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PAPER

Effects-driven chemical design: the acute toxicity of CO_2-triggered switchable surfactants to rainbow trout can be predicted from octanol-water partition coefficients†

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For both environmental protection and improved energy efficiency, CO_2-triggered switchable surfactants have been developed to change surface activity and solubility upon command. Surfactant activity is turned on by introduction of one atmosphere of CO_2 and reversed by purging with air or nitrogen. These surfactants have numerous potential industrial applications related to their ability to stabilize and destabilize emulsions upon command. To assess their potential environmental impacts, we tested the acute toxicity of nine switchable surfactants to rainbow trout (*Oncorhyncus mykiss*) at pH ~8.0, typical of natural surface waters. The surfactants were synthesized in several variations, differing in the structure of the hydrophobic tail group, the hydrophilic head group, or both. A strong correlation between the log of the estimated octanol/water partition coefficients (log *P*) and the toxicity of eight switchable surfactants formed the basis of a structure–activity relationship that was used to design a ninth compound. That new compound had the lowest toxicity of all of the switchable surfactants tested. The effect of log *P* on acute toxicity was similar to that reported in the literature for other organic compounds. This model shows that despite the addition of varying functional groups, switchable surfactant toxicity remains largely dependent on log *P* and differs little from traditional non-switchable surfactants. The log *P* relationship developed provides a very useful tool for screening new compounds for acute toxicity.

Introduction

A primary goal of green chemistry is to develop compounds that are less environmentally-damaging than chemicals that they might replace. Characteristics that are important in reducing environmental impacts of chemicals include manufacturing and use properties such as low embodied energy content, use of substrates or feedstocks that have low environmental impacts, and a low potential for persistence, bioaccumulation, and toxicity (PBT). An equally important characteristic is that a new compound, when used in a process, would make that process less environmentally damaging. For aquatic environments, the past environmental impacts of detergents and surfactants have been associated with persistence and high toxicity. Changes to detergents in use before the 1970s were made to increase biodegradability and reduce persistence, so that waste treatment plants could reduce concentrations in effluent and avoid toxicity and foaming in receiving waters.

Alternatives to traditional surfactants are switchable surfactants, which are compounds designed with surfactant properties that can be changed by a simple adjustment to solution chemistry. To reverse the properties of surfactants at will, structural designs are needed that integrate optimal surfactant characteristics with structures and functional groups that are responsive to triggers such as light, heat, oxidants, *etc.*[1–7] A recent example is a class of surfactants that are pH sensitive and specifically designed to respond to the pH changes triggered by the carbonation of water with 1 atm of CO_2.[8–10] The hydrophobic alkyl 'tail' of each molecule is similar to that of traditional surfactants, while the head group is hydrophilic when protonated and hydrophobic when unprotonated. The switchable hydrophilicity of the head group gives the surfactant its switchable surfactancy. Because the head groups of these molecules are amidines, they are sufficiently basic to be protonated in carbonated water at only 1 atm. Thus, addition of carbon dioxide to reduce solution pH can activate the surfactant, and aeration with inert gases such as nitrogen, argon, or air

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will inactivate the surfactant by driving off carbon dioxide and raising pH.

The advantages of switchable surfactants are many, but the most important is their ability to make or break emulsions at will. Switchable surfactants are useful in applications where an emulsion is needed for one step of a process but is unwanted in the next step. These include, but are not limited to: micro-suspension polymerizations, viscous oil transportation through pipelines, separating oil from a solid substrate, *e.g.* oil from tar sands or soybean oil from soybean flakes, and enhancement of oil recovery over existing methods. However, while switchable surfactants are 'greener' than their traditional alternatives due to energy savings, are they any more or less toxic than traditional surfactants if lost to surface waters? Even more importantly, can ecotoxicity studies be used to guide the design of even greener switchable surfactants?

The mechanism of toxicity of traditional surfactants involves both the hydrophilic and the hydrophobic moiety of each molecule. The hydrophobic end will interact with fish gill epithelial cells just as it does with liquid oil, while the hydrophilic end enables the lipidic components of the membrane to be solubilized in water, destroying cell membranes and causing suffocation.[11,12] In theory, switchable surfactants should have little effect on gills. While the hydrophobic end will interact with cell membranes, the hydrophilic end should be 'switched off' when added to most surface waters (6.0 to 8.5). However, fish gills are the site of gas exchange, with carbon dioxide being released as oxygen is taken up. As a consequence, there is a surface or boundary microlayer of water flowing over the gills that may be at a lower pH, perhaps sufficient to activate the switchable surfactant.

Ecological risk assessment is crucial for novel chemicals destined for commercial or industrial applications. Because a variety of switchable surfactants have been produced, toxicity assessment and life cycle assessment, as well as efficacy as a surfactant will be important factors in determining which, if any, are best suited for industry. We measured the acute lethality to rainbow trout of a series of CO_2-triggered switchable surfactants characterized by differing structures of the switchable head group and differing chain lengths and compositions of the tail. We tested two alternative hypotheses to explain the variations in observed toxicities: first, that toxicity would vary primarily with changes to the structure of the switchable head group; and second, that toxicity would be determined largely by the degree of hydrophobicity of the entire molecule, as expressed by the log octanol-water partition coefficient (log *P*). The relationship between toxicity and log *P* was used to design a surfactant that was switchable and of low toxicity.

Materials and methods

The acute toxicity of eight novel switchable surfactant compounds (Fig. 1) to 0.5–3 g rainbow trout (*Oncorhynchus mykiss*) was evaluated by 96 h static daily renewal lethality tests at 15 °C

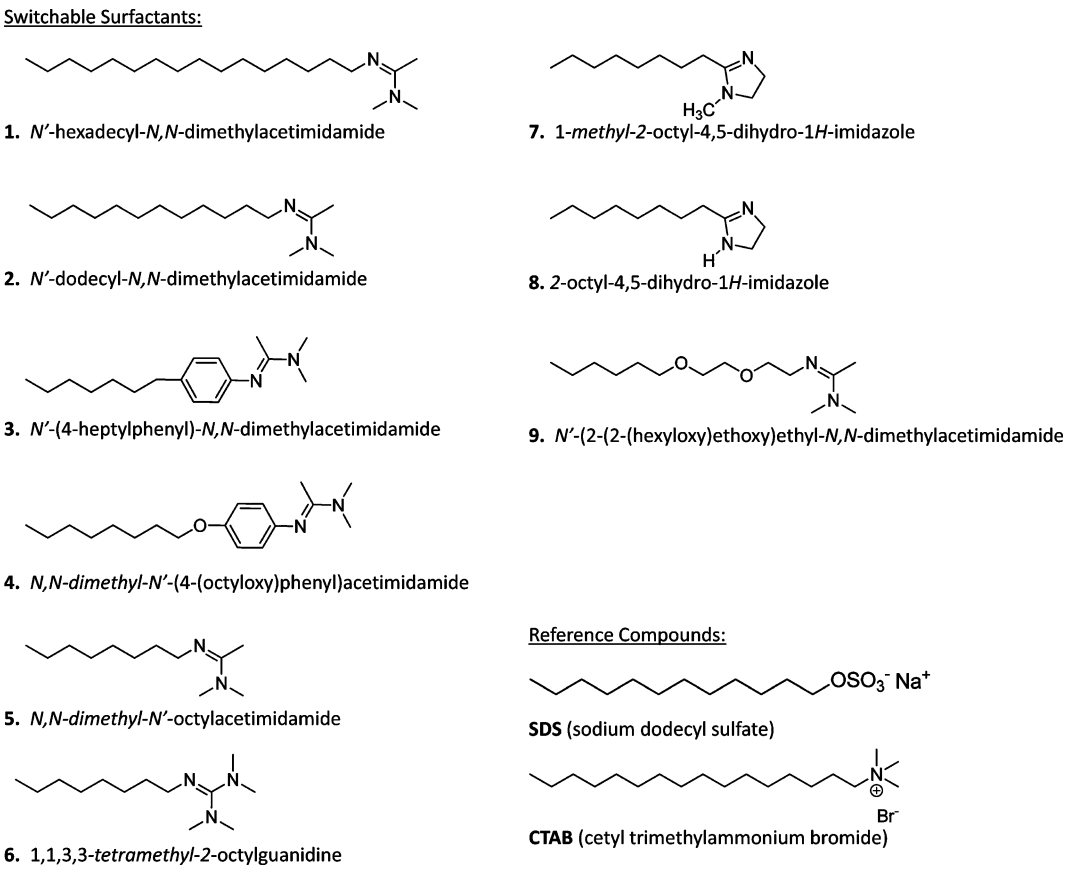

Fig. 1 Structures and IUPAC names of nine switchable surfactants.

and a 16 h : 8 h light : dark photoperiod. The relationship between the toxicity data and log *P* directed the synthesis of a ninth compound which was designed to be less toxic by virtue of its low log *P*. Tests followed Environment Canada test guidelines[13] with some minor modifications depending on the availability of test compounds. Animals and test methods used were approved by the Queen's University Animal Care Committee (UACC), the Canadian Council on Animal Care (CCAC) and the Ontario Ministry of Agriculture, Food and Rural Affairs (OMAFRA).

Test chemicals

All switchable surfactants (Fig. 1) were synthesized as described previously.[10] Their purity was verified using ^{1}H NMR spectroscopy; purity ranged from 70% to >99% (purity data were not available for compounds **1**, **2** and **5**). These compounds had from 7 to 16 carbon alkyl tails, and some tail groups contained embedded ethoxy groups within the alkyl chain. The switchable hydrophilic functional groups included amidine, guanidine and imidazole structures. Two reference toxicant positive controls, cetyl trimethylammonium bromide (CTAB; CAS # 57-9-0, Lot # 019K00241) and sodium dodecyl sulfate (SDS; CAS # 151-21-3, Lot # MKBC3000) were obtained from Sigma Aldrich Canada, Oakville, ON. Each was added with or dissolved in 1 mL of HPLC grade methanol (Lot # K1552, Fisher Scientific, Ottawa, ON).

Test solutions

For preliminary toxicity tests, a series of dilutions was prepared with ten-fold decrements in concentration, starting near the solubility limit. Stock solutions were prepared so that the amount of methanol for each dose was either 100 µl or 1 mL. For subsequent full-scale tests, a series of doses was prepared using methanol as a diluent, and the amount of methanol added varied according to the test concentration. In some cases, water bath sonication was needed to dissolve chemicals (switchable surfactants **3**, **4** and **8**; reference compounds SDS and CTAB). Water was used as a solvent in place of methanol for SDS due to its low solubility in alcohol – methanol was added to the test solutions of SDS to mimic the conditions of the tests in which methanol was used as a carrier. For liquid surfactants, the mean density (n = 5) was determined with an analytical balance (Denver Instrument Co., model TR-204) to express concentrations gravimetrically. In addition to positive controls, negative controls included methanol at the highest concentration used to deliver test compounds, and water only.

Fish

Rainbow trout (0.54–2.22 g) were obtained from a commercial trout farm (Rainbow Springs, Thamesford, ON) and were acclimatized to 12–15 °C, allowing at least 1 day per °C of change from the hatchery temperature. Prior to testing, fish were held in 260 L tanks with a continuous flow of carbon-filtered municipal water (Lake Ontario) (>1.5 L/g fish/day) under a 16 h : 8 h light : dark photoperiod. Measured water quality parameters during the test period were: pH 7.04–9.36, conductivity 221–292 µS cm^{-1}, dissolved oxygen 11.1–13.6 mg L^{-1}, and chlorine 0–10 µg L^{-1}. Lake Ontario water is very stable and has a hardness and alkalinity of 135 and 90 mg L^{-1} $CaCO_3$, respectively.[14]

Test methods

Initially, full scale toxicity tests were preceded by a range-finder test, in which two fish were exposed to each of a broad range of concentrations to provide a first estimate of the LC50. These preliminary tests were conducted with 2 L of test solution in 3 L stainless steel bowls. The final toxicity tests were performed in 5–10 L of solution, depending on the amount of chemical available, in 20 L plastic buckets lined with a food-grade polyethylene bag, with 10 fish per treatment and a narrower range of test concentrations. After three complete tests, we avoided preliminary tests by estimating the LC50 from log *P*. All test solutions were aerated and each test was accompanied by a water and a 1 mL methanol control test of 10 fish.

Treatments were inspected multiple times each day for dead or dying fish (gasping, loss of equilibrium, lack of swimming). Dying fish were counted towards the next time interval and euthanized humanely by an overdose of the anesthetic MS-222 (ethyl-3 aminobenzoate methanesulfonate lot: MKAA2400, Sigma Aldrich Canada, Oakville, ON) at the test temperature and pH.

Experiments were terminated after 96 h or when acute toxicity ceased. Fish remaining alive through the duration of toxicity tests, as well as fish exhibiting severe signs of toxicity during tests were euthanized humanely. In early trials, a small number of exposed fish were dissected to identify external and internal pathologies, and freshly excised gill tissue was inspected by microscopy.

Statistics

LC50′s were calculated by the probit method or trimmed Spearman-Karber method (5% alpha) using the LC50 Calculation Program version 2.0.[15] Log P values were estimated using ALOGPs 2.1,[16] and structure data were entered as SMILES notation (simplified molecular input line entry specification). These were verified using the Depict imaging tool by Daylight Chemical Information Systems, Inc. Simple linear regressions of switchable surfactant log LC50 concentrations and log *P* values were performed for both nominal gravimetric concentrations and molar concentrations. The R^2 values for these regressions were 0.94 and 0.95 respectively.

Results and discussion

All surfactants were toxic to rainbow trout, but only one mortality was observed among 190 fish tested in methanol and water control treatments. It occurred in the methanol control treatment during the test of compounds **3** and **6**. The remaining fish in this tank appeared normal. In surfactant tests, toxic effects included inflammation of exposed tissues, primarily the gills, skin and eyes. Excess mucous production around the gills was observed in treatments of compounds **1**, **2**, **5** and CTAB. Blood was visible around the gills in treatments of **1** and **2**. Glossy eyes were observed in **1** and **2** and CTAB treatments. The skin of fish treated with toxic concentrations of **1**, **2**, **3**, **5**, SDS and CTAB was blanched white, and excess mucous was common. In extreme

Table 1 The measured acute lethality to rainbow trout and the estimated log *P* values for nine switchable surfactants and two reference surfactants

Compound	log *P*[b]	96 h LC50 (mg L^{-1})	Upper C.L.	Lower C.L.	96 h LC50 (μmol L^{-1})
Switchable surfactants					
1	7.3 ± 1.1	0.08	0.08	0.07	0.2
2	5.8 ± 0.8	0.43	0.49	0.37	1.7
3[a]	5.5 ± 0.6	0.53	n/a	n/a	2.0
4	5.1 ± 0.9	0.73	1.50	0.36	2.5
5[a]	4.1 ± 0.6	8.94	n/a	n/a	45.1
6	3.9 ± 0.5	17.35	18.96	15.88	76.3
7	3.8 ± 0.5	6.89	9.43	5.03	35.1
8[a]	3.4 ± 0.5	8.95	n/a	n/a	49.1
9	2.6 ± 0.5	52.34	68.80	39.84	202.5
Reference Compounds					
SDS	3.4 ± 1.3	15.48	19.16	15.51	53.7
CTAB[a]	5.9 ± 2.8	0.14	n/a	n/a	0.4

[a] No confidence intervals defined. [b] Estimated from ALOGPs 2.1 (Tetko & Tanchuk, 2002).[16]

cases of skin blanching, the tensile strength of the skin was lost, and the skin would rupture. This was observed primarily in **1**, **2**, and CTAB treatments. Hyperactivity was observed in compound **4** and CTAB treatments. Decreased activity and fish lying on the bottom of the test vessel were observed in compound **6** treatments. Loss of equilibrium and death were observed for all compounds at various concentrations. With some compounds, certain effects were less pronounced or absent.

Time to death varied by compound, as measured at the lowest concentration causing 100% mortality. Tests with compounds **5** and **6** had the longest times to 100% mortality at 72 h. Compounds **1** and **2** caused 100% mortality at 48 h, CTAB at 24 h and compounds **3** and **4** in 4 h. Compound **9** and SDS caused 100% mortalities after 2 h and **7** and **8**, within 1 h. Mortality (and 96 h LC50s) spanned about three orders of magnitude of test concentrations, increasing with octanol-water partition coefficients which spanned about 4.5 orders of magnitude (Fig. 2, Table 1).

The mechanism of toxicity for switchable surfactants suggested by our observations is similar to that of other common ionic and nonionic surfactants. The signs of toxicity for switchable surfactants were congruent with those of the reference compounds sodium dodecyl sulfate and cetyl trimethylammonium bromide and corresponded to previous observations of surfactant effects on fish gills *in vivo* or *in vitro*.[5,17]

Each switchable surfactant tested was toxic to rainbow trout within 96 h. Calculated 96 h LC50's ranged from 0.08 mg L^{-1} to 52.3 mg L^{-1}, and these extreme values correspond to surfactants with the highest and lowest log *P* values, respectively. For all compounds, toxicity varied with log *P* in a very predictable manner, and the relationship between log *P* and LC50 (Fig. 3) was very strong ($R^2 = 0.95$). These results are based on nominal concentrations, rather than actual concentrations, as methods for measuring these compounds in water at the concentrations tested are not yet available. After the first eight compounds were tested, the relationship between switchable surfactant toxicity and log *P* guided the development of a ninth switchable surfactant with lower log *P* and lower toxicity.

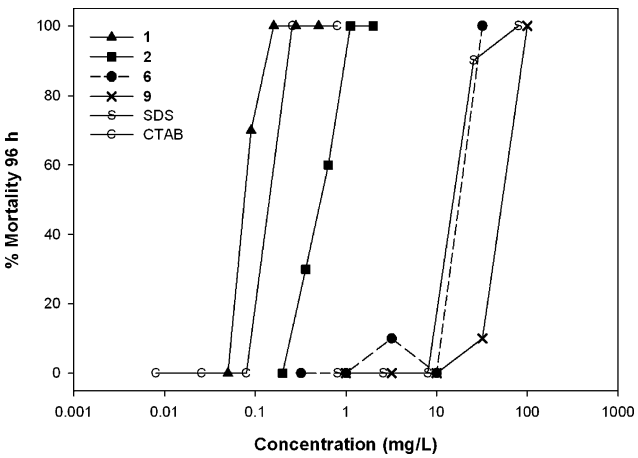

Fig. 2 Mortality curves for four switchable surfactants and two reference compounds. The exposure-response curves for the remaining six switchable surfactants appeared similar within the range presented, but were omitted for clarity due to extensive overlap.

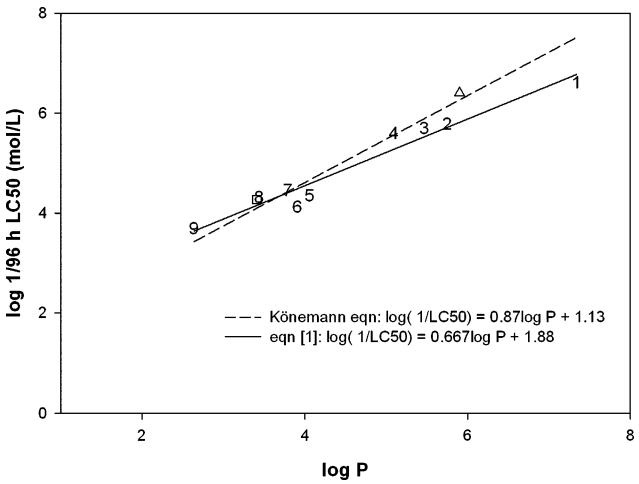

Fig. 3 The effect of log *P* on the toxicity of nine switchable surfactants (numbers 1 to 9 and solid black line; eqn (1); $n = 9$, $r = 0.98$, $s = 0.234$, $p < 0.001$). The relationship developed by Könemann[18] for 50 organic compounds is shown as a dashed line (eqn (2); $n = 50$, $r = 0.99$, $s = 0.237$; p not reported in ref. 18). 96 h LC50s for sodium dodecyl sulfate (square) and cetyl trimethylammonium bromide (triangle) are presented as reference points but are not included in the regression.

The relationship that was developed between log P and toxicity for the switchable surfactants was sufficiently predictable that a Quantitative Structure Activity Relationship (QSAR) could be developed. The linear equation relating log P and the log of the inverse of 96 h LC50's for the switchable surfactants (Fig. 3) was:

$$\log(1/\mathrm{LC50}) = 0.667\log P + 1.88 \quad (n = 9,\ r = 0.98,\ s = 0.234,\ p < 0.001) \tag{1}$$

where s is the standard error of the estimate and p is the probability that the regression is not significant. This equation is very similar to that developed by Könemann[18] for a wide array of organic compounds:

$$\log(1/\mathrm{LC50}) = 0.87\log P + 1.13 \quad (n = 50,\ r = 0.99,\ s = 0.237;\ p \text{ not reported in ref. 18}) \tag{2}$$

Although derived for compounds that are not surfactants, Könemann's equation (2)[18] predicts the toxicity of switchable surfactants with great accuracy (Fig. 3). This suggests that the bioaccumulation (and hence exposure and toxicity) of the surfactants tested in this study, as well as other non-ionic surfactants, are controlled to the same extent by water-lipid partitioning as the aromatic compounds and alcohols that Könemann[18] used to develop eqn (2). This does not mean that they would have the same proposed general narcotic mechanism of toxicity,[18] because surfactants clearly degraded exposed tissues by solubilizing lipid membranes.

Eqn (2) was useful only for log $P < 6$;[18] above this value, compounds were often less toxic than predicted from the equation. This has been observed previously and is attributed to deterioration of the log P-bioaccumulation relationship due to steric hindrance, so that toxicity within 96 h is limited by uptake kinetics.[19] As shown by Fig. 4, removing the switchable surfactant with the highest log P (**1** – log P 7.34) increases the slope of the resulting equation:

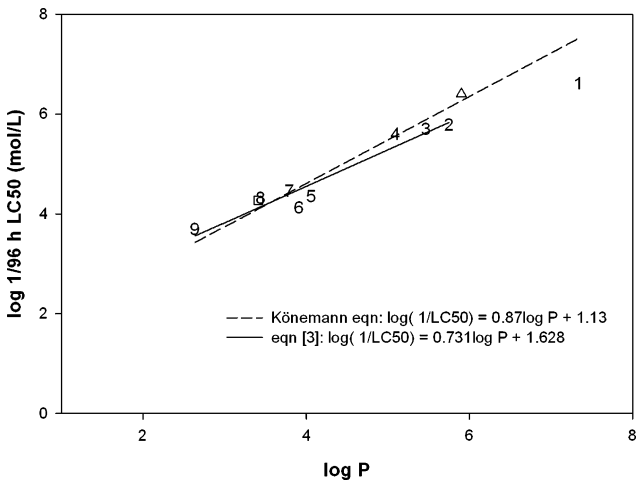

Fig. 4 The effect of log P on surfactant toxicity for switchable surfactants with log $P < 6$ (eqn (3); $n = 8$, $r = 0.97$, $s = 0.230$, $p = <0.001$). The relationship developed by Könemann[18] for 50 organic compounds is shown as a dashed line (eqn (2); $n = 50$, $r = 0.99$, $s = 0.237$; p not reported in ref. 18). The 96 h LC50s for sodium dodecyl sulfate (square) and cetyl trimethylammonium bromide (triangle) are presented as reference points but are not included in the regression.

$$\log(1/\mathrm{LC50}) = 0.731\log P + 1.628 \quad (n = 8,\ r = 0.97,\ s = 0.230,\ p = <0.001) \tag{3}$$

This new slope is closer to the slope of 0.87 observed by Könemann (eqn (2)).[18] The 96 h LC50s for the reference compounds SDS and CTAB also fall nearly in line with eqn (2).

Functional groups

The switchable surfactants tested are mostly comprised of a switchable-hydrophilicity amidine head group with a hydrophobic alkane tail group. One exception is a guanidine head group in place of the amidine (compound **6**). If the lone guanidine compound is omitted from the regression, the relationship between log P and toxicity becomes even stronger ($r = 0.99$). Thus, the guanidine version of the switchable surfactant appears somewhat less toxic than the other versions tested (Fig. 5) because the LC50 fell outside the 95% confidence limits of the amidine regression. Since there was only one guanidine test compound, it is uncertain whether this class of compounds is less toxic in general or if this compound is the exception.

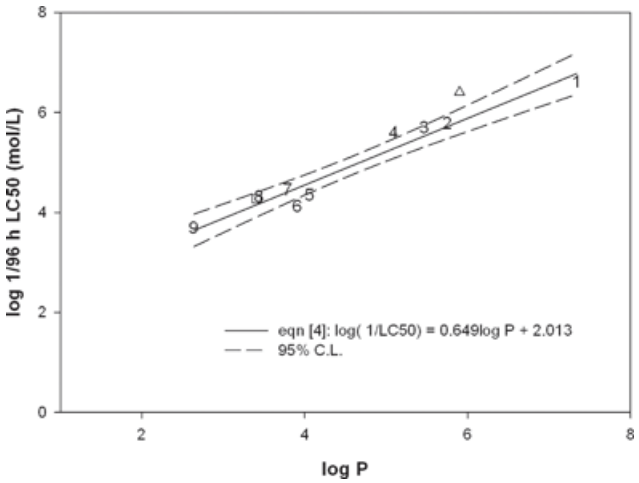

Fig. 5 The effect of log P on the toxicity of switchable surfactants to rainbow trout (Eq 4; $n = 8$, $r = 0.98$, $s = 0.192$, $p < 0.001$). The single guanidine compound tested (compound **6**) is not included in the regression, and falls outside of the 95% C.L. of a regression of the other switchable surfactants. Two of the amidine compounds (**4** and **5**) also fall slightly outside of the confidence limits, but to a lesser degree.

Aside from the guanidine group, no other functional group seemed to cause a variation in toxicity independent of their effect on log P. These additional functional groups include various 7–16 carbon alkyl, ethoxy, and octyloxy groups, as well as cyclic groups such as aniline, phenyl and imidazoline rings. The relationship developed between log P and toxicity for this largely heterologous series suggests that the only apparent way of decreasing toxicity of the switchable surfactants is by modifying the structural design to achieve a reduction of log P. The simplest and most effective way of doing this is by the addition of ethylene oxide units to the hydrophobic alkyl tail group,[20] a strategy used for traditional non-ionized surfactants.[19] After the toxicity tests were performed with the first 8 compounds, the lessons learned guided the design of a ninth switchable surfactant. The addition of two ethylene oxide groups to amidine compound

2 (log P 5.8 ± 0.8) produced compound **9** (log P 2.6 ± 0.5), a reduction of approximately 1.6 log P units per ethylene oxide group and a reduction in toxicity of two orders of magnitude. In results to be published separately, we have found that this new less-toxic switchable surfactant, in the presence of CO_2, stabilizes emulsions of water in heavy crude oils.[21]

Environmental impacts

These switchable surfactants were designed to activate in carbonated water, while in natural surface waters they would act as demulsifiers. Our tests however, show that this is not an advantage in alleviating toxic effects when compared with common commercial surfactants.

As with many other surfactants, switchable surfactant toxicity is driven by the compound's affinity for a hydrophobic phase over an aqueous one. The greater affinity for lipids means higher accumulation of compounds in the tissues of the fish, and increased toxicity. Additions or subtractions of any of the functional groups contained within the switchable surfactants tested did not markedly affect toxicity independent of their effect on the value of log P. The switchable surfactants have the same toxicity as conventional surfactants of equal log P. This does not automatically exclude switchable surfactants from being classified as 'green chemicals' because their switchability could lead to substantial savings in energy and materials during the breaking of emulsions or suspensions.

By using switchable surfactants during industrial processes, manufacturers requiring separation of chemicals could reduce waste by creating and breaking emulsions without addition of emulsion-breaking additives such as salts, demulsifiers, or strong acids. Additionally, the application of switchable surfactants to recover waste oil from products before it reaches landfills, waterways, or groundwater surely warrants their designation as a green chemical. In addition to their applications, the nature of these surfactants could provide a viable method for removing them entirely from any effluent or discharge in which they may be entrained.

The observed 96 h LC50s for switchable surfactants are not a complete account of the toxicity, nor of associated potential environmental impacts. They do, however, provide a sound metric for comparison between novel switchable surfactants and traditional surfactants of similar log P, and a basis for designing a switchable surfactant of lower toxicity that is worth pursuing as an industrial 'green' chemical.

Acknowledgements

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