ACUTE TOXICITY AND STRUCTURE–ACTIVITY RELATIONSHIPS OF NINE ALCOHOL ETHOXYLATE SURFACTANTS TO FATHEAD MINNOW AND DAPHNIA MAGNA

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Abstract—Aquatic toxicity of nine commercial-grade alcohol ethoxylate surfactants was studied in acute exposures to fathead minnow (Pimephales promelas) and Daphnia magna. All studies were conducted in accordance with USEPA TSCA Good Laboratory Practice Standards. Mean measured surfactant concentrations in the exposure solutions showed good agreement with nominal concentrations for both fathead minnow ($R^2 = 0.98$, $p = 0.05$) and daphnid ($R^2 = 0.99$, $p = 0.05$) tests. Surfactant recoveries ranged from 59 to 97% and 67 to 106% in the fathead minnow and daphnid solutions, respectively. Response of both species to the surfactants was generally similar. Surfactant toxicity tended to increase with increasing alkyl chain lengths. The effect of low average ethylene oxide (EO) groups on increased surfactant toxicity was more evident in the daphnid exposures. Quantitative structure–activity relationship (QSAR) models were developed from the data, which relate surfactant structure to toxicity. The models predict increasing toxicity with decreasing EO number and increasing alkyl chain length. The models also indicate that average alkyl chain length has a greater effect on toxicity than average EO groups. Further, the models indicate that both species did not differ markedly in their response to alkyl chain length effects, while the number of EO groups had a stronger effect on daphnids than fathead minnow. Model-predicted toxicity agreed well with toxicity estimated from USEPA alkyl ethoxylates SARs and with toxicity values reported in the literature for several surfactants previously studied.

Keywords—Nonionic surfactant toxicity  Alcohol ethoxylate toxicity  Surfactant acute toxicity  Quantitative structure–activity relationship

INTRODUCTION

Alcohol ethoxylates (AE) are nonionic surfactants that are used widely in household products and commercial applications. AEs are the largest volume nonionic surfactants produced with the greatest single end-use in household laundry detergents [1]. Linear primary AEs are the preferred nonionic surfactants due to their rapid biodegradability [1]. The rapid growth in AE usage in household laundry products over the past 20 years has been driven by trends toward low to no phosphate formulations, more synthetic fibers in the wash load, and cooler washing temperatures. Household usage usually results in down-the-drain disposal with the discharge entering septic systems or municipal sewage treatment plants and ultimately the environment. AEs are extensively removed in wastewater treatment systems through both degradation (>90% for activated sludge plants) and adsorption to sludge [1].

Alcohol ethoxylates are composed of a hydrophobic alcohol backbone having a carbon chain length ranging from $C_8$ to $C_{18}$ that combines, via an ether linkage, with a hydrophilic polyoxyethylene chain containing an average of 1 to 12 ethylene oxide units per mole of alcohol [1]. Commercial AE products are usually mixtures that are identified by the range of alkyl chain lengths and the average molar number of ethylene oxide (EO) groups across all carbon chain lengths. For example, NEODOL® 23-5 is a Shell commercial product having a mixture of alcohols with $C_{12}$ to $C_{13}$ alkyl chain lengths and an average of five ethylene oxide units per mole of alcohol.

The majority of work on aquatic safety assessment of surfactants has been carried out on the anionic surfactant, linear alkylbenzene sulfonate (LAS) [2–4]. Only recently has interest been shown on the aquatic safety of AEs [5]. Several previous studies on the aquatic toxicity of AEs to fish and invertebrates have been reported [6–10] along with two recent reviews on fate and effects of surfactants [1,11]. In 1994, the Dutch government began to assess environmental safety of AE and a review was published summarizing available data [5]. The database for AE is not comprehensive in describing the acute and chronic effects of these surfactants, especially the effects of alkyl chain length and average ethoxylate distribution on toxicity.

Acute aquatic toxicity data for nine AEs using fathead minnow (Pimephales promelas Rafinesque) and Daphnia magna Strauss were developed in the present study. All studies were conducted in accordance with U.S. Environmental Protection Agency (USEPA) Toxic Substances Control Act (TSCA) Good Laboratory Practice (GLP) Standards [12]. This paper presents the acute toxicity data and the quantitative structure–activity relationship (QSAR) models developed from the data relating surfactant structure and toxicity for each species.

MATERIALS AND METHODS

Surfactants

The nine surfactants tested were commercial-grade products obtained from Shell Chemical, Houston, TX, USA, and Shell Chemicals UK, Chester, UK. The structure of a model...
Table 1. Physical and chemical properties of the nine alcohol ethoxylate surfactants studied

<table>
<thead>
<tr>
<th>Surfactant trade name</th>
<th>Surfactant chemical structure</th>
<th>Molecular weight</th>
<th>Average ( \text{CH}_2 )</th>
<th>Average EO</th>
<th>EO content (% wt.)</th>
<th>Active content (% wt.)</th>
<th>Hydroxyl no. (mg KOH/g)</th>
<th>HLB no.</th>
</tr>
</thead>
<tbody>
<tr>
<td>NEODOL 23-5</td>
<td>C12-13 EO5</td>
<td>413</td>
<td>12.5</td>
<td>5</td>
<td>53.3</td>
<td>100</td>
<td>136</td>
<td>10.7</td>
</tr>
<tr>
<td>DOBANOL 23-4.5/6</td>
<td>C12-13 EO4.5-6</td>
<td>421</td>
<td>12.5</td>
<td>5.25</td>
<td>55.6</td>
<td>100</td>
<td>136</td>
<td>11.1</td>
</tr>
<tr>
<td>NEODOL 23-6.5</td>
<td>C12-13 EO6.5</td>
<td>484</td>
<td>12.5</td>
<td>6.5</td>
<td>60</td>
<td>100</td>
<td>116</td>
<td>12</td>
</tr>
<tr>
<td>NEODOL 91-6</td>
<td>C9-11 EO6</td>
<td>425</td>
<td>10</td>
<td>6</td>
<td>62.1</td>
<td>100</td>
<td>132</td>
<td>12.4</td>
</tr>
<tr>
<td>DOBANOL 91-8</td>
<td>C9-11 EO8</td>
<td>554</td>
<td>10</td>
<td>8</td>
<td>69.7</td>
<td>100</td>
<td>107</td>
<td>13.9</td>
</tr>
<tr>
<td>NEODOL 1-7</td>
<td>C11 EO7</td>
<td>479</td>
<td>11</td>
<td>7</td>
<td>64.3</td>
<td>100</td>
<td>117</td>
<td>12.9</td>
</tr>
<tr>
<td>NEODOL 1-9</td>
<td>C11 EO9</td>
<td>569</td>
<td>11</td>
<td>7</td>
<td>69.6</td>
<td>100</td>
<td>99</td>
<td>13.9</td>
</tr>
<tr>
<td>NEODOL 25-12</td>
<td>C12-15 EO12</td>
<td>729</td>
<td>13.5</td>
<td>12</td>
<td>71.8</td>
<td>100</td>
<td>77</td>
<td>14.4</td>
</tr>
<tr>
<td>NEODOL 45-13</td>
<td>C14-15 EO13</td>
<td>790</td>
<td>14.5</td>
<td>13</td>
<td>71.8</td>
<td>100</td>
<td>71</td>
<td>14.4</td>
</tr>
</tbody>
</table>

Linear alcohol ethoxylate is RO\((\text{CH}_2\text{CH}_2\text{O})_n\)H, R = CH\(_3\)\((\text{CH}_2)_m\). The alkyl chain length, R, ranged from C\(_9\) to C\(_15\) in this study, while the average EO groups, n, ranged from 4.5 to 13. Some physical/chemical properties of the surfactants are listed in Table 1 [10]. The range of EO distribution for each surfactant is shown in Figure 1. The concentrated surfactants were stored at ambient room temperature at the testing laboratories.

Toxicity tests

Toxicity testing was conducted by two contract laboratories. Laboratory 1 conducted tests with the following surfactants: DOBANOL® 23-4.5/6, NEODOL® 91-6, DOBANOL 91-8, NEODOL 25-12, and NEODOL 45-13, while laboratory 2 conducted tests with NEODOL 23-5, NEODOL 23-6.5, NEODOL 1-7, and NEODOL 1-9. Testing was based on USEPA-TSCA procedures [13] and American Society for Testing and Materials (ASTM) guidelines [14]. The acute toxicity tests conducted were a 96-h static renewal test using fathead minnow and a 48-h static renewal test using D. magna. A daily renewal schedule was used. The test organisms were cultured at the contract laboratories.

Surfactant concentrations used in the definitive tests were based on results from range-finding tests. One or two percent primary stock solutions were prepared at test initiation and for each renewal of test solutions. Dilution water used varied with the laboratory and was either filtered well water or fortified laboratory water. Organisms were exposed to a geometric series of at least five concentrations and an untreated dilution water control in the definitive tests. Two replicates with 10 fish per replicate were used in the fathead minnow tests, while four replicates with five daphnids per replicate were used in the D. magna tests. All test chambers used were made of glass or were Teflon®-lined polyethylene tanks in some fish studies. Observations on fish mortality and daphnid immobilization were made after each 24-h period.

The fathead minnow and daphnid studies were conducted at 22 ± 2°C and 20 ± 2°C, respectively, under a photoperiod of 16 h of light and 8 h of darkness. Temperature, pH, and dissolved oxygen were measured at approximately 24-h intervals. Dilution water chemical parameters (e.g., hardness, alkalinity, acidity, conductivity, total organic carbon) were also measured at test initiation using the appropriate instruments or Standard Methods procedures [15].

Samples from the exposure solutions were collected to verify surfactant concentrations. Replicates within a treatment were pooled and preserved with 1% formalin (final concentration) for analysis. For the fathead minnow test, the day-0
solutions and day-3 renewal (new) solutions and the corresponding 24-h-old solutions (day-1-old and day-4-old) were collected. For the daphnid test, the day-0 solutions and day-1 renewal solutions and the corresponding 24-h-old solutions (day-1-old and day-2-old) were collected. The new-old paired solutions were collected to determine the extent of surfactant loss over 24 h. The mean measured concentration for each treatment was used in data analyses.

Surfactant analysis

The concentration verification samples were analyzed by a third contract laboratory using a method developed by Shell Development Company for the determination of total AE surfactants in aqueous samples [16]. The surfactant was isolated and concentrated from the aqueous samples using solid-phase extraction (SPE) with C8 reverse-phase cartridges. C2 cartridges were used for surfactants with low number of EO groups (NEODOL 23-5, DOBANOL 23-4.5/6). Methyl and isopropyl alcohol were used to elute the surfactant from the SPE cartridges. The surfactant was then separated on an octyl HPLC column using a methanol/water gradient and detected and quantified by an evaporative light-scattering detector (ELSD). This method quantified total AE without distinguishing between the various individual AE or carbon chain distributions. Blank and matrix-spiked quality control (QC) samples were included with each batch of water samples analyzed. Surfactant recoveries in the matrix-spiked samples ranged from 66 to 103% with the lower recoveries associated with surfactants having low number of EO groups. The measured concentrations in the exposure solutions were not normalized to the recoveries in the QC matrix-spiked samples.

Data analysis

Toxicity endpoints determination. Measured toxicity endpoints were immobilization for daphnids and mortality for fathead minnow. Statistical analyses of the concentration–response data were accomplished using EPA computer programs. The fathead minnow 96-h 50% lethal concentration (LC50), daphnid 48-h 50% effective concentration (EC50), and 95% confidence intervals were estimated using probit analysis if there were at least two partial responses in the survival data. If the data were not suited for probit analysis, then moving average angle analysis or nonlinear interpolation was used.

QSAR models derivation. A quadratic model \( y = c + \alpha_1x_1 + \alpha_2x_2 + \alpha_1_1x_1^2 + \alpha_2_2x_2^2 + \alpha_1_2x_1x_2 \) was used to relate surfactant toxicity (\( y \)) to average EO number (\( x_1 \)) and alkyl chain length (\( x_2 \)). The full quadratic regression model was used to fit the data in order to test (a) if there is significant interaction between EO and alkyl chain length in their toxicity and (b) if EO and alkyl chain toxicity show curvature. Toxicity endpoints, expressed in molar concentration of the surfactants, were used in model derivation. The model was fitted by stepwise regression using STATGRAPHICS® Plus statistical program [17]. Variable selection was done using both forward and backward selection. In forward selection, terms in the model are added one at a time until no other significant term is available. Backward selection starts with all the terms in the model and then deletes one nonsignificant term at a time until there is no more such term for deletion. Choice between the two selection methods is based on comparing coefficient of determination (\( R^2 \)), mean squared error (MSE), and model parsimony (simpler model is better).

Assessment of the model assumptions of normality and randomness using the residuals of the fitted model indicated that a logarithmic transformation of the response variable (\( y \)) was more suitable than the original response variable. Use of a criterion of ±3 standardized residuals indicated no outliers for exclusion from modeling.

In addition, response surface plots of models were used to show that the model-predicted results were interpretable and consistent with the observed data. In the case for D. magna, the quadratic term for EO was replaced by a linear term for model simplicity as both models performed equally well and produced essentially the same results.

RESULTS

Measured surfactant concentrations

The mean measured surfactant concentrations in the exposure solutions showed good agreement with nominal concentrations for both fathead minnow and daphnid tests (Fig. 2). Surfactant recoveries ranged from 59 to 97% and 67 to 106% in the fathead minnow and daphnid solutions, respectively. In general, the measured concentrations indicate no appreciable surfactant loss between solution renewals in the daphnid tests. In the fish tests, however, a 30 to 40% decrease in concentration between the new and old solutions was found for some surfactants, particularly in the low concentration treatments.

Surfactant toxicity

The responses of fathead minnow (mortality) and D. magna (immobilization) to the nine surfactants were similar (Fig. 3). The concentration–response curves appear steeper for surfactants with C12 and greater alkyl chain lengths than for surfactants with C9–11 and C11 chain lengths. Because the majority of the concentration–response curves were steep, only 5 of the 18 toxicity tests yielded more than one partial response to
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Fig. 3. Concentration response of fathead minnow and Daphnia magna to the nine alcohol ethoxylate surfactants studied.

Table 2. Acute toxicity endpoints for fathead minnow and Daphnia magna exposed to nine alcohol ethoxylate surfactants

<table>
<thead>
<tr>
<th>Fathead minnow 96-h LC50</th>
<th>Daphnia magna 48-h EC50</th>
</tr>
</thead>
<tbody>
<tr>
<td>mg/L</td>
<td>µmol/L</td>
</tr>
<tr>
<td>NEODOL 23-5</td>
<td>1.0 (0.84–1.3)c</td>
</tr>
<tr>
<td>DOBANOL 23-4.5/6</td>
<td>0.96 (0.73–1.6)c</td>
</tr>
<tr>
<td>NEODOL 23-6.5</td>
<td>1.3 (0.72–2.7)c</td>
</tr>
<tr>
<td>NEODOL 91-6</td>
<td>8.5 (6.0–12)c</td>
</tr>
<tr>
<td>DOBANOL 91-8</td>
<td>11 (8.5–17)c</td>
</tr>
<tr>
<td>NEODOL 1-7</td>
<td>3.9 (3.2–5.0)c</td>
</tr>
<tr>
<td>NEODOL 1-9</td>
<td>7.1 (6.3–8.1)c</td>
</tr>
<tr>
<td>NEODOL 25-12</td>
<td>1.4 (1.2–1.5)c</td>
</tr>
<tr>
<td>NEODOL 45-13</td>
<td>1.0 (0.62–1.9)c</td>
</tr>
</tbody>
</table>

a The 95% confidence intervals are given in parentheses.
b Endpoint calculated by nonlinear interpolation, confidence intervals estimated by binomial probability.
c Endpoint calculated by probit analysis.

A relationship between surfactant structure to toxicity can be seen from the toxicity endpoints. The data show that for both species surfactant toxicity tended to increase with increasing alkyl chain length and appeared to level off at the C\textsubscript{14–15} chain length. Toxicity also tended to increase with decreasing EO groups as evident in the C\textsubscript{9–11} and C\textsubscript{11} surfactants. Within the present data set, the highest toxicity was seen in the C\textsubscript{12–13} surfactants, likely due to the effects of low EO groups. This observation is not conclusive because C\textsubscript{12–13} surfactants with higher EO groups and C\textsubscript{12–15} and C\textsubscript{14–15} surfactants with low EO groups were not tested. Although this study was not designed to address specifically the influence of EO groups on toxicity, the data suggest an increase in surfactant toxicity with low EO groups. In addition, there is no obvious pattern of statistically significant interaction between alkyl and EO chain length on toxicity.

Daphnids appeared to be slightly more sensitive than fathead minnow to the effects of EO groups. The difference in toxicity between 91-6 and 91-8 and between 1-7 and 1-9 is greater for the invertebrate than the fish. The C\textsubscript{12–13} Surfactants also appeared slightly more toxic to the daphnids.

QSAR models for surfactant toxicity

The observed relationships between surfactant structure and toxicity in the data can be formally verified by QSAR models (log base 10). The full quadratic model used initially to fit the data showed that the quadratic and interaction terms were small or not significant, leading to the adoption of linear models. The model for fathead minnow is

\[
\log \text{LC50} = 4.35 - 0.34(\text{alkyl}) + 0.05(\text{EO}),
\]

\[n = 9, \quad R^2 = 0.99\]  

The fit of model 1 to the data is very good because the p-value from the F-test for model adequacy is very small (\(F = 324.7, \ p < 0.01\), with a standard error of estimate \[s = 0.05\]).

The model for the \(D. \text{magna}\) toxicity data is

\[
2\log \text{EC50} = 4.89 - 0.40(\text{alkyl}) + 0.01(\text{EO})^2,
\]

\[n = 9, \quad R^2 = 0.96\]  

Because the curvature represented by the quadratic EO term is small, a linear EO term can be used in its place for model

allow for probit analysis in spite of a 50 or 60% dilution factor used to prepare the exposure concentrations. The general similarity in response of both species is also reflected in their LC\textsubscript{50} or EC\textsubscript{50} values (Table 2). Daphnia magna appeared to be more sensitive than fathead minnow in the 23-5 and 1-7 exposures. For the fathead minnow, the 24-h : 96-h LC\textsubscript{50} ratios ranged from 1.0 to 1.6, indicating that the toxic effects of the surfactants were essentially achieved after 24 h. Surfactant toxicity to the daphnids was more gradual over time for 23-5, 23-6.5, 1-7, and 45-13 with 24-h : 48-h EC\textsubscript{50} ratios of \(\geq 2\). Toxic effects to the daphnids were apparent after 24 h for the other surfactants.

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\[
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\]

\[n = 9, \quad R^2 = 0.96\]  

Because the curvature represented by the quadratic EO term is small, a linear EO term can be used in its place for model
simplicity and for allowing direct comparison with the fathead minnow model. Thus, the model, with linear terms, for *D. magna*

\[
\log \text{EC}50 = 4.23 - 0.38(\text{alkyl}) + 0.10(\text{EO}),
\]

\( n = 9, \quad R^2 = 0.96 \)  

(3)

Again, the fits for models 2 and 3 are very good because the *p*-values from the *F*-tests are very small (model 2: *F* = 108.8, *p* < 0.01, *s* = 0.10; model 3: *F* = 100.7, *p* < 0.01, *s* = 0.10). Both models 2 and 3 predict comparable surfactant toxicity to *D. magna*.

The models show that the LC50 or EC50 value is directly related to EO number (positive coefficient) and inversely related to alkyl chain length (negative coefficient). In other words, toxicity increases (lower LC50 or EC50 values) with decreasing EO number and increasing alkyl chain length. Further, the models show that for both species, alkyl chain length has a greater effect on toxicity than average number of EO groups. The slopes for the alkyl term in models 1 and 3 are not statistically different (*p* > 0.2), implying that both species did not differ markedly in their response to the effects of carbon chain length. However, the number of EO groups had a stronger effect on daphnids than fathead minnow. The regression coefficients for the EO term are significantly different (*p* < 0.01). The absence of an interaction term in the models seems to be consistent with the data at hand, although it seems logical to suspect some degree of interaction between alkyl and EO chain lengths and toxicity. Response surface plots of these models (Figs. 4 and 5) show greatest surfactant toxicity at high alkyl chain length and low EO groups.

**DISCUSSION**

The surfactants studied are chemically heterogeneous because they are mixtures of homologs with different alkyl chain lengths and each homolog has a range of EO distributions including some free alcohol (no EO group). The toxicity determined for these surfactants and the QSARs developed are representative of the average structure of these mixtures. Although QSARs based on pure compounds are preferred, commercial-grade surfactants are typically mixtures that ultimately enter the environment, and thus, toxicity studies of these surfactants are appropriate and necessary for risk and hazard assessments.

In general, results of this study showed that fathead minnow and *D. magna* responded similarly to the effects of the surfactants. The daphnids appeared to be slightly more sensitive to two surfactants (23-5, 1-7) than fathead minnow. Additionally, effects of EO group, as seen in the toxicity difference between 91-6 and 91-8 and 1-7 and 1-9 were greater for the daphnids. The increased susceptibility in *D. magna* was also observed previously in other studies conducted by Shell. Daphnid EC50 values of 2.5 and 0.14 mg/L and rainbow trout LC50 values of 6.0 and 1.5 mg/L were obtained for NEOHIDOL 91-2.5 and NEOHIDOL 25-3, respectively (Shell Oil Company, unpublished data). Macek and Krzeminski [18] also reported an LC50 of 1.5 mg/L for bluegills exposed to 25-3. Similarly, for NEOHIDOL 45-7, Lewis and Perry [19] obtained EC50 values ranging from 0.29 to 0.40 mg/L in a series of tests in which water hardness was varied. In comparison, the toxicity of 45-7 to fathead minnows was reported to range from 1.2 to 2.5 mg/L (LC50) by Maki et al. [20].

The results of this study also showed that toxicity to fathead minnow and *D. magna* tended to increase as the surfactant structure becomes more hydrophobic (high alkyl or low EO chain length). Relationships between surfactant structure (particularly EO groups) and toxicity to aquatic organisms have been observed previously. Maki and Bishop [9] found decreasing toxicity with increasing EO chain length for *D. magna*, while Hall et al. [21] found that ethoxylated alkylphenols with EO molar ratios of 30 to 50 were consistently of low toxicity to *Mysidopsis bahia*. Guhl and Gode [22] also found decreasing toxicity with increasing EO number for an unidentified fish and *Daphnia* sp. in acute exposures to three AE surfactants (C<sub>12-18</sub> EO10, C<sub>16-18</sub> EO14, C<sub>12-14</sub> EO30). Wildish [23] related surfactant liposolubility to fish toxicity and found that lipid solubility and toxicity of polyoxyethylene esters, ethers, and amines, used in oil dispersants, decreased with increasing EO number.

In the surfactant structure–toxicity relationships reported
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Previously, no interaction between EO and alkyl chain length effects has been indicated. Although we attempted to test for presence of an interaction with the full quadratic model, the data at hand suggest no statistically significant interaction present. Future studies with larger data sets may shed more light on this issue.

Toxicity of anionic and nonionic surfactants have been previously modelled using Königemann [24] general narcosis QSARs for chemically unreactive compounds using log \( P \) (log \( K_{ow} \), octanol/water). Schüürmann [25] used a two-variable (#EO-1, log \( P \)) regression model on Hall et al. [21] data and found that the toxicity of polyoxyethylene surfactants to *Mysidopsis* was mainly due to narcotic-type mechanisms. Roberts [26] analyzed a range of surfactant acute toxicity data to determine whether QSARs can be developed relating toxicity to calculated log \( P \) values. He found that the log \( P \)-based QSARs for surfactants were very similar to QSARs established for nonsurfactant organic compounds with nonspecific modes of action. More recently, Feijtel and van de Plassche [5] compared experimental no-observed-effect concentrations (NOECs) for AE (normalized to \( C_{13.3} \) EO8.2) for fathead minnow, *D. magna*, and two algal species to values calculated using QSARs based on log \( P \) for nonspecific acting compounds derived by van Leeuwen, et al. [27]. The Dutch Soap and Detergent Association (Nederlandse Vereniging Van Zeepfabrikanten) environmental review of AEs [28], however, was unable to distill a clear SAR from the compiled data, but three clusters were differentiated. The first cluster contained the most lipophilic combination of chain lengths with a weight percent EO <50% and with an EC50 of 1.2 mg/L. The second category contained substances with a weight percent EO between 50 and 70% with alcohol chain length between \( C_9 \) and \( C_{18} \) and with an average EC50 of 2.7 mg/L. The third cluster consisted of the most hydrophilic substances with long ethoxylate chains and weight percent EO >70% and with an average EC50 of 14 mg/L [28].

The QSARs developed in this study directly relate acute toxicity to surfactant hydrophobicity in terms of alkyl and EO chain lengths rather than estimated using log \( P \). The US EPA has also developed SARs for AEs based on alkyl and EO chain lengths from measured test data that have been submitted by industry. In EPA’s ECOSAR program [29], equations to determine the acute toxicity of nonionic surfactants to fish and daphnids are provided. The models for fish and daphnids are identical, but separate equations based on carbon number are used with the number of ethoxylate groups entered as a variable. To obtain some validation of our models, we used them to estimate the toxicity of six other AEs that have been studied previously [18–20,30]. The alkyl and EO chain lengths of these surfactants were within the range used in the present study. The predicted values from QSAR models are compared to reported values in the literature for fathead minnow and *D. magna* (Tables 3 and 4). Predicted toxicity using the EPA models are also shown for comparison. In general, the predicted values show good agreement with the values obtained from toxicity tests. The predicted and reported values for *D. magna* are highly correlated \( (r = 0.97, \ p < 0.01) \) using the model from the present study and less well correlated using EPA models \( (r = 0.85, \ p < 0.05) \). For fathead minnow, predicted values from our models are less well correlated \( (r = 0.76, \ p = 0.08) \) with reported values than a similar comparison using predicted values from EPA models \( (r = 0.82, \ p < 0.05) \). Using the model from the present study, the predicted toxicity of NEODOL 1-5 and 25-7 to fathead minnow was slightly lower than the observed toxicity, in contrast to higher predicted toxicity for the other surfactants. Excluding these two surfactants improved the correlation of the predicted and observed toxicities for fathead minnow \( (r = 0.98, \ p < 0.05) \). In general, the predicted toxicity is higher than the reported toxicity for both species. The higher reported values may be due to non-specific methods used to quantify surfactant concentration or nominal concentrations were used. The lower observed toxicity for surfactants with low EO groups may, additionally, be related to decreased water solubility or physical phase separation.

QSARs have become scientifically acceptable tools in aquatic toxicology for predicting toxicity of chemicals when laboratory data are few or lacking. The USEPA uses such models for predicting toxicity of new chemicals that are sub-

**Table 3. Comparison of predicted surfactant toxicity to fathead minnow using QSAR model from present study, EPA SAR models and observed toxicity values from literature**

<table>
<thead>
<tr>
<th>Surfactant</th>
<th>QSAR-predicted LC50 (present study)</th>
<th>EPA SAR-predicted LC50</th>
<th>Observed LC50</th>
</tr>
</thead>
<tbody>
<tr>
<td>NEODOL 91-2.5</td>
<td>3.5</td>
<td>8.4*</td>
<td>6.0*</td>
</tr>
<tr>
<td>NEODOL 1-5</td>
<td>2.9</td>
<td>6.0</td>
<td>1.6, 2.0*</td>
</tr>
<tr>
<td>NEODOL 25-3</td>
<td>0.28</td>
<td>0.59*</td>
<td>1.5*</td>
</tr>
<tr>
<td>NEODOL 25-7</td>
<td>0.69</td>
<td>1.3</td>
<td>1.48*</td>
</tr>
<tr>
<td>NEODOL 25-9</td>
<td>0.97</td>
<td>1.9</td>
<td>1.6*</td>
</tr>
<tr>
<td>NEODOL 45-7</td>
<td>0.32</td>
<td>1.0</td>
<td>1.2, 1.4, 2.5*</td>
</tr>
</tbody>
</table>

* LC50 values are in mg/L.
* EPA SAR recommended for ethoxylates ≥5 and <20.
* Trout value, Shell internal report, 1979.
* EPA SAR recommended for ethoxylates ≥5 and <40.
* Trout and bluegill values, Shell internal report, 1978, and Macek and Krzeminski [18], respectively.
* [30].
* Shell unpublished data, 1968.
* [20].

mitted for product registration under TSCA. Additionally, the use of QSARs to estimate toxicity of existing chemicals has helped to prioritize chemicals to be tested. The models developed in this study are based on surfactants with average carbon chain lengths ranging from C_{10} to C_{14} and average EO groups from 4.5 to 13. Although the QSARs allow estimates of effects to be made for AEIs in the absence of laboratory data, care should be taken when using these models to predict toxicity of surfactants with structures that are outside the range of those tested.

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REFERENCES