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Toxicity of Ionic and Nonionic Surfactants to Six Macrobes Found in Agra, India

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Surfactants or surface active agents are substances that when dissolved in a liquid especially water can reduce its surface tension and thus can allow it to foam or penetrate solids. Surface active means they tend to adsorb on surfaces. They are helpful in wetting. Commercial surfactants are synthetic organic chemicals used in large amounts in detergents, soaps, shampoos, fabric softeners, mining and oil field chemicals, leather and food additives, food emulsifiers, pesticides, herbicides, defoliants, insecticides, antiseptics, disinfectants etc. They are present in several combinations such as detergents and household cleaning products. Granular and liquid detergents are complex mixtures containing as many as 20-30 constituents including 4-5 surfactants, dyes, enzymes, builders and stabilizers. Dispersants that are added to oil spills may contain as much as 75% surfactant (Crossland et al. 1992). Thus, they find applications in almost every chemical industry. They are also used for environmental protection. Surfactant enhanced subsurface remediation is being evaluated as an innovative technology for expediting groundwater remediation. Surfactants generally consist of polar or hydrophilic groups, like $—COO^-$, $—SO_3^-$, NH_4^+, soluble in water and oily or lipophilic groups soluble in lipids. They are of four types: anionic, nonionic, cationic and zwitterionic. Alkyl sulphates (AS), linear alkylbenzene sulphonates (LAS), alkyl ethoxy sulphates (AES), alkyl ethoxylates (AE), alkyl phenol ethoxylates (APE) and quaternary ammonium compounds are commonly used commercial surfactants.

Due to their widespread use surfactants have been measured at various concentrations in river water, drinking water, sediment and sludge-amended soil. Their toxicities to aquatic life have been summarized previously (Feijtel and de Plassche 1995; Gillespic et al. 1996; Lewis 1991). However, environmental assessment based on reviews (Belanger et al. 1995; Newsome et al. 1995) is outdated considering the constant development of new surfactants and reformation of their existing products particularly detergents. The available toxicity data base is largely laboratory derived for a few surfactants, predominantly LAS and single species such as *Daphnia magna*. Toxicity data for high volume surfactants other than LAS such as AS, AES, AE, APE and cationic surfactants are fewer (McAvoy et al. 1993). Furthermore, the complete toxicity evaluation of a compound needs to include data for several test organisms (alga, invertebrate, fish) representing the trophic levels contained in the planktonic and benthic habitats of the particular environment to which the compound is discharged. Currently toxicity data are required to provide the most realistic exposure scenario that can reflect recent usage rates, biodegradation and removal from the water body (Dorn et al. 1993). Surfactants enter aquatic environments (Raopaport and Eckhoff 1990) due to their presence in wash water as wastewater or byproducts of production processes or simply as results of normal use and disposal. They often enter a water body following wastewater treatment. Many laws governing the production and distribution of chemicals require physicochemical and toxicological data on these chemicals prior to production and marketing. One of the first steps commonly taken in generating

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ecotoxicity data is to conduct acute, single-species toxicity tests (Bressan et al. 1991). Since it is not economical to test all chemicals individually, increasing attention has been given to the use of quantitative structure activity relationship (QSAR) to screen for likely chemical toxicity (Roberts 1991; Rodgers et al. 1996). The purpose of this study was to assess the acute toxicity (Hofer et al. 1995; Wong et al. 1997; Yang et al.1999) of different types of commonly used surfactants on aquatic macro-organisms available locally in Agra. To the best of our knowledge such a study has never been conducted.

Four fish and two invertebrate species were chosen as test organisms for toxicological studies. The choice of organisms is based on availability, regional ecological importance and sensitivity to chemicals. In this study, the retardation of immobility effects on macrobes due to surfactants, have been measured within a short period of cultivation (not more than 3 days). Immobility concentration (IC_{50}) is the concentration of a chemical that immobilises 50% of the organisms tested in a specified time. Lethality concentration (LC_{50}) is the concentration of a toxicant which is lethal (fatal) to 50% of the organisms tested under the test conditions in a specified time. We always avoid LC_{25} or LC_{50} because we do not want to kill test organisms unless it is extremely essential. This is an ecofriendly effort. 48 hour tests instead of more convenient 96 hour were used because for some animals, the mortality might continue with increased time of exposure.

MATERIALS AND METHODS

Surfactants having a wide range of physicochemical characteristics and applications were obtained from different commercial suppliers. They were three anionic surfactants sodium dodecyl sulfate (SDS, an AS), sodium dodecyl benzene sulphonate (SDBS, an LAS), and sodium dodecyl ethoxy sulfate (SDES, an AES); two non-ionic surfactants dodecyl hexaethoxylate (DHE, an AE) and iso-octylphenoxy-hexaethoxyethanol (trade name Criton X-100 or CTX, an APE); and two cationic surfactants n-cetyl trimethyl ammonium bromide (CTAB) and ditallow dimethyl ammonium chloride (DTDMAC).

Six surfactant concentrations of 1, 10, 25, 50, 75 and 100 mg/L were prepared from 1g/L surfactant stock solution. There was a control for each surfactant concentration. The control container contained the same amount of medium (water + feed) but no surfactant. The stock solutions were prepared in double distilled water. The series of working concentrations were chosen to include the IC_{50} value between any consecutive concentrations in the series. The IC_{50} values were calculated graphically by plotting the surfactant concentrations against the numbers of immobilized organisms. The test solutions were prepared in duplicate for each concentration.

The following adult organisms *Salmo gairdneri* (rainbow trout), *Gammbusia affinis* (mosquito fish), *Carassius auratus* (goldfish) and *Cirrhina mrigala*, a fresh water shrimp *Gammarus pulex* and a diptera larva *Chironomous bharati* were obtained from commercial sources and reared in standard aquaria under laboratory conditions (Versteeg and Shorter 1992; Tolls et al. 1994; Snell and Carmona 1995). Trout and *C. mrigala* are used as food. They were reared in 100 liter and other organisms in 40 liter aquaria. Test vessels were 10 liter plastic vessels for trout and *Cirrhina* and 5 liter buckets for other organisms. *Gambusia* and trout are used for controlling insect larvae. *G. affinis* is called mosquito fish because it is very sturdy in growth and eats mosquito larvae to eradicate malaria. *Chironomous* larva is benthic but the midge (adult insect) flies away from water and is terrestrial. The larva is red in colour and is called red worm. It was easily collected from the bottom of a pond. *Gammarus* is a zooplankton. This arthropod was collected from the water surface. The test species can be arranged in the decreasing order of their body weight and size as follows:

Trout > *Cirrhina* > Goldfish > *Gambusia* > *Gammarus* > *Chironomus*

All holding water had been filtered through 25 μ mesh filters and analysed for various physicochemical parameters prior to introduction to 120 liter aquaria. The holding water characteristics were adjusted to attain the following conditions:
Temperature 24 ± 2°C, pH 7.70 to 8.33, dissolved oxygen > 90% saturation, alkalinity 92 to 120 mg/L $CaCO_3$; NO_2 was non-detectable to 0.27 mg/L. Temperature was determined with a thermometer and pH with an Orion model SA 250 pH meter. Dissolved oxygen, total alkalinity and NO_2 were measured by following the standard methods (APHA 1998).

The fish were fed live *Tubifix* and also commercial fish feed. Prior to testing the test organisms were held under the above conditions in round, clear plastic holding vessel, having 63 or 202 micro mesh Nitexo net windows to allow a continuous supply of water. The holding vessels contained tubifix for easy capture by fish and an airstone in each holding vessel ensured high levels of dissolved oxygen while providing a current to which macrobes orient to feed.

The organisms were not fed for 24 hours prior to testing were introduced separately in the plastic containers and treated with various concentrations of the surfactants in the geometric ratio of 1, 10, 25, 50, 75 and 100 mg/L by keeping all other factors constant. No food was given during the test period. There were four fishes per aquarium and two aquaria per concentration. The immobility concentration (IC_{50}) for 48 hours was determined in all toxics exposures containing two replicates of four organisms per concentration. The controls also contained two replicates of four organisms per concentration. 4 trouts or *Cirrhinas* were taken in 5 liters of water, whereas 4 each of the other organisms in 2 liters of the medium. The temperature, pH, dissolved oxygen and alkalinity of the water used were estimated at the start, after 24 hours and at the termination of the experiment. The above experiments were repeated four times, results were averaged and the standard deviation was calculated.

RESULTS AND DISCUSSION

Among the seven surfactants tested for toxicity (Table 1 & 2), DTDMAC was found to be the most toxic and SDS to be the least toxic. The general order of toxicity was :

	DTDMAC >	CTAB >	SDBS >	CTX >	SDES >	DHE >	SDS
Mol. wt.	543.5	364	348	646	316	450	288

Table 1. Toxicity (48-h IC_{50}, mg/L) of surfactants against freshwater fishes

Surfactants	*Trout*		*Gambusia*		*Goldfish*		*Cirrhina*	
	IC_{50}	SD	IC_{50}	SD	IC_{50}	SD	IC_{50}	SD
SDS	33.61	0.32	40.15	0.26	38.04	0.81	30.81	0.47
SDES	10.84	0.11	13.64	0.10	12.35	0.20	7.42	0.05
SDBS	3.63	0.03	8.81	0.09	5.10	0.07	2.69	0.005
DHE	22.38	0.10	29.26	0.16	28.02	0.22	17.21	0.09
CTX	6.44	0.04	9.65	0.04	9.24	0.06	4.03	0.05
CTAB	1.21	0.01	8.24	0.03	3.58	0.02	0.92	0.01
DTDMAC	0.74	0.009	7.91	0.16	2.37	0.09	0.49	0.002

Table 2. Toxicity (48-h IC_{50}, mg/L) of surfactants against *Gammarus* and *Chironomas*

Surfactants	*Gammarus*		*Chironomas*	
	IC_{50}	SD	IC_{50}	SD
SDS	93.88	0.91	56.23	0.63
SDES	39.24	0.41	18.57	0.14
SDBS	28.62	0.34	15.46	0.09
DHE	84.31	0.72	41.31	0.64
CTX	31.53	0.32	17.44	0.22
CTAB	24.36	0.09	12.51	0.09
DTDMAC	18.62	0.29	10.94	0.11

Thus cationics are more toxic than anionics and non-ionic surfactants are the least toxic, but SDS is an exception. Therefore, the products of non-ionic surfactants should be promoted.

CTX is non-ionic, but it is more toxic than anionics SDES and SDS, probably due to high molecular weight and presence of benzene ring. Surfactant toxicity usually increases with increasing alkyl chain length that has a greater effect on toxicity than average EO (ethylene oxide or ethoxylate) groups (Lewis 1991; Roberts 1991)

Toxicity of anionic surfactants to the macrobial population of an activated sludge is a very complex phenomenon involving several mechanisms such as adsorption, precipitation and biodegradation. For the commercial single homologues C_{10}-C_{14} of LAS, C_{12}-LAS showed the highest toxicity of all homologues tested, as a consequence of the equilibrium among all the above three processes taking place simultaneously. In the range C_{10}- C_{12}, the lower the molecular weight the lower the toxicity. For C_{12}-C_{14}, the relation is opposite; the higher the molecular weight the lower the toxicity due to higher adsorptivity and lower bioavailability of the higher homologues. C_{12} represents the minimum in this relationship. In the C_{10}- C_{14} LAS homologues series, the longer the alkyl chain the faster the biodegradation kinetics. C_{12} is the least toxic of all LAS homologues. The 48-h IC_{50} values of SDBS were 28.62 mg/L for *Gammarus*, 15.46 mg/L for *Chironomas*, 8.81 mg/L for *Gambusia,* 5.10 mg/L for *Goldfish,* 3.63 mg/L for *Trout,* and 2.69 mg/L for *Cirrhina.*

AS and AES are less toxic than LAS, possibly due to their higher adsorption, precipitation and biodegradation taking place simultaneously. CTX presents toxicity values slightly lower than SDBS possibly due to a strong adsorption and precipitation. Chronic and sublethal toxicities of cationic surfactants occur at concentration less than that of other surfactant groups. Thus, cationic surfactants DTDMAC and CTAB are most inhibitory on aquatic life. DTDMAC was the most toxic among the seven surfactants tested for the four microbes in the present study.

The effects of surfactants on organisms, particularly macrobes, are not only compound specific but also species specific. The 48-h IC_{50} values of SDS, SDES and SDBS for *Gammarus* were 93.88 ± 0.91, 39.24 ± 0.41 and 28.62 ± 0.34, but for *Chironomas* were 56.23 ± 0.63, 18.57 ± 0.14 and 15.46 ± 0.09 respectively.

The general structure of surfactants (both branched or linear, aromatic or aliphatic) is not a main factor controlling their toxicity to macrobes. Toxicity of the ethoxylated alkylphenol,

both branched (tp-NPEO, OPEO) and linear APEO, increases with decreasing EO molar ratio which corresponds to decreasing solubility in water. This is an expected finding, since decreasing solubility in water reflects increasing biophilic character of a molecule which in turn indicates that the molecule is more likely to adsorb on lipid membranes and cause disruption of the membrane functions (Funasaki et al. 2000; Moren et al. 2000). Both aromatic and aliphatic based products with EO molar ratios of 30 or higher are relatively nontoxic. Toxicity of linear APEO (with the average alkyl chain length equal to that of nonylphenol) is similar to the toxicity of the corresponding (in terms of EO molar ratio) branched nonylphenol ethoxylates (tp-NPEO), indicating that the structure of the alkyl group is not an important factor in pure product toxicity. Substitution of an anionic active group (OSO_3^- or OPO_3^{2-}) in place of the hydroxyl group at the end of the polyethoxy chain apparently decreases surfactant toxicity, most likely due to the increased solubility in water caused by the dissociating active group. Surfactant base structure (aromatic or aliphatic) does not appear to be an important factor in altering the toxicity of the chemicals tested. The ethylene oxide molar ratio is a good indicator of surfactant toxicity and would be a good parameter for evaluating surfactant toxicity when conducting hazard assessments on these chemicals. However, biological degradation, toxicity and surface microlayer enrichment (SML) of surfactants would have to be considered in evaluating possible environmental impacts of these chemicals.

Differentiating among an arthropoda, *Gammarus*, an insect, *Chironomus* and four species of fish, no clear picture holds regarding which of these six is the most sensitive one. With different species the effective concentrations being more or less equal according to their body weight. Invertebrates are more tolerable than fish and so they are able to survive even in highly polluted waters. Among all fish species tested *Gambusia* has the maximum resistance to toxic chemicals. Among the six organisms tested fishes were found to be the most sensitive.

In conclusion, cationic surfactants were found to be more toxic than anionic surfactants and anionics more toxic than non-ionic surfactants. Therefore, the products of non-ionic surfactants should be promoted. The ethylene oxide molar ratio is a good indicator of surfactant toxicity, and would be a good parameter for evaluating surfactant toxicity when conducting hazard assessments on these chemicals. However, biological degradation, toxicity and SML enrichment of surfactants would have to be considered in evaluating possible environmental impacts of these chemicals.

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