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# Acute Toxicity Studies of Surfactants to *Daphnia magna* and *Daphnia pulex*

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**Abstract.** Several experiments were designed to examine the acute toxicity of surfactants to *Daphnia*. Specific tests were designed to develop comparisons between existing acute toxicity data for fish and similar data for *Daphnia,* and to provide data on the effects of various environmental factors on resultant toxicity of surfactants to *Daphnia*.

Acute toxicity data for a series of homologous linear alkyl benzene sulfonates (LAS) demonstrate increases of up to one order of magnitude in toxicity for each increase of two alkyl carbons. LC 50's obtained with *Daphnia magna* are similar to those obtained with bluegills, *L. Macrochirus*. Comparative tests with *D. magna* and *D. pulex* indicate no statistical differences in 48-hr LC 50 values for three anionic and two nonionic surfactants. A 50 mg/L concentration of suspended, naturally-occurring kaolin significantly reduced the toxicity of longer chain length LAS homologs and had no effect on nonionic surfactant toxicity.

In tests with variable hardness concentrations, the acute toxicity of LAS to *D. magna* is a combined function of both culture and test water hardness. The toxicity of a nonionic surfactant to *D. magna* was higher in soft water and was not affected by culture water hardness levels.

Unlike previously published data for fish, the results of acute toxicity tests with *D. magna* cultures previously exposed to 0.4 mg/L LAS for periods up to seven generations indicated no significant difference in LAS susceptibility compared to simultaneously tested unexposed controls.

The use of laboratory toxicity tests with aquatic species serves the important function of predicting the effects on aquatic life of chemical substances potentially released to surface water environments. As such, toxicity data for aquatic species is integral to the development of aquatic safety assessments for new or expanded-use chemicals and for the establishment of relevant water quality criteria. Traditionally, acute tests have been done with representative fish species since they are the most visible and economically significant component of most aquatic communities. Recently, as the utility of acute toxicity data for aquatic safety programs has been realized and the number of chemicals requir-

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ing the submission of acute data in support of use or registration has increased, the demand for such data has produced increased interest in the use of invertebrate species for the development of needed toxicity information. Representatives of the genus *Daphnia* have emerged as some of the more important test organisms and as such they typify the advantages of employing macroinvertebrates for development of toxicity data: ease of laboratory culture and maintenance in a strictly defined media, small culture space and water requirements when compared to fish, ease of handling and counting, small test volume requirements, significantly shorter life cycles, and greater disease resistance.

This program was designed to examine the application of *Daphnia* acute toxicity data to aquatic safety programs for the evaluation of effects of residual concentrations of surfactants potentially reaching surface water communities. Specific objectives were to:

1. Examine the effects of changes in surfactant chemical structure on the biological activity as measured by acute toxicity to *Daphnia,* and compare effect concentrations with those published for fish species.
2. Test the relative susceptibility of *D. magna* and *D. pulex* to surfactant toxicity.
3. Examine the effects of environmental variables such as presence of suspended solids, variable water hardness, and previous surfactant exposures or acclimation on the acute toxicity of several classes of surfactants.

## Materials and Methods

### *Test Materials and Analyses*

The test materials employed in these investigations included both anionic and nonionic surfactants. The identifications and chemical descriptions are listed in Table 1. Since the surfactants were of varying degrees of activity, a determination of percent active material was performed on each material immediately prior to toxicity test initiation. A standard curve for each anionic homolog was obtained utilizing the analysis for methylene blue active substances (Amer. Pub. Health Assoc. 1971).

**Table 1.** Chemical characterization of the test surfactants. Generic names are used throughout the text

| Generic Name | Structure | Chemical Characterization |
|---|---|---|
| $C_{10}$ LAS through $C_{18}$ LAS | $CH_3-(CH_2)_X-CH_3$ / $SO_3Na^+$ | Anionics, alkyl chain length range: $C_{10}-C_{18}$, molecular weight range: 341–453 |
| Linear alkyl ethoxylates $C_{14}AE_1$ through $C_{14}AE_9$ | $CH_3-(CH_2)_X-(C_2H_4O)_YH$ | Nonionics<br>X = 13<br>Y = 1 to 9 |

**Table 2.** Dilution water quality for all *Daphnia* toxicity tests with surfactants

| Parameter | Concentration (mg/L) |
|---|---|
| Hardness | 120 mg/L as $CaCO_3$ |
| pH | 7.4 ± 0.2 |
| Dissolved oxygen | 8.5 – 9.5 |
| Nitrate | <0.05 |
| Nitrite | <0.05 |
| Copper | <0.001 |
| Iron | <0.05 |
| Lead | <0.01 |
| Sodium | 11.6 |
| Zinc | <0.001 |

Water samples were taken from each test concentration at the termination of the 48-hr exposure period, preserved with 1% formaldehyde, analyzed by the MBAS procedure and compared with the standard curve for confirmation of expected nominal test concentrations. Results for the nonionic surfactants are based on nominal concentrations since accurate analytical methodologies for quantification of dilute aqueous concentrations of these materials are not available.

### *Test Procedure*

The methods used for culture procedures and acute toxicity tests followed the guidelines established by the USEPA (EPA-660/3-75-009, 1975). The quality of the carbon-filtered well water used as dilution water for all tests is listed in Table 2. All tests were carried out at a constant temperature of 21 ± 1°C under a 16-hr illumination period.

Prior to a test, adult *Daphnia,* sorted by size, were isolated in separate aquaria and the young produced overnight were tested the following day. In this manner, known age individuals, 24-hr old or less, were used to initiate all tests. The test containers were 250 ml Pyrex beakers with a total solution volume of 200 ml. The beakers were cleaned and sterilized after each test. Prior to use, the beakers were rinsed in hot tap water, brushed in 95% reagent grade alcohol, and rinsed in deionized water and allowed to dry. All tests were of 48-hr duration and all concentrations were done in triplicate. Five *Daphnia* were added with a glass pipet to each beaker at the test initiation. Mortality was recorded after 24 and 48-hr intervals. The individuals were not fed during the test. Results were analyzed using a computerized probit analysis program providing for calculation of LC 50 values and associated 95% confidence intervals (Finney 1971).

Deviations from this standard method were employed for tests incorporating kaolin clay (Georgia origin, mean particle size 4$\mu$). For these tests, 50 mg/L kaolin was added to a 4 L volume of dilution water and vigorously mixed. Five 1-L beakers were then filled with 600 ml of this suspension, placed on magnetic stirrers, and the required amounts of test surfactant added to achieve the desired test dilutions. The solutions were then vigorously mixed for 30 min and the three 200 ml replicates of each test concentration were decanted into the test containers.

For tests designed to determine the effects of prior exposure or acclimation to sublethal concentrations of the anionic surfactant $C_{11.8}$ LAS, *D. magna* was cultured in a 0.4 mg/L LAS concentration for periods of 24-hr (short-term exposures) up to seven continuous generations (long-term exposures) prior to comparative toxicity testing with unexposed individuals. During the long-term acclimation period, the 0.4 mg/L LAS concentrations in the culture chambers were replaced three times weekly to minimize degradation of the LAS. All tests were run simultaneously with unexposed individuals to examine the significance of the prior exposure on acute toxicity of LAS.

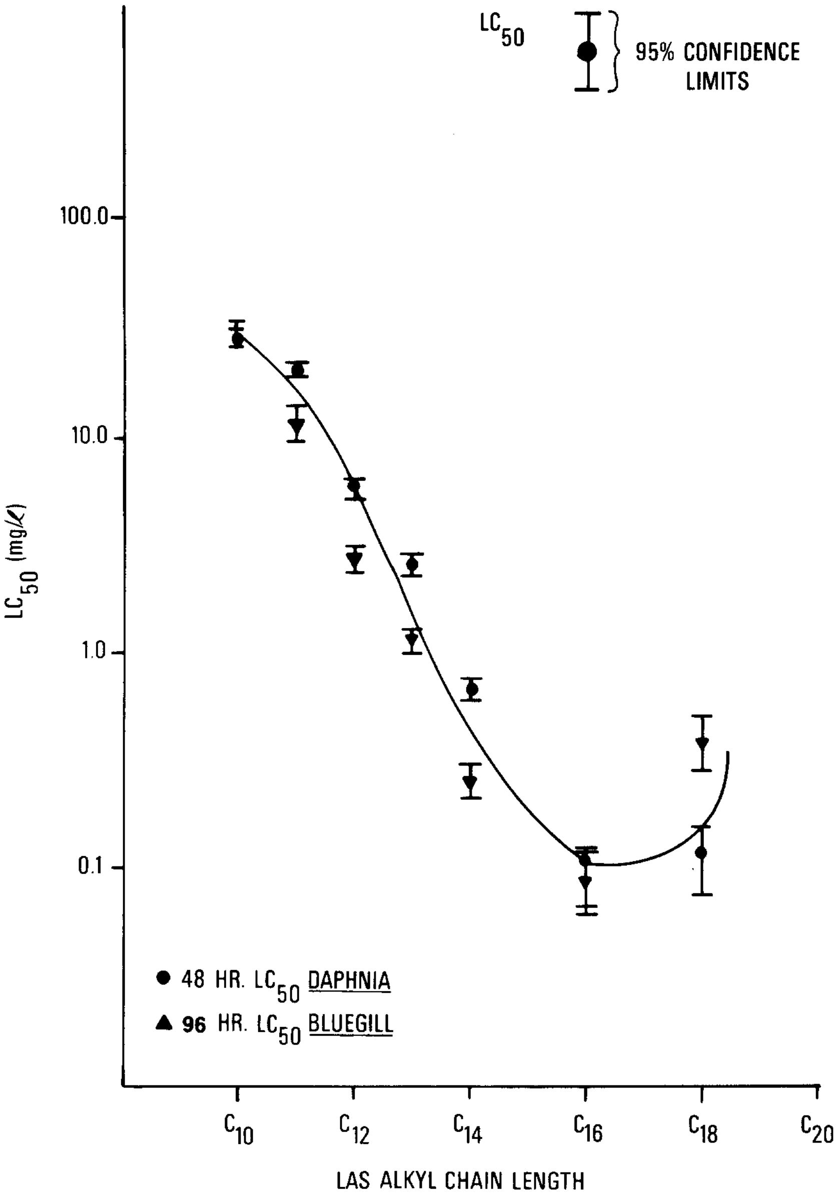


**Fig. 1.** The effect of variations in the alkyl carbon chain length of linear alkyl benzene sulfonate on resultant acute toxicity to *Daphnia magna*.

## Results and Discussion

### *Structure: Activity Correlations*

The effect of an increase in the alkyl carbon chain length of the anionic surfactant, LAS, on resulting acute toxicity is summarized in Figure 1. An increase in

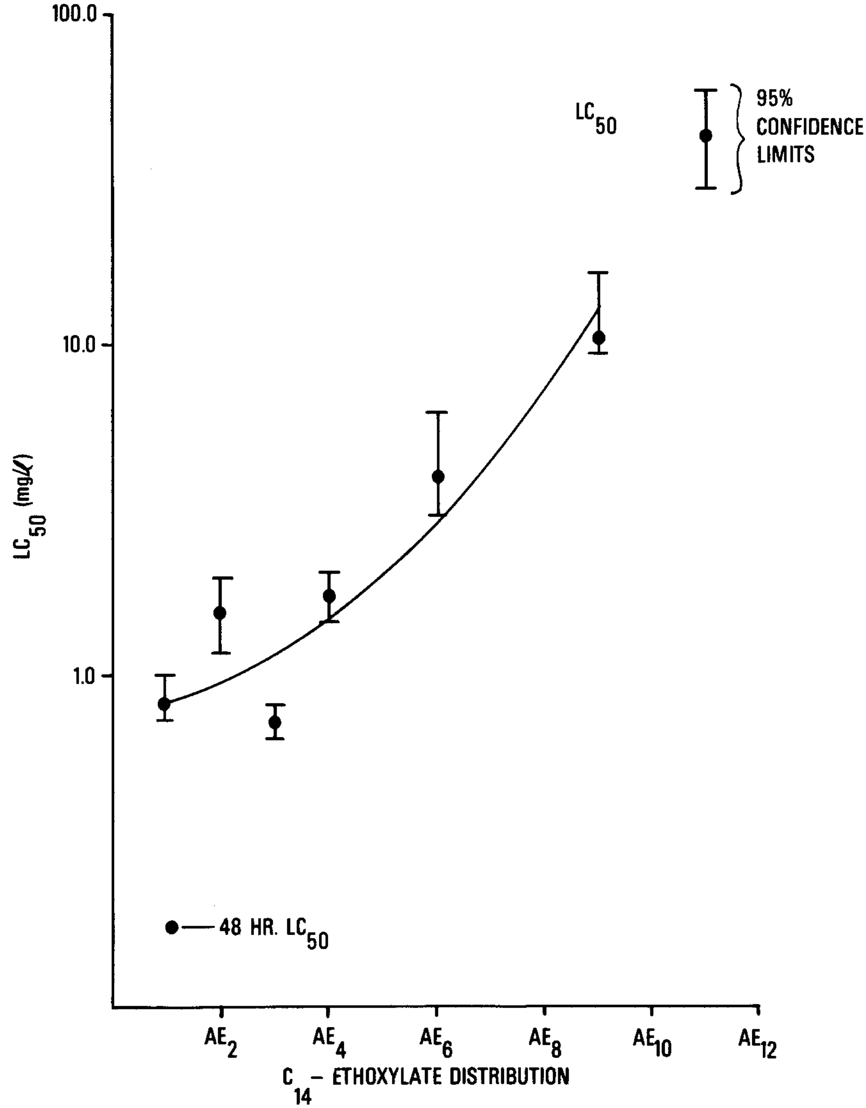


**Fig. 2.** The effect of variations in the ethoxylate distribution of nonionic surfactants on the resultant acute toxicity to *Daphnia magna*.

toxicity of approximately one order of magnitude is demonstrated for each additional two carbons between $C_{10}$ and $C_{16}$, with a subsequent slight decrease at $C_{18}$. The acute toxicity measured as 48-hr LC 50 values drops consistently from 29.5 mg/L at $C_{10}$ to 0.11 mg/L at $C_{16}$, demonstrating a slight but statistically insignificant increase to 0.12 mg/L at $C_{18}$.

Also plotted in Figure 1 are the bluegill 96-hr LC 50 values for the same homologous series of anionic surfactants (A.D. Little 1977). These data demonstrate a similar relationship between increased toxicity and increasing alkyl carbon chain length. Acute toxicity values for bluegill and *Daphnia* indicate that anionic surfactant toxicity data obtained for representatives of one trophic level may be extended to species of different trophic status within aquatic communities.

Similar *Daphnia* toxicity tests completed with nonionic, linear alcohol ethoxylate surfactants with a constant alkyl carbon chain length of $C_{14}$ and an ethoxylate distribution ranging from $AE_1$ to $AE_9$ indicate that increasing ethoxylate distribution causes a decrease in acute toxicity to *Daphnia* (Figure 2). The 48-hr LC 50 value for $C_{14}AE_1$ is 0.83 mg/L, while the corresponding value for $C_{14}AE_9$ is 10.1 mg/L, demonstrating greater than one order of magnitude decrease in toxicity over this increased ethoxylate composition (Table 3).

Consistent changes in biological activity, measured as acute toxicity to *Daphnia*, resulted from variation in chemical composition within these homologous series of anionic and nonionic surfactants. These relationships demonstrate that it may be possible to predict subsequent toxicity of formulation changes for new materials by knowing precise chemical structure and basic toxicity data for representatives of the homologous series. Thus, individuals attempting to design safety assessment programs for new chemicals or individuals attempting to establish water quality criteria have much to gain from an examination of data for similarly structured chemical substances.

### *Species Comparison Testing*

Five surfactants representing three anionics and two nonionics were utilized during comparative acute toxicity tests with *D. magna* and *D. pulex*. All tests with a particular material were run simultaneously under similar conditions with young daphnids 24-hr old in an attempt to control any extraneous variables

**Table 3.** Acute toxicity of homologous series of surfactants to *Daphnia magna*

| Anionic Surfactants | 48 hr LC50 (ppm) | 95% Confidence limits (ppm) |
|---|---|---|
| $C_{10}$ LAS | 29.55 | 27.94 – 31.05 |
| $C_{11}$ LAS | 21.15 | 18.49 – 22.25 |
| $C_{12}$ LAS | 5.88 | 5.24 – 6.49 |
| $C_{13}$ LAS | 2.63 | 2.37 – 2.85 |
| $C_{14}$ LAS | 0.68 | 0.58 – 0.77 |
| $C_{16}$ LAS | 0.11 | 0.068 – 0.126 |
| $C_{18}$ LAS | 0.12 | 0.074 – 0.154 |
| **Nonionic Surfactants** | **48 hr LC50 (ppm)** | **95% Confidence Limits (ppm)** |
| $C_{14}AE_1$ | 0.83 | 0.73 – 0.91 |
| $C_{14}AE_2$ | 1.53 | 1.18 – 1.97 |
| $C_{14}AE_3$ | 0.73 | 0.64 – 0.81 |
| $C_{14}AE_4$ | 1.76 | 1.43 – 2.03 |
| $C_{14}AE_6$ | 4.17 | 3.05 – 6.02 |
| $C_{14}AE_9$ | 10.07 | 9.46 – 10.66 |

**Table 4.** Results of 48 hr acute toxicity tests with surfactants and two species of *Daphnia*

| | 48 hr LC50 (mg/L) | |
|---|---|---|
| Test Material | Daphnia magna | Daphnia pulex |
| $C_{12}$ LAS | 6.84<br>(5.29 – 8.46) | 8.62<br>(7.28 – 9.91) |
| $C_{14}$ LAS | 0.80<br>(0.69 – 0.91) | 0.59<br>(0.51 – 0.69) |
| $C_{16}$ LAS | 0.20<br>(0.17 – 0.22) | 0.15<br>(0.13 – 0.17) |
| $C_{14}AE_1$ | 0.14<br>(0.11 – 0.16) | 0.10<br>(0.07 – 0.12) |
| $C_{14}AE_4$ | 0.24<br>(0.20 – 0.27) | 0.21<br>(0.18 – 0.23) |

otherwise influencing directional susceptibility of the two test species. Results of these comparative tests with anionics ranging from $C_{12}$ to $C_{16}$ LAS, and the nonionics $C_{14}EA_1$ and $C_{14}AE_4$, indicate that no statistically significant difference can be shown between the 48-hr LC 50 values for the two species (Table 4). Slight differences existing between the two species are most likely due to the sample size—15 individuals from three replicates of each test concentration. These differences would most likely be normalized with a higher number of replicates.

Representatives of the genus *Daphnia* have become some of the more important test organisms for aquatic safety evaluation programs, due to the relative ease of laboratory culture and short generation times of approximately eight days. Traditionally, the most popular *Daphnia* test species has been *D. magna;* however, recently, questions have arisen over the most appropriate *Daphnia* species for testing. *Daphnia magna* has a somewhat limited geographical distribution, being typically found in small, hard water lakes (Brooks 1957). It has been argued that the more cosmopolitan species, *D. pulex,* is a more valid test organism for water quality demonstrations (Buikema et al. 1976). Such a conclusion, however, is not warranted by the available data. Using LC 50 values as a measure of acute toxicity, *D. magna* and *D. pulex* have been reported to exhibit virtually identical sensitivities to a wide variety of organic and inorganic compounds (Canton and Adema 1978; Winner and Farrell 1976; Buikema et al. 1976). The similarity of response for *D. magna* and *D. pulex* demonstrated in this investigation indicates that both species are equally sensitive to anionic and nonionic surfactants.

### *Effects of Suspended Solids on Surfactant Toxicity*

Numerous investigators have examined the effects of various physical and chemical environmental factors on the toxicity of surfactants to aquatic life. These studies have demonstrated that acute toxicity thresholds are generally observed at lower concentrations of nonionic surfactants (Swedmark et al. 1971) and anionic surfactants (Hokanson and Smith 1971) when test temperature is increased. Similar studies examining the effects of variable concentrations of dissolved oxygen (Herbert et al. 1957; Hokanson and Smith 1971) and salinity concentrations (Eisler 1965) on the acute toxicity of surfactants have shown effect levels to be significantly lower at reduced oxygen concentrations and when tested at high and low salinity extremes. Little information, however, concerning the interactions of suspended solids with surfactant toxicity is currently available. Since natural surface waters typically contain measurable concentrations of suspended inorganic sediments and intact surfactants are known to interact with these solids, it is reasonable to assume that toxicity tests completed with the presence of suspended sediments would closely model the chemical/physical complex likely to exist in surface waters to which undegraded surfactants may have been discharged.

The results of the present tests with *Daphnia magna* demonstrate variable toxicity, with interactive effects between the 50 mg/L suspension of kaolin clay, the chemical class of surfactant tested, and individual alkyl chain lengths (Table 5). For the anionic surfactants tested, the 48-hr LC 50 values were observed at significantly higher concentrations of both the $C_{14}$and $C_{18}$ homologs, but no significant difference was observed with $C_{11}$ LAS (Figure 3). The acute toxicity of the nonionic surfactants tested varied as a function of alkyl carbon chain length; however, there was no effect on the 48-hr LC 50 values when kaolin clay was present (Table 5).

The results of these tests with surfactants demonstrate that the presence of suspensions of purified, naturally occurring kaolin clay, significantly alters the

**Table 5.** Effects of a 50 mg/L suspension of kaolin clay on the acute toxicity of a homologous series of anionic surfactants to *Daphnia magna*

| | | 48 hr LC50 and Associated 95% Confidence Limits (ppm) | |
|---|---|---|---|
| Anionic Surfactants | | Without Kaolin | With Kaolin |
| $C_{11}$ LAS | | 19.3 (14.3 – 23.5) | 13.0 (5.1 – 17.4) |
| $C_{14}$ LAS | | 1.0 (0.94 – 1.1) | 1.4 (1.2 – 1.5) |
| $C_{18}$ LAS | | 0.09 (0.07 – 0.11) | 0.18 (0.12 – 0.24) |
| Nonionic Surfactants | | | |
| $C_{10}AE_3$ | | 1.9 (1.0 – 2.6) | 1.7 (1.1 – 2.3) |
| $C_{14}AE_3$ | | 0.12 (0.08 – 0.16) | 0.12 (0.05 – 0.18) |
| $C_{18}AE_3$ | young | 5.0 – 20.0 | <5.0 |
| | adults | >80.0 | >80.0 |

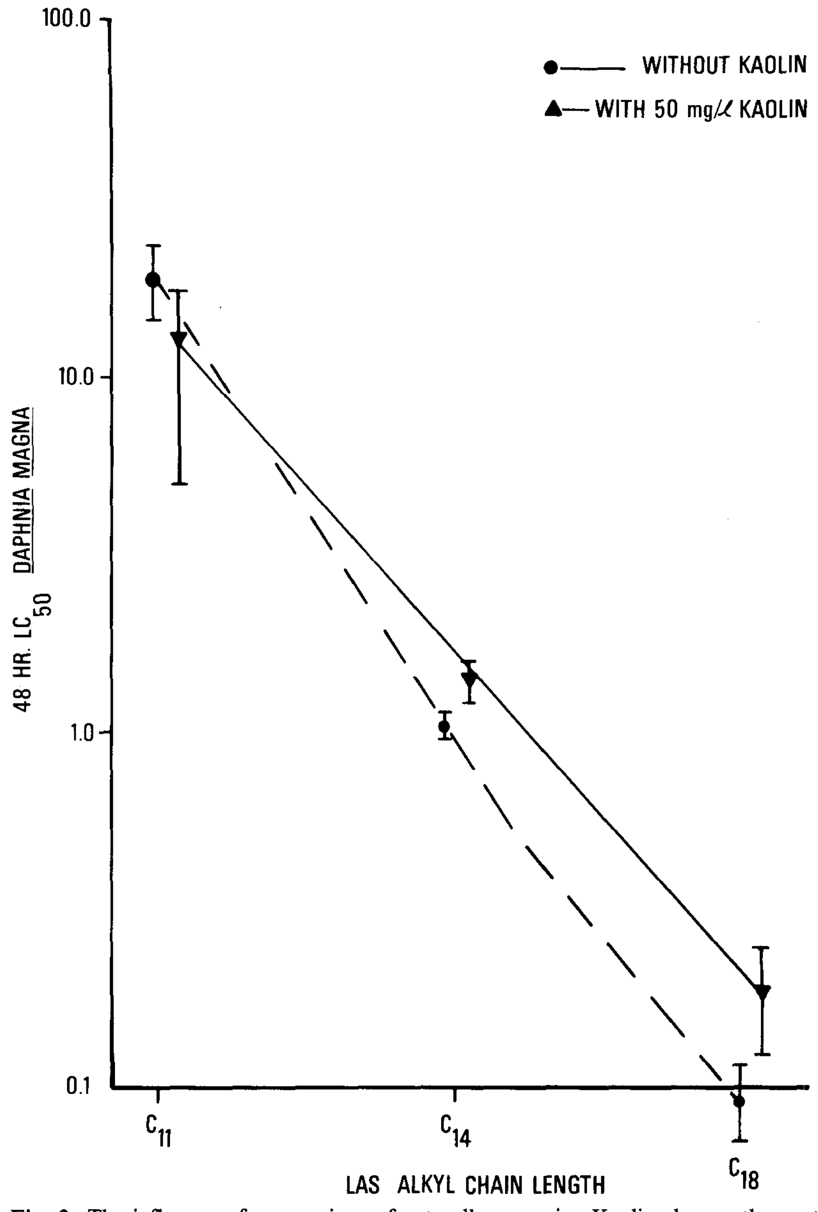


**Fig. 3.** The influence of suspensions of naturally-occurring Kaolin clay on the acute toxicity of linear alkyl benzene sulfonate to *Daphnia magna*.

observed acute toxicity threshold and LC 50 concentration. The proposed mechanism by which toxicity is reduced is believed to result from absorption of the surfactant on the clay and subsequent settling of this suspension in the test container, with resultant loss of active surfactant from solution. The degree of adsorption and subsequent observed loss of toxicity is dependent on the chemical class and specific composition of the surfactant. In general, the longer alkyl chain length homologs of anionic surfactants demonstrate significant reductions in toxicity in the presence of kaolin, while nonionic surfactants representing a wide range of alkyl chain lengths appear unaffected by the suspended solids additions. These results lead to the conclusion that the addition of suspended solids to a toxicity test chamber can be important to testing designed to determine acceptable or nonhazardous concentrations of surfactants to aquatic life, but that the decision to employ solids during the testing procedure should be predicated on the demonstrated ability of the material in question to strongly adsorb to natural solids. In these instances, the addition of known solids is advisable to more closely simulate the physical/chemical form of test material existing in the environment.

### *Effects of Hardness Ions*

Water hardness affects the toxicity of many chemicals in solution and the toxicity of surfactants has been variously reported to increase, decrease, or be unaffected by water hardness. (Abel 1974). Henderson et al. (1959) found nonionic toxicity to be unaffected by water hardness, but reported the anionic sodium alkyl sulphate to be more toxic in soft water. Hokanson and Smith (1971) reported the toxicity of LAS to be significantly greater in waters of 290 mg/L $CaCO_3$ than in waters of 15 mg/L $CaCO_3$.

The toxicity and bioconcentration of the anionic surfactant, sodium lauryl sulphate, were directly proportional to the hardness of the test solution and to the hardness of the water in which the fish were acclimated. (Tovell et al. 1974). Toxicity and bioconcentration were related to the concentration of divalent cations ($Ca^{+2}$ of $Mg^{+2}$) in the test solution. The toxicity and bioconcentration of a nonionic surfactant, $C_{12}$ alkyl ethoxylate, were inversely proportional to the test water hardness, and not related to the cation composition of the test solution or to the hardness of the water in which the fish were acclimated (Tovell et al. 1975).

The acute toxicity of LAS to *D. magna* is a function of both culture and

**Table 6.** Effects of culture and test water hardness on the acute toxicity of an anionic and nonionic surfactant on *Daphnia magna*

| Culture Water | | Test Water | | 48 hr LC50 (95% Confidence Interval)[b] | |
|---|---|---|---|---|---|
| Type | Hardness[a] | Type | Hardness | $C_{11.8}$ LAS | Neodol 45-7 |
| Soft | 50 | Soft | 25 | 7.1 (5.4-16.9)[b] | .36 (.28-.45) |
| Soft | 50 | Very hard | 350 | 3.5 (2.9-4.1) | .65 (.52-.79) |
| Moderately hard | 125 | Soft | 25 | 4.2 (3.2-6.0) | .56 (.52-.74) |
| Moderately hard | 125 | Very hard | 350 | 4.0 (3.4-4.8) | .62 (.48-.77) |
| Hard | 225 | Soft | 25 | 2.0 (.5-2.9) | .36 (.28-.45) |
| Hard | 225 | Very hard | 350 | 3.2 (2.6-3.8) | .88 (.64-1.90) |
| Very hard | 350 | Soft | 25 | 1.8 (.6-2.4) | .36 (.28-.45) |
| Very hard | 350 | Very hard | 350 | 4.0 (3.1-5.0) | .90 (.72-1.30) |

[a] mg/L as $CaCO_3$
[b] mg/L, based on nominal concentrations

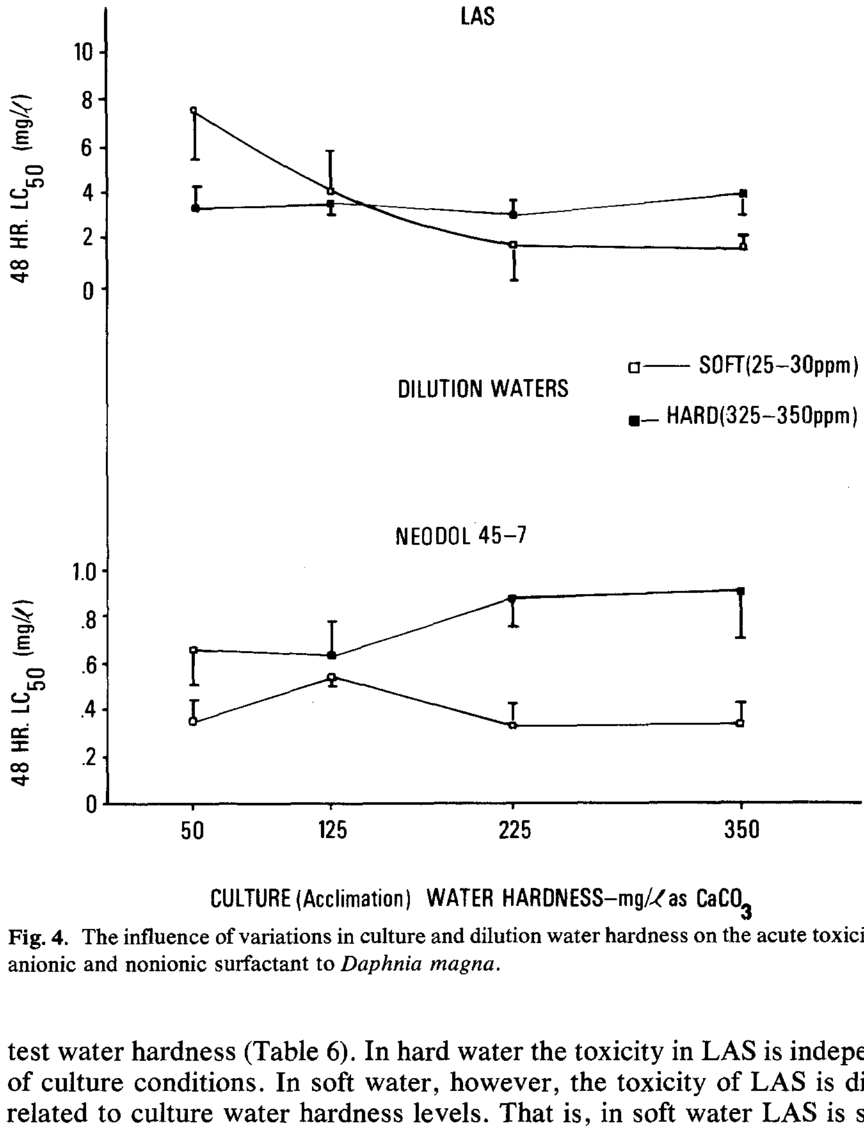


**Fig. 4.** The influence of variations in culture and dilution water hardness on the acute toxicity of an anionic and nonionic surfactant to *Daphnia magna.*

test water hardness (Table 6). In hard water the toxicity in LAS is independent of culture conditions. In soft water, however, the toxicity of LAS is directly related to culture water hardness levels. That is, in soft water LAS is significantly more toxic to *Daphnia* cultured at high hardness levels (figure 4). This apparent discrepancy may indicate a significant additional physiological stress induced by testing high hardness acclimated *Daphnia* in low hardness test solutions. By allowing a sufficient time for acclimation to low hardness, a significant reduction of LAS toxicity in soft water may be affected (Table 6).

The nonionic surfactant, Neodol[1] 45-7, was more toxic to *Daphnia* in soft water (Table 6). The compound has a mixed alkyl chain length of linear $C_{14}$ and $C_{15}$ and an average of seven moles of ethylene oxide per mole of alcohol. Furthermore, the sensitivity of *Daphnia* to Neodol was not conditioned by the

[1] Neodol is a registered trademark of the Shell Chemical Company

Table 7. Acute toxicity of $C_{11.8}$ LAS to acclimated and unacclimated *D. magna*

| Acclimated[a] | | Unacclimated |
|---|---|---|
| Acclimation Period | 48 hr LC50[b] (95% Confidence Interval) | 48 hr LC50[b] (95% Confidence Interval) |
| 24 hr | 2.4 (2.1 − 2.7) | 2.9 (2.5 − 3.2) |
| 24 hr | 2.8 (2.1 − 3.3) | 2.5 (1.7 − 3.1) |
| 5 generations | 3.2 (2.0 − 4.0) | 4.3 (3.7 − 4.9) |
| 7 generations | 2.6 (1.8 − 3.3) | 3.0 (2.0 − 3.8) |

[a] Nominal LAS concentration = .4 mg/L
[b] mg/L

hardness of the water in which the organisms were cultured (Figure 4). The results of these investigations underscore the importance of documenting the quality of test dilution waters and the culture conditions of aquatic species used in toxicity evaluation programs.

*Effects of Prior Surfactant Exposures*

Existing data indicate that fish are able to acclimate to surfactant concentrations. A 30-day exposure of bluegill, *L. macrochirus,* to 3 mg/L ABS (alkyl benzene sulphonate) produced a 48-hr LC 50 63% higher than that of unacclimated control fish tested simultaneously (Lemke and Mount 1965). Hokanson and Smith (1971) also found that acclimation of bluegill to 0.5 mg/L LAS increased lethal threshold concentrations by 50 to 400% that of unexposed controls at several levels of oxygen saturation. If aquatic life is able to acclimate to previous surfactant exposure with no effects on survival or reproductive parameters, this capability could have significance to natural surface water communities existing in receiving waters continuously exposed to low background concentrations of the surfactant, indicating that results of laboratory toxicity tests may yield unnecessarily restrictive values when projected to these real world populations.

To determine if this acclimation effect also occurs in *D. magna,* cultures were exposed to $C_{11.8}$ LAS at a residual concentration of 0.4 mg/L, selected as approximately 1/10 the 48-hr LC 50 concentration, for up to seven generations (approximatley 14 weeks). Subsequent tests with unexposed controls indicate no significant difference in the acute toxicity of $C_{11.8}$ LAS to acclimated or unacclimated cultures (Table 7), leading to the conclusion that previous exposure of *D. magna* to concentrations of LAS as high as 1/10 the 48-hr LC 50 does not alter the sensitivity of the organisms to acute effects. Caution should be used, therefore, in extrapolating results of acclimation reported for fish, to aquatic life in general.

## Conclusions

1. Data for a series of linear alkyl benzene sulfonates and a series of linear alkyl ethoxylates show regular and predictable effects of variation in chemical structure on subsequent acute toxicity to *D. magna*.
2. Comparative toxicity testing with *D. magna* and *D. pulex* and several anionic and nonionic surfactants indicated that both species have statistically similar 48-hr LC 50 values and that both species are equally valid test organisms for investigators attempting to develop water quality information.
3. Acute toxicity tests with *D. magna* and two surfactant types carried out in solutions with and without suspended kaolin clay, indicate that observed loss of toxicity is dependent on the chemical class and specific composition of the surfactant. While nonionics were generally unaffected, the $C_{14}$ and $C_{18}$ alkyl chain lengths of LAS have reduced toxicity in the presence of kaolin.
4. In hard water, the acute toxicity of LAS to *D. magna* is independent of culture conditions, while in soft water toxicity is directly related to culture water hardness. The acute toxicity of a nonionic surfactant was greater in soft water and was not affected by culture conditions.
5. Acute toxicity tests with *D. magna* cultures previously exposed to 0.4 mg/L LAS for periods of up to seven generations indicated no significant difference in LAS susceptibility compared to simultaneously tested unexposed controls.

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