NPEO to *M. bahia*.

than initially indicated by the above effluent concentrations.

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