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Ecotoxicology and Environmental Safety 64 (2006) 75–84
Ecotoxicology and Environmental Safety
www.elsevier.com/locate/ecoenv

Ecotoxicity quantitative structure–activity relationships for alcohol ethoxylate mixtures based on substance-specific toxicity predictions ☆

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Received 18 February 2005; received in revised form 18 August 2005; accepted 21 August 2005
Available online 26 October 2005

Abstract

Traditionally, ecotoxicity quantitative structure–activity relationships (QSARs) for alcohol ethoxylate (AE) surfactants have been developed by assigning the measured ecotoxicity for commercial products to the average structures (alkyl chain length and ethoxylate chain length) of these materials. Acute *Daphnia magna* toxicity tests for binary mixtures indicate that mixtures are more toxic than the individual AE substances corresponding with their average structures (due to the nonlinear relation of toxicity with structure). Consequently, the ecotoxicity value (expressed as effects concentration) attributed to the average structures that are used to develop the existing QSARs is expected to be too low. A new QSAR technique for complex substances, which interprets the mixture toxicity with regard to the "ethoxymers" distribution (i.e., the individual AE components) rather than the average structure, was developed. This new technique was then applied to develop new AE ecotoxicity QSARs for invertebrates, fish, and mesocosms. Despite the higher complexity, the fit and accuracy of the new QSARs are at least as good as those for the existing QSARs based on the same data set. As expected from typical ethoxymer distributions of commercial AEs, the new QSAR generally predicts less toxicity than the QSARs based on average structure.

Keywords: QSAR; Alcohol ethoxylate; Mixture toxicity; Additivity; Toxic units; Ecotoxicity; Nonionic surfactant; Risk assessment

1. Introduction

Quantitative structure–activity relationships (QSARs) for ecotoxicity are mathematical relationships between molecular structure descriptors (Roberts, 1991; Morrall et al., 1999), and ecotoxicological effects values of these structures. For well-defined single substances, both the ecotoxicity and the molecular descriptors can be determined "exactly". Hence, QSARs for such substances can be good descriptions of reality—albeit limited by inaccuracies in the underlying experimental data and potential "lack of fit" of the applied mathematical model.

Complex substances, as described here, are not well-defined single chemical structures but are mixtures containing multiple structurally similar chemicals. Ecotoxicity data are rarely available for the individual chemicals but are usually available for the commercial multi-component substances. The ecotoxicity of a complex substance can be highly dependent on the shape of the distribution of its

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doi:10.1016/j.ecoenv.2005.08.009

different components. This is because toxicity is usually not linearly related to molecular descriptors. For example, for surfactants, ecotoxicity will typically increase logarithmically with a linear increase in alkyl chain length. Hence, based on the principle of additive mixture toxicity (Loewe, 1953), the measured ecotoxicity of a complex substance may be largely driven by a limited number of components, i.e., those that are orders of magnitude more toxic than the others. The presence of these highly toxic components is not necessarily reflected in the calculated average structure of a complex substance. As a consequence, for complex substances, there is not always a meaningful relationship between the measured toxicity and the molecular descriptors of the average structure.

In such cases of nonlinearity, the most highly toxic components have an impact on toxicity that is disproportionate to their molar abundance, whereas their impact on the calculation of the substance's average structure is proportionate to molar abundance. Hence, it is possible that a complex mixture will in reality be significantly more ecotoxic than the single substance representing its average structure. Consequently, when for the purpose of QSAR derivation a mixture's measured ecotoxicity is assigned to the average structure (as if it were a single substance), this is expected to result in a QSAR that will—on average—overpredict the toxicity of most individual components of the mixture.

The mode of ecotoxic action for surfactants is generally accepted to be nonspecific, with exposure resulting in disruption of biological membrane integrity (Roberts, 1991; Roberts and Marshall, 1995). Roberts and Marshall (1995) state that the assumption of additivity (concentration addition model) for nonionic surfactants, specifically alcohol ethoxylates (AEs), is valid. Escher and Hermens (2002), Escher et al. (2002), and Dyer et al. (2000) demonstrated that baseline toxicants and related alcohol-based surfactants also follow a concentration addition model.

Alcohol ethoxylates are a class of nonionic surfactants that are complex substances. An AE molecule consists of a fatty alcohol, which is ester-linked to a polyethylene glycol (or ethoxylate) chain. The general formula for AE is CH_3–$(CH_2)_x$–O–$(CH_2CH_2O)_y$–H. For typical commercial AE materials, x can range from 8 to 17 and y can range from 0 to >20. Thus, an AE mixture could contain over 100 individual components ("ethoxymers") due to all possible combinations of alkyl chain lengths and ethoxylation degrees. The notation C_xEO_y will be used below to denote AEs with alkyl chain lengths of x and polyethylene glycol chain lengths of y. Different commercial materials may have different distribution shapes (e.g., narrow or wide) in accordance with the starting alcohols and the routes of synthesis. Logically, the "fingerprint" of AE in the environment also consists of a matrix with all possible ethoxymers. Further, the shape of the environmental distribution is likely to be different from the distribution shape of any of the commercial mixtures of AE.

Several QSARs have been developed to describe the ecotoxicity of AE surfactants. Wong et al. (1997) have derived AE-specific QSARs for acute toxicity to *Daphnia magna* and *Pimephales promelas*, using C length and EO number as molecular descriptors. Willing (2000) presented a QSAR to determine the acute algal toxicity of AE, using C length and EO number. Wind and Belanger (2005) used the same underlying data set to develop a QSAR for chronic algal toxicity (E_bC_{20}). Belanger et al. (2000) calculated an AE-specific QSAR to describe mesocosm NOEC and LOEC as a function of log K_{ow}. Finally, Morrall et al. (2003) developed a chronic ecotoxicity QSAR, based on log K_{ow}, for *D. magna*. An overview of these existing QSARs is given in Table 1.

Except for the algal work, the toxicity of commercial AE materials has previously always been related to the average structure of the exthoxymer distribution to develop the above QSARs. This approach is relevant for interpolation between commercial AE mixtures with distributions strongly centered around the average structures. However, it may not be appropriate for mixtures that have radically different distributions, such as those measured in environmental matrices, or for single ethoxymers. Based on the above reasoning, it can be suspected that these published QSARs overpredict ecotoxicity in those cases.

The research described in this paper aims (1) to verify whether the toxicity of a complex substance (in the case of AE) is indeed inadequately represented by the toxicity of the average structure, (2) to develop (based on the mixture toxicity concepts) a method for deriving QSARs specifically for complex substances, and (3) to apply this new method to derive new QSARs for AE ecotoxicity.

Table 1
Existing AE-specific ecotoxicity QSARs

Ecotoxicological endpoint	QSAR	Unit	Ref.
Forty-eight-hour EC_{50} *Daphnia magna*	$\log(EC_{50}) = -0.38\,C + 0.1\,EO - 1.77$	(mol/L)	1
Ninety-six-hour LC_{50} Fathead minnow	$\log(LC_{50}) = -0.34\,C + 0.05\,EO - 1.65$	(mol/L)	1
Seventy-two-hour ErC_{50} algae	$\log(EC_{50}) = -0.314\,C + 0.237\,EO + 2.96$	(mg/L)	2
Seventy-two-hour EC_0 algae	$\log(EC_0) = -0.168\,C + 0.182\,EO + 0.9$	(mg/L)	3
Twenty-one-day NOEC *Daphnia magna*	$\log(NOEC) = -0.84 \log K_{ow} - 2.0$	(mol/L)	4
NOEC mesocosm	$\log(NOEC) = -0.66 \log K_{ow} + 2.41$	(mg/L)	5
LOEC mesocosm	$\log(LOEC) = -0.748 \log K_{ow} + 3.16$	(mg/L)	5

Refs: (1) Wong et al. (1997); (2) Willing (2000); (3) Wind and Belanger (2005) based on Willing (2000); (4) Morrall et al. (2003); (5) Belanger et al. (2000).

2. Materials and methods

2.1. Experimental

To determine whether the mixture toxicity theory applies to AE, the acute toxicity (48 h) to *D. magna* of two binary AE mixtures was determined (following OECD guidelines; OECD, 1993) and compared with the acute toxicity of the mixtures' single-ethoxymer components and the single-ethoxymer "average structure". The two (1:1 w/w) mixtures in this experiment consisted of individual model components having exactly $C_8EO_4 + C_{16}EO_8$ and $C_{14}EO_8 + C_{10}EO_8$. Hence, the corresponding average structures were $C_{12}EO_6$ and $C_{12}EO_8$.

Test solutions for the mixtures were prepared by combining individual chemicals in equal concentrations (w/w) to give a geometric series of test solutions. The concentrations of these test solutions were selected from the individual measured EC_{50}s using the toxic unit approach so that an effect equivalent to an EC_{50} would be expected toward the center of the concentration range. Test solutions were renewed after 24 h. Actual concentrations were determined using solvent extraction derivatization and high-performance liquid chromatography (HPLC). However, due to the poor recovery of $C_{16}EO_8$, the effects for the first mixture are based on nominal concentrations.

The following analytical methodology was used. All samples were preserved with 3% (v/v) formaldehyde and stored at 4 °C until analyzed. A series of calibration standards for each AE was prepared by diluting 100 mg/L stock solutions with acetonitrile. A spiked sample was prepared by adding 0.25 ml of each of the 100-mg/L solutions to 100 ml of preserved Elendt medium. C18 (1 g/6 ml) Isolute cartridges were conditioned with 10 ml methanol followed by 10 ml Milli-Q water. Sample vessels were rinsed with 2 × 5 ml aliquots of Millipore water. The washings were added to the reservoirs. After extraction of the sample the cartridges were allowed to dry under vacuum for a minimum of 30 min. The AEs were eluted with 10 ml methanol. The samples were evaporated to dryness at 60 °C under a gentle stream of nitrogen and the vials were removed immediately after the methanol was evaporated. The dried residues were dissolved in 1 ml acetonitrile with the aid of 5 min sonication and "Whirli" mixing. The samples were then derivatized with 20 µl napthoyl chloride in the presence of 50 µl 1-methyl imadazole. Derivatization was performed at 60 °C for 30 min. After derivatization the vials were allowed to cool and 100 µl of methanol was added. The samples were then analyzed by HPLC. Calibration standards were derivatized as described above.

On each day of analysis a calibration including test samples, spikes, and standard checks was run at the beginning and end of the sequence. Analysis was performed using the following instrumentation and conditions:

Injector:	Perkin–Elmer ISS 100	
	Injection volume 20 µl	
Pump:	Perkin–Elmer series 200	
Eluent A 75:25 0.05 mol L^{-1} ammonium acetate in Millipore water: Methanol		
Eluent B 50:50 methanol: Acetonitrile (v/v)		
Gradient programme:	Time	% B
	0 min	50
	6 min	75
	30 min	100
	Flow 1.0 ml/min	
Column:	150 × 4.6 mm i.d. Hypersil Elite C18, 5 µm	
Detector:	Perkin–Elmer LC240 Fluorescence Detector	
	Excitation wavelength 300 nm	
	Emission wavelength 385 nm	
Data handling:	Perkin–Elmer Turbochrom	

Quantitation was by an external standard calibration of the AE peak area. The calibration curve was generated with Turbochrom software using a first-order fit. Corrections were made to account for sample volume. To monitor the performance of the HPLC system standard checks (2.5 or 10 mg/L) were run at least every five samples.

2.2. Theoretical

In a mixture of which the components have the same mode of action, these components are expected to exert additive ecotoxic action. To predict the overall ecotoxicity of such mixtures, the additive mixture toxicity concept can be applied (Loewe, 1953). In this approach, it is assumed that the ecotoxicity of a mixture is equal to the sum of the ecotoxicity of its individual components. For each component i, a toxic unit contribution (TU_i) is calculated as the reciprocal of the toxicity number (TOX_i, e.g., EC_{50}, NOEC, etc.) multiplied by the abundance of the component in the mixture (f_i, fraction). Subsequently, the reciprocal of the sum of all the toxic units in the mixture represents the total ecotoxicity number of the mixture ($TOX_{mixture}$) (expressed, like TOX_i, as EC_{50} or NOEC—i.e., the more toxic the mixture is, the lower its TOX_i value will be):

$$\mathrm{TOX}_{\mathrm{mixture}} = \frac{1}{\sum_i \mathrm{TU}_i} = \frac{1}{\sum_i f_i/\mathrm{TOX}_i}.$$

This equation can be directly applied to calculate the expected ecotoxicity of binary mixtures, as described under Experimental. However, this equation can also be applied in the development of ecotoxicity QSARs for complex substances. In the mixture toxicity equation, the toxicity value for each component of the mixture (TOX_i) can be substituted by a QSAR expression, predicting single-substance ecotoxicity as a function of single substance molecular descriptors ($QSAR_i$):

$$\mathrm{TOX}_{\mathrm{mixture}} = \frac{1}{\sum_i f_i/\mathrm{QSAR}_i}.$$

When a series of experimental data is available for different mixture compositions of the complex substance, a series of such equations can be developed. The technique of minimizing the sum of squared errors (SSE) can then be applied to determine the QSAR parameters. To deal with the fact that ecotoxicological data of different substances in the training set may be spread over several orders of magnitude, the SSE should be calculated based on the logarithms of the toxicity values,

$$\begin{aligned}\mathrm{SSE} &= \sum_j (\log(\mathrm{TOX}^{\mathrm{QSAR}}_{\mathrm{mixture},j}) - \log(\mathrm{TOX}^{\mathrm{measured}}_{\mathrm{mixture},j}))^2 x \\ &= \sum_j \left(\log\left(\frac{1}{\sum_i f_i/\mathrm{QSAR}_i}\right) - \log(\mathrm{TOX}^{\mathrm{measured}}_{\mathrm{mixture},j})\right)^2,\end{aligned}$$

where $TOX^{measured}_{mixture,j}$ is measured toxicity for mixture j and $TOX^{QSAR}_{mixture,j}$ is QSAR estimated toxicity for mixture j.

The measured ecotoxicological data, the molecular descriptors, and the fraction of each component in the different mixtures are known. Hence, the only variables that influence the SSE are the QSAR parameters. Consequently, the SSE can be minimized by optimizing the QSAR parameters in the SSE equation. This approach is illustrated schematically in Fig. 1. Given the high mathematical complexity, it is preferable to do this using numerical optimization methods rather than analytical solution techniques (e.g., linear regression) which are more common in single-substance QSAR work.

The above approach of minimizing the SSE of additivity-based toxicity predictions was implemented into a spreadsheet model for AE surfactants. For each complex mixture in the data set, the toxic units calculation was performed via a matrix with carbon chain length (C) and ethoxylation degree (EO) as the two dimensions.

As with the existing AE QSARs, two different models were used to describe AE ecotoxicity: one model based on $\log K_{ow}$ and a second model based on C and EO numbers,

$$\log K_{ow}\ \text{model}: \quad \mathrm{TOX}_i = 10^{A_1 \log K_{ow_i} + I_1}$$

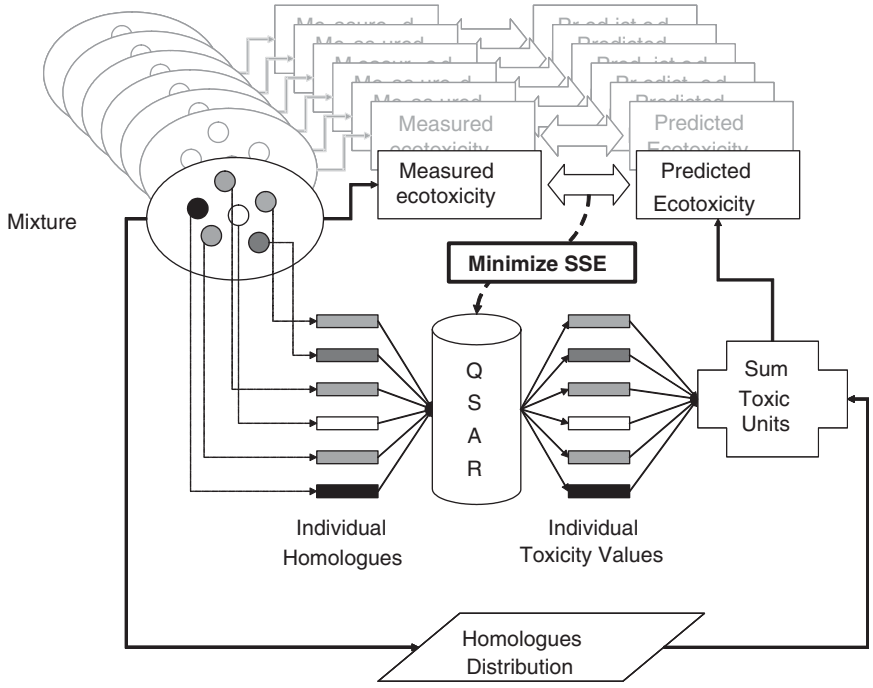

Fig. 1. Principle of mixture-toxicity-based QSAR fitting.

and

$$C/\mathrm{EO}\ \text{model}: \quad \mathrm{TOX}_i = 10^{A_2\, C_i + B_2\, \mathrm{EO}_i + I_2},$$

where A_1 and I_1 are the slope and intercept for the log K_{ow} model and A_2, B_2, and I_2 are the slopes and intercept for the C/EO model. The C/EO model is a (linear) simplification of the full quadratic model, which was shown by Wong et al. (1997) to be appropriate to describe AE ecotoxicity.

For the log K_{ow} model, hydrophobicity was calculated following the method by Leo and Hansch (1979) and Roberts (1991). Specifically for AEs (and nonethoxylated alcohol), this resulted in the following equations:

$$\mathrm{EO} > 0: \quad \log K_{ow} = -1.15 + 0.54C - 0.1\mathrm{EO}$$

and

$$\mathrm{EO} = 0: \quad \log K_{ow} = -1.29 + 0.54C.$$

For each studied ecotoxicity endpoint, the SSE for each QSAR model was minimized by optimizing the slope and intercept parameters. The Microsoft Excel Solver routine (GRG2 optimization) (Excel 2000, Microsoft Corp., 1999) was used for this numerical procedure. The resulting SSE could be compared with the SSE obtained with the existing QSARs, to compare the goodness of fit with the underlying data.

3. Results

3.1. Experimental verification of the mixture toxicity concept for AE

The results of the single-ethoxymer acute *D. magna* studies for C_8EO_4, $C_{16}EO_8$, $C_{14}EO_8$, $C_{10}EO_8$, $C_{12}EO_6$, and $C_{12}EO_8$, together with the results for the binary mixtures [$C_8EO_4 + C_{16}EO_8$] and [$C_{10}EO_8 + C_{14}EO_8$], are shown in Table 2. The EC_{50} values for the binary mixtures, predicted by the toxic units method, are also given in Table 2.

Table 2
48 h *Daphnia magna* EC_{50} of alcohol ethoxylates

	Measured EC_{50} (mg/L)	Calculated EC_{50} (mg/L)
C_8EO_4	24	
$C_{16}EO_8$	0.36	
$C_{12}EO_6$	2.7	
Mixture $C_8EO_4 + C_{16}EO_8$	0.56	$(0.5/24 + 0.5/0.36)^{-1} = 0.71$
$C_{10}EO_8$	50.5	
$C_{14}EO_8$	1.0	
$C_{12}EO_8$	7.7	
Mixture $C_{10}EO_8 + C_{14}EO_8$	3.0	$(0.5/50.5 + 0.5/1.0)^{-1} = 1.96$

For [$C_8EO_4 + C_{16}EO_8$] the measured EC_{50} is 0.56 mg/L. This is close (within 25%) to the prediction by the additivity method. On the other hand, the measured EC_{50} of the average structure $C_{12}EO_8$ is nearly a factor of 5 higher than the measured value for the mixture.

The EC_{50} for [$C_{10}EO_8 + C_{14}EO_8$] is 3.0 mg/L, which is 50% higher than predicted by the additivity method. On the other hand, the EC_{50} of the average structure $C_{12}EO_8$ is a factor of 2.5 higher than that measured for the mixture. Due to the variability in the analytical recovery (69.9–101% for $C_{10}E_8$, 58.7–76.8% for $C_{14}E_8$) it is possible that the corrected measured concentrations are overestimates, which would lead to an overestimation of the EC_{50} in the mixture. This would suggest that, in reality, the toxicity of the mixture would be more consistent with concentration addition than observed here.

These findings indicate that an average structure is a less reliable predictor for the toxicity of AE mixtures than the sum of the toxicity from all of the components in the mixture. As expected, the mixtures were observed to be more ecotoxic than the single ethoxymers representing the average structures of the mixtures.

3.2. Development of mixture-toxicity-based QSARs for AE

Using the method described above, the acute QSARs for AE toxicity to *Daphnia* and fathead minnow (Wong et al., 1997) were recalculated. The existing chronic QSAR for *Daphnia* (Morrall et al., 2003) and for mesocosm toxicity (Belanger et al., 2000) were also revised. In addition, a new chronic QSAR for fathead minnow was developed.

3.2.1. Acute QSARs

Detailed mixture characterization data (C number and EO number distributions) were available for several samples used in acute toxicity tests. These "training set" data were used to develop the QSARs listed below. Next to these, several other acute studies were available for *Daphnia* and fathead minnow with a less reliable characterization of the *C*/EO distribution. These studies were used as a "test set".

3.2.2. Acute daphnia magna QSAR

The existing acute *D. magna* toxicity QSAR for AE by Wong et al. (1997) was used as a reference. This QSAR was derived from an ecotoxicity data set for nine AE commercial materials:

$$\text{Wong}: \quad EC_{50} = 10^{-0.38\,C+0.1\,EO-1.77} \quad (\text{mol/L}).$$

Based on the same underlying data, the revised QSAR was developed. Considering the size of the training data set (nine data points), both the *C*/EO and the $\log K_{ow}$ could be used according to a rule of thumb on QSAR complexity which requires that for each parameter to be fitted (additional to the intercept) about five data points should be available (J. Jaworska, Procter & Gamble, personal communication):

$$\text{New } (\log K_{ow}): \quad EC_{50} = 10^{-0.58\log K_{ow}-2.70} \quad (\text{mol/L}),$$

$$\text{New } (C/EO): \quad EC_{50} = 10^{-0.32\,C+0.12\,EO-2.26} \quad (\text{mol/L}).$$

Both revised QSARs are acceptable from a mechanistic point of view in that it is expected that an increasing K_{ow} or alkyl chain length leads to a lower EC_{50}, whereas increasing ethoxylation leads to lower toxicity. It can be observed that the contribution of EO units to toxicity in the new *C*/EO QSAR is almost identical to that in the Wong QSAR.

Additional to the training data used for QSAR development, Wong et al. (1997) also used a set of test data to assess the QSAR (10 data points). The fit of the different QSAR models with both the training and the test data is shown in Table 3 and illustrated in Fig. 2 (top). As indicated by the larger SSE and the lower R^2, the goodness

Table 3
Fit of the revised and existing acute QSARs

		SSE	R^2	Avg. relative error[a]	Max. relative error[a]
Daphnia magna					
Training data set					
New ($\log K_{ow}$)	EC_{50}	0.304	87.4%	1.5	1.9
New (C/EO)	EC_{50}	0.233	85.0%	1.4	2.2
Wong	EC_{50}	0.098	97.7%	1.3	1.4
Test data set					
New ($\log K_{ow}$)	EC_{50}	1.82	94.4%	1.5	2.0
New (C/EO)	EC_{50}	1.74	98.3%	1.5	2.2
Wong	EC_{50}	2.33	92.7%	2.2	3.0
Single ethoxymers					
New ($\log K_{ow}$)	EC_{50}	1.06	62.2%	2.6	5.1
New (C/EO)	EC_{50}	0.39	92.7%	1.7	3.0
Wong	EC_{50}	2.40	81.5%	4.4	6.8
Pimephales promelas					
Training data set					
New ($\log K_{ow}$)	EC_{50}	0.055	91.4%	1.1	1.5
New (C/EO)	EC_{50}	0.058	91.2%	1.2	1.5
Wong	EC_{50}	0.017	98.2%	1.1	1.2
Test data set					
New ($\log K_{ow}$)	EC_{50}	1.98	50.5%	3.0	6.4
New (C/EO)	EC_{50}	1.95	49.8%	2.9	6.3
Wong	EC_{50}	2.22	65.5%	3.2	8.0

[a]Relative error expressed as a factor calculated as follows: measured/predicted if measured <predicted and predicted/measured if predicted <measured.

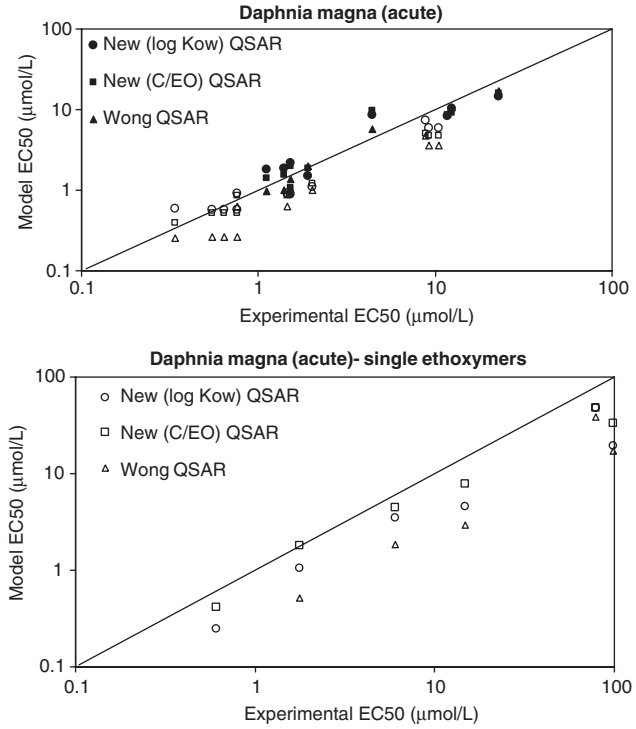

Fig. 2. Acute toxicity for *Daphnia magna*: predictions using new and existing QSARs versus experimental data. Closed symbols represent training data and open symbols represent test data.

of fit of the new QSAR to the training data is worse than that of the existing QSAR. Conversely, for the test data, a better correlation and SSE are seen with the new QSARs. Further, both the maximum and the average deviation between the model predictions and the measured data are lower with the revised QSARs compared to the existing QSAR. At worst, the predictions of the revised QSARs were found to be within a factor of 2.2 from the measurements. For the existing QSAR, this was a factor of 3.

When applied to individual ethoxymers in the C_{9-18} and EO_{0-20} matrix, the new $\log K_{ow}$ QSAR predicts a higher EC_{50} than the existing QSAR for about two thirds of the ethoxymers. The new C/EO QSAR predicts a higher EC_{50} than the existing QSAR for all ethoxymers. For a further evaluation of the accuracy of the existing and new QSARs at predicting single-ethoxymer ecotoxicity, the QSARs were applied to the single-ethoxymer data presented in Table 2. The results are given in Table 3 and Fig. 2 (bottom). Both new QSARs were consistently more accurate than the Wong QSAR, which always predicted lower EC_{50} values (i.e., higher toxicity), as was expected based on theoretical considerations. The highest accuracy was found with the new C/EO QSAR, for which the maximum relative error was below a factor of 3. For the Wong QSAR, the maximum error was nearly a factor of 7. There is no difference between the development of the Wong QSAR and the new C/EO QSAR except for the inclusion of the additive mixture toxicity concept in the latter. Hence, the consistently higher accuracy provides evidence of the higher reliability of the new approach.

3.2.3. *Acute pimephales promelas QSAR*

Wong et al. (1997) also developed an acute fathead minnow (*P. promelas*) toxicity QSAR for AE, based on a data set covering the same AE materials used for the *D. magna* QSAR (i.e., nine data points in the training data):

$$\text{Wong}: \quad EC_{50} = 10^{-0.34\,C+0.05\,EO-1.65} \quad (\text{mol/L}).$$

A revised QSAR was developed based on the same underlying data. As discussed for *Daphnia*, both the $\log K_{ow}$ model and the *C*/EO model could be used. This resulted in the following QSARs:

$$\text{New}\,(\log K_{ow}): \quad EC_{50} = 10^{-0.60\,\log K_{ow}-2.48} \quad (\text{mol/L}),$$

$$\text{New}\,(C/EO): \quad EC_{50} = 10^{-0.32\,C+0.05\,EO-1.78} \quad (\text{mol/L}).$$

As with *D. magna*, both revised QSARs are acceptable from a mechanistic point of view, and the contribution of EO units in the new *C*/EO QSAR is identical to the existing QSAR, but the intercept is different.

Also for *P. promelas*, Wong et al. (1997) used an additional test data set. The fit of the QSARs to both training and test data is illustrated in Fig. 3 and quantified in Table 3. The goodness of fit for the new QSAR is lower than that for the existing QSAR. The deviation between model predictions and measured data is similar with both QSARs. It is striking that the overall QSARs' fit and accuracy are very good for the training data set but poor for the test data set (with a maximum relative error over a factor of 6 for the new and even a factor of 8 for the existing QSAR). A possible explanation for this could be the variable analytical recoveries for the underlying test data. These varied from 59% to 97%, the lower recoveries being associated with the lower ethoxylation degrees. Possibly, these analytical complications may have had less impact on the existing QSAR approach, since this does not consider individual ethoxymers.

When applied to individual ethoxymers in the C_{9-18} and EO_{0-20} range, the new $\log K_{ow}$ QSAR predicts a higher

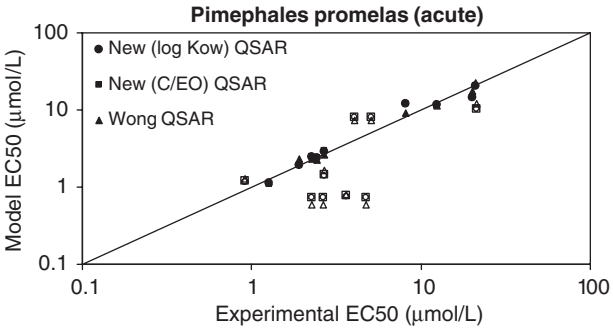

Fig. 3. Acute toxicity for *Pimephales promelas*: predictions using new and existing QSARs versus experimental data. Closed symbols represent training data and open symbols represent test data.

EC_{50} than the existing QSAR for all ethoxymers. The range of differences across the AE matrix is much smaller than that with the *Daphnia* $\log K_{ow}$ QSAR, with a maximum difference of a factor of 2. The new C/EO QSAR for fathead minnow also predicts a higher EC_{50} than the existing QSAR across all ethoxymers. The pattern across the AE matrix is similar to the $\log K_{ow}$ QSAR, and the range of differences across the matrix is always less than a factor of 2.

3.3. Chronic QSARs

3.3.1. Chronic Daphnia magna QSAR

A QSAR for chronic ecotoxicity (21-day reproduction) to *D. magna* of AE was developed by Morrall et al. (2003) based on six data points:

$$\text{Morrall}: \quad \text{NOEC} = 10^{-0.84 \log K_{ow}+4.0} \quad (\mu\text{mol/L}).$$

A new QSAR was derived using the same data used by Morrall et al. (2003) and an additional data point for $C_{14-15}EO_7$. Since the data set included two points for $C_{14-15}EO_7$, a geometric mean of these two data was used. Reproduction was the most sensitive endpoint in the series of studies and so was used as the basis for the QSAR. As the effects levels in the data set were expressed in mass concentrations (mg/L), a mass-to-molar conversion was required. The molecular weight of each complex substance was calculated as the weighted average of the molecular weights of each ethoxymer—in line with the general thinking applied in this new QSAR approach.

To allow a more direct comparison of the reference versus the new QSAR, the former was refitted using exactly the same data as the new QSAR (i.e., using the different conversion of the effect levels from mg/L to mol/L and using the geometric mean of two studies for the $C_{14-15}EO_7$ AE), based on the 'average structure approach' (referred to as Morrall2).

Only the $\log K_{ow}$ QSAR equations were applied, because the size of the data set (six data points) was judged too limited to justify fitting a QSAR with more than one parameter additional to the intercept. This resulted in the following:

$$\text{New (NOEC)}: \quad \text{NOEC} = 10^{-0.803 \log K_{ow}+4.078} \quad (\mu\text{mol/L}),$$

$$\text{Morrall2}: \quad \text{NOEC} = 10^{-0.723 \log K_{ow}+3.424} \quad (\mu\text{mol/L}).$$

For the chronic *D. magna* studies which were used to develop the NOEC QSARs, EC_{20} information was also available. Hence, an EC_{20} QSAR could also be fitted using the same approach:

$$\text{New } (EC_{20}): \quad EC_{20} = 10^{-0.532 \log K_{ow}+2.975} \quad (\mu\text{mol/L}).$$

The fit of these QSARs is presented in Table 4 and illustrated in Fig. 4. Overall the new NOEC QSAR has a better fit than Morrall and Morrall2 (higher correlation, lower SSE). The new NOEC QSAR is also more accurate: both its average and maximum relative error are lower than Morrall and Morrall2. For the training set, the new NOEC QSAR was always accurate within close to a factor of 3. The new EC_{20} QSAR has a fit similar to that of the new NOEC QSARs (better SSE but worse correlation, slightly smaller maximum relative error).

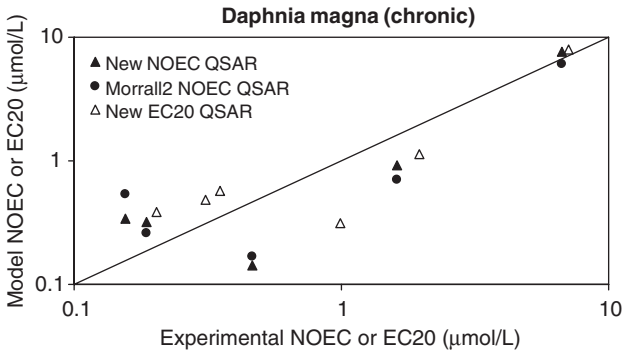

Fig. 4. Chronic toxicity for *Daphnia magna*: predictions using new and existing QSARs versus experimental data.

Table 4
Fit of the revised and existing chronic (and mesocosm) QSARs

		SSE	R^2 (%)	Avg. relative error[a]	Max. relative error[a]
Daphnia magna					
New ($\log K_{ow}$)	NOEC	0.508	98.1	1.9	3.2
Morrall2	NOEC	0.659	97.2	2.1	3.5
New ($\log K_{ow}$)	EC_{20}	0.467	96.6	1.9	3.1
Pimephales promelas					
New ($\log K_{ow}$)	EC_{10}	0.005	97.5	1.1	1.2
New ($\log K_{ow}$)	EC_{20}	0.001	99.6	1.0	1.1
Mesocosm					
New ($\log K_{ow}$) (mass)	NOEC	0.760	93.5	2.4	4.9
New ($\log K_{ow}$) (molar)	NOEC	0.792	95.1	2.4	4.7
Belanger (mg/L)	NOEC	0.716	92.9	2.3	4.4

[a]Relative error expressed as a factor calculated as follows: measured/predicted if measured < predicted and predicted/measured if predicted < measured.

3.3.2. Chronic Pimephales promelas QSAR

Until now, no chronic AE ecotoxicity QSARs for fish have been published. Based on a limited data set (with three early life stage studies), a QSAR for *P. promelas* was derived following the method of this paper. Due to the insufficient number of data points, this cannot be considered a reliable QSAR, but it is given for completeness. In line with the chronic *Daphnia* QSAR, only the log K_{ow} QSAR was applied. Survival was found to be the most sensitive endpoint. QSARs are presented for the survival EC_{10} and EC_{20} but not for the NOEC as no good fit could be obtained for the latter:

$$\text{New } (EC_{10}): \quad EC_{10} = 10^{-0.280 \log K_{ow} + 1.90} \quad (\mu mol/L).$$

$$\text{New } (EC_{20}): \quad EC_{20} = 10^{-0.307 \log K_{ow} + 2.08} \quad (\mu mol/L).$$

The results are presented in Table 4 and Fig. 5. Considering that only three data points were used, it is not relevant to discuss the goodness of fit of these QSARs.

3.4. Mesocosm QSAR

A QSAR for mesocosm ecotoxicity (NOEC) of AE was developed by Belanger et al. (2000). This work was based on a data set consisting of four studies in experimental stream ecosystems (University of Mississippi, Shell Research, and Procter & Gamble):

$$\text{Belanger}: \quad NOEC = 10^{-0.66 \log K_{ow} + 2.41} \quad (mg/L).$$

Based on the same underlying data, but complemented with an extrapolated NOEC for Neodol 23–6.5 (as derived in the original work by Dorn et al. (1997)), a revised QSAR was calculated, using the log K_{ow} model. For comparison with Belanger et al. (2000), a mass-based QSAR was developed next to the molar-concentration-based QSAR:

$$\text{New (mass)}: \quad NOEC = 10^{-0.663 \log K_{ow} + 2.51} \quad (mg/L),$$

$$\text{New (molar)}: \quad NOEC = 10^{-0.740 \log K_{ow} + 3.22} \quad (\mu mol/L).$$

The results and the comparison with the existing QSAR are presented in Table 4 and illustrated in Fig. 6. The goodness of fit of the revised QSARs is very similar to the existing one, as shown by the SSE and R^2 numbers. Also, the maximum and average deviation between the model predictions and the measured data are of the same order for the old and new QSARs. For the training set, predicted values are within a factor of 4–5 of the actual data.

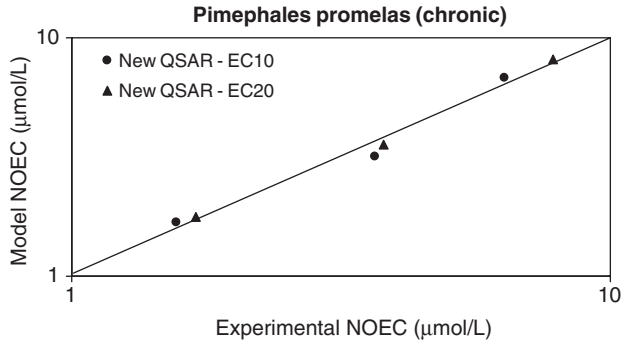

Fig. 5. Chronic toxicity for *Pimephales promelas*: predictions using new and existing QSARs versus experimental data.

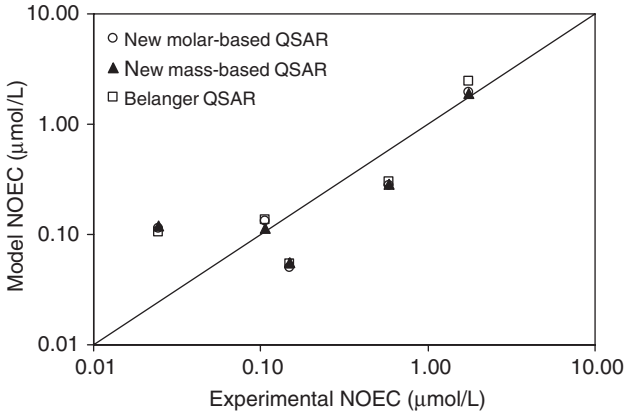

Fig. 6. Mesocosm toxicity: predictions using new and existing QSARs versus experimental data.

4. Discussion

4.1. QSAR selection for aquatic risk assessment for AE

The choice of a QSAR for use in environmental risk assessment is dependent upon the goals and objectives of the assessment. Many relevant variables have been considered in this paper including the choice of effect concentration (EC_{10}, EC_{20}, and EC_{50}), the taxa to be considered (daphnid, algae, fish), and even single-species versus ecosystem-level responses. Scientists engaged in environmental risk assessment are currently actively debating how to make these choices. Chronic effects data and QSARs for alcohol ethoxylates are available for three taxonomic groups (algae, invertebrates, fish) and can be used to determine the predicted no effect concentration (PNEC) in the aquatic risk assessment of alcohol ethoxylates. An application factor (AF) of 10 is generally accepted for extrapolation of the most sensitive of three chronic data points to the PNEC. The QSAR fitting results described in this paper show that the deviation of the QSAR predictions from the measurements is less than a factor of 3, which is similar to the normal experimental variability in different ecotoxicological studies. Additionally, the revised QSARs developed in this paper are not biased by mixture toxicity effects and thus are applicable to all ethoxymer distributions irrespective of their shape. Hence, no additional uncertainty is expected to be introduced to the effects assessment by the application of the QSARs.

Based on the available chronic ecotoxicity data for alcohol ethoxylates (as used and referred to in this paper),

D. magna was identified as the most sensitive species. *Daphnia* is also the organism for which the most reliable chronic QSAR is available. Hence, in the context of a traditional deterministic single-species-based effects assessment, the revised *D. magna* chronic QSARs with an AF of 10 are suitable for the derivation of PNECs for AE.

The *Daphnia* (and fish) EC_{20} QSARs have the features of representing chronic effects, and because they summarize these effects as an EC_{20} (using exposure expressed in μmol/L) they are more robust than NOEC or EC_{10} QSARs. By definition, the EC_{50} QSAR would be most robust, as described in Morrall et al. (2003), because the center point of the regression has the smallest confidence interval. However, the EC_{50} for chronic predictions is not sufficiently conservative.

Additionally, a deterministic multispecies PNEC can be derived from the mesocosm NOEC QSAR. For mesocosms, the appropriate application factor is to be determined on a case-by-case basis, and typically ranges 1–3. For alcohol ethoxylates, five high-quality mesocosm studies are available, which justifies an AF in the lower end of this range. Expression of mesocosm responses as an EC_x is problematic because the mesocosm conclusion is based on a combination of statistical findings, expert judgments, and the particular conditions of the test (species composition, for example). Therefore, the mesocosm QSAR conclusions are given as NOECs.

Alternatively, a more sophisticated probabilistic effects assessment approach can be applied, based on the species sensitivity distribution (SSD) concept. In Belanger et al. (2005), QSARs are used for the processing of individual species effects data, to allow the development of SSDs for individual AE ethoxymers. In this case, the most appropriate QSAR was identified for each species. Hence, different QSARs were used for different species.

It is important to note that all three chronic QSARs have fairly similar slopes (ranging from −0.31 for fish to −0.74 for mesocosm) and are built from the same structural descriptor ($\log K_{ow}$), making their implementation into the eventual environmental risk assessment straightforward (Belanger et al., 2005).

4.2. Comparison of the new and revised chronic Daphnia QSARS

In this paper, two methods to derive QSARs for complex substances were discussed. It is clear that, starting from identical training data sets, different QSAR models can be obtained. These may all have acceptable fits to the complex substance ecotoxicological data but may lead to noticeably different results for individual ethoxymers. As explained in the introduction, from a theoretical mechanistic point of view the new approach based on the ethoxymer distribution of the training set's data points should be preferred over the existing method using average structures. This theoretical view is supported in general by the goodness of fit information for the different QSARs presented in this paper, specifically for the *D. magna* chronic QSAR. Using identical underlying training data, the correlation of the new QSAR is higher than that of the existing method (98.1% versus 97.2%), and the SSE is about 25% lower. Also, the accuracy (measured by the average and maximal relative error of the predictions versus the training data) is better for the new QSAR. The average error is about 25% lower, and the maximal error is over 50% lower.

The predictions by the new *D. magna* chronic QSAR were compared to those by the analogous QSAR obtained via the existing approach (referred to as Morrall2). In Fig. 7, (top) it can be seen that the new QSAR leads to higher NOEC predictions (i.e., lower toxicity) for nearly all ethoxymers. The ratio between the predicted NOECs ranges from slightly below 1 to a factor of 2.7. On average (across all ethoxymers, no weighting), the new QSAR will predict NOEC values that are approximately 70% higher. Interestingly, the new QSAR indicates that increasing ethoxylation reduces toxicity and increasing carbon chain length increases toxicity relatively more than is indicated by the old QSAR.

In of Fig. 7 (bottom) the ratio between the NOECs as predicted by the new versus the existing QSAR are plotted as a function of the NOEC. This shows graphically that, for the most toxic components (i.e., those with the lowest

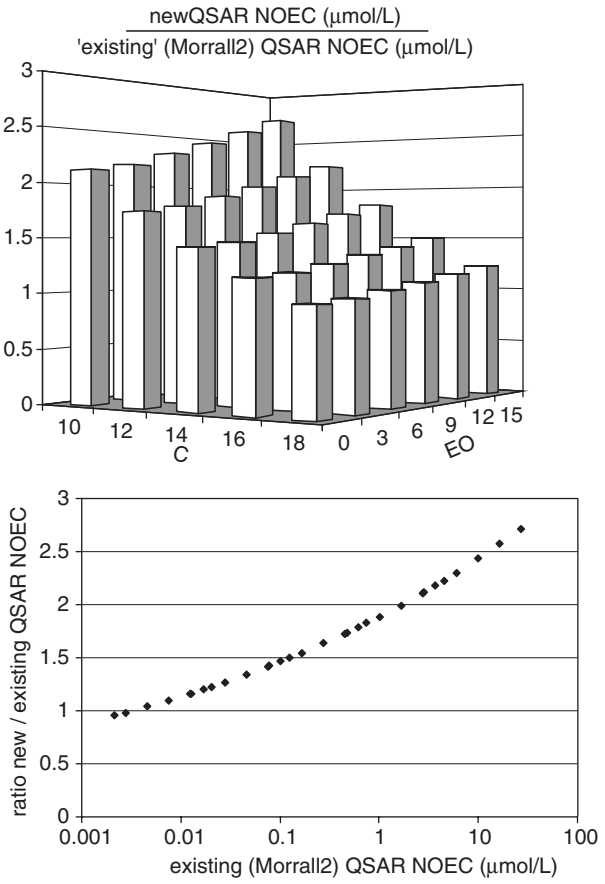

Fig. 7. Comparison of new versus existing (Morrall2) QSAR for chronic *Daphnia magna* toxicity.

NOECs), the new QSAR predicts nearly the same NOEC as the old QSAR (ratio close to 1), whereas, for components with decreasing toxicity (increasing NOEC), the ratio between the new and the old NOEC values increases, reaching close to a factor of 3 for the least toxic components.

This observation is in line with the theoretical considerations. Indeed, the QSARs based on average structure are biased toward the toxicity of the most toxic components in a complex substance, because these components drive the overall measured toxic effects. Hence, the existing QSARs were expected to overpredict toxicity in general and more so for the less toxic components.

5. Conclusion

The new method described in this paper allows the development of ecotoxicity QSARs for complex substances that take into account the available information on the distribution of the materials in the QSAR training data set. This leads to QSARs that fit the data equally well or better and are more accurate from a mechanistic point of view.

The revised QSARs for AEs are especially suitable for the prediction of the ecotoxicity of single components or—via toxic units addition—of environmental fingerprints. It was shown that these QSARs do not introduce more uncertainty than the usual experimental variability in ecotoxicological studies. Given the breadth of information available on AE and the desire to be consistent and to maintain an appropriate balance of conservatism and pragmatism, the following chronic QSARs are recommended for deterministic PNEC derivation in environmental effects assessments:

Daphnia EC_{20} QSAR:

$$EC_{20} = 10^{-0.532 \log K_{ow}+2.975} \quad (\mu mol/L).$$

Mesocosm NOEC QSAR:

$$NOEC = 10^{-0.740 \log K_{ow}+3.22} \quad (\mu mol/L).$$

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