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Effects of nonionic and ionic surfactants on survival, oxidative stress, and cholinesterase activity of planarian

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Abstract

Eight widely used surfactants (cetyltrimethylammonium bromide; CTAB, benzethonium chloride; Hyamine 1622, 4-nonylphenol; NP, octylphenol ethoxylate; Triton X-100, dodecylbenzene sulfonate; LAS, lauryl sulfate; SDS, pentadecafluorooctanoic acid; PFOA, and perfluorooctane sulfonate; PFOS) were selected to examine their acute toxicities and effects on oxidative stress and cholinesterase (ChE) activities in *Dugesia japonica*. The differences in acute toxicity among eight surfactants to planarians were at least in the range of three orders of magnitudes. The toxicity rank of surfactants according to estimated 48-h LC_{50} was SDS > NP > LAS > Hyamine 1622 > CTAB > Triton X-100 > PFOS > PFOA. The toxicity rank of surfactants according to 96-h LC_{50} was as follows: SDS > CTAB > NP > LAS > Hyamine 1622 > Triton X-100 > PFOS > PFOA. There were significant increases in catalase activities in planarians exposed to LAS at nominal concentrations of 0.5 or 1 mg l^{-1} and to PFOS at nominal concentrations of 5 or 10 mg l^{-1} after 48-h exposure. Inhibitions of ChE activities were found in planarians exposed to Hyamine 1622 at all concentrations tested, to PFOS at nominal concentration of 10 mg l^{-1}, to PFOA at nominal concentrations of 50 or 100 mg l^{-1} and to NP at nominal concentration of 0.5 mg l^{-1}. A significant increase in ChE activities was also observed in planarian exposed to Triton X-100 at nominal concentration of 5 mg l^{-1}. The implication of ChE inhibition by NP, PFOS and PFOA on neurological and behavioral effects in aquatic animals requires further investigation.

Keywords: *Dugesia japonica*; Lipid peroxidation; Cholinesterase activity; Antioxidant enzyme activity; Nonionic surfactants; Ionic surfactants

1. Introduction

Surfactants are widely used in everyday personal care and household products as well as in a variety of industrial applications. As a result, large amounts of surfactants are commonly discharged in large quantities to sewage treatment plants or directly to the aquatic environment in areas where there is no sewage treatment. In fact, many surfactants and their degradation products have been found worldwide in wastewater discharges, sewage treatment plant effluents, natural water and sediments (Ying, 2006). Because many surfactants are ubiquitous (Ying et al., 2002; Venhuis and Mehrvar, 2004), the potential toxic effects of these chemicals have attracted much research attention in the past several decades (Abel, 1974; Lewis and Suprenant, 1983; Lewis, 1991). However, previous investigations have concentrated mainly on anionic surfactants, and there is limited toxicological information on other types of surfactants or some new emerging surfactants such as 4-nonylphenol (NP), pentadecafluorooctanoic acid (PFOA), or perfluorooctane sulfonate (PFOS).

Many different mechanisms of toxicities exist for different types of surfactants and one single surfactant can produce its toxicity through more than one mechanism. In general, toxic effects of surfactants are observed via damage on gills and epidermis of aquatic vertebrate or disruption of cellular membrane of aquatic invertebrate (Abel, 1974). The toxicity of surfactants is primarily determined by the ability of the surfactants to adsorb and penetrate

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the cell membrane of aquatic organisms (Rosen et al., 2001). However, the molecular mechanisms of toxicities of surfactants are not well understood after surfactant adsorption on the membrane surface. What is known is that an interaction with lipid membranes appears to disrupt membrane integrity, thus causing toxic effects (Abel, 1974). The disruption of membrane integrity is possibly caused by interference with membrane permeability or membrane proteins. One possible mechanism of disruption of membrane integrity was oxidative stress which has detrimental effects on membrane integrity, leading to a loss of fluidity and increased ion permeability (Livingstone, 2003).

Most surfactants have an ionic or polar head group connected to a hydrophobic tail with a straight or branched hydrocarbon chain. Hydrocarbon metabolisms of surfactants by aquatic animals might be produced highly reactive oxygen species and to cause oxidative stress in organisms. Information of surfactant-induced oxidative stress in aquatic organisms is still very limited and effects of different classes of surfactants *in vivo* need further investigations. In addition, some studies have recently indicated that some surfactants may inhibit cholinesterase (ChE) activity in aquatic animals (Guilhermino et al., 1998, 2000a; Garcia et al., 2000). Indeed, the chemical properties of surfactants can alter enzyme activities by binding or disrupting enzyme structure (Cserháti et al., 2002). However, most ChE inhibitions were observed *in vitro* experiments (Guilhermino et al., 1998; Garcia et al., 2000) but very few *in vivo* experiments (Guilhermino et al., 2000a). Because of their common occurrence in aquatic environment, it will be of important to examine effects of different surfactant types on ChE inhibition in aquatic animals.

Freshwater free-living planarians are distributed worldwide in unpolluted streams and an important component of the aquatic ecosystem. Traditionally, planarians have been a favored animal model in developmental biology (Newmark and Alvarado, 2002) and neuroscience research (Pagan et al., 2006). Furthermore, they have been suggested as test organisms for various types of short-term toxicity bioassays because they are sensitive to different classes of environmental pollutants (Horvat et al., 2005; Pra et al., 2005). In addition, they can be easily collected in large numbers, require only low culture and test medium volumes, and can be kept inexpensively in laboratory for toxicological testing. These characteristics make planarian a suitable organism for studying the effects of environmental pollutants in aquatic environment.

The objective of this study was to evaluate aquatic toxicity of surfactants using a freshwater planarian, *Dugesia japonica*, as an animal assay. Eight commonly used surfactants were selected. Their adverse toxicities on aquatic invertebrates were determined by examining the effects of these surfactants on survival, oxidative stress and ChE activity in *D. japonica*. These different surfactants were selected in view of their known widespread human exposure and environmental occurrence (Venhuis and Mehrvar, 2004; Lehmler, 2005). Cetyltrimethylammonium bromide (CTAB) and benzethonium chloride (Hyamine 1622) are used primarily in cosmetics and shampoos for its antimicrobial and cationic surfactant properties. Octyl phenol ethoxylate (Triton X-100) and NP are nonionic surfactants widely used in industrial and household products. Of four anionic surfactants chosen, sodium dodecylbenzene sulfonate (LAS) and lauryl sulfate (SDS) are commonly used as active ingredients in household and personal care products as well as in specialized applications, while PFOS and PFOA are two fluorinated surfactants with growing environmental concerns and are widely applied to fabrics, carpets and paper (Renner, 2005).

2. Materials and methods

2.1. Chemicals

NP was purchased from Riedel-de Haën (Sigma–Aldrich, USA), with a chemical purity of 94%. PFOS (>98%) was obtained from Fluka. Hyamine 1622, Triton X-100, LAS (80%), PFOA (>98%), SDS (99%) and CTAB (99%) were obtained from Sigma–Aldrich. The properties of surfactants and the range of nominal testing concentrations for each surfactant are listed in Table 1. In this study, all stock solutions and test beakers for surfactants tested were used glass containers, except for PFOS and PFOA treatments which used polyprolene containers for stock solutions and test vessels because these two chemicals have potential to be adsorbed onto glass surface. In addition, all biochemical materials for enzyme assays were purchased from Sigma–Aldrich. HPLC grade acetone was purchased from Mallinckrodt. NP was dissolved in acetone for preparing the test stock solution. All other stock solutions for testing chemicals were prepared in dechlorinated tap water.

2.2. Test organisms

D. japonica was collected from Nan-shi stream located in Wu-lai, Taipei County, Taiwan in 2004. Since then, the planarians have been maintained in dechlorinated tap water at our laboratory. Animals were fed with raw chicken liver once a week. Culture medium was renewed weekly after feeding.

2.3. Acute toxicity test

The planarians (body length $= 0.9 \pm 0.1$ cm) were exposed to different surfactants with at least five different concentrations or dechlorinated tap water as a control group. For each concentration, five animals were kept in 50 ml of test solution in a beaker and each treatment was replicated five times during the experiment. All acute toxicity experiments were conducted in a temperature incubator at 25 ± 1 °C with a 12 L:12 D illumination. The animals were not fed and inspected every 24 h for mortality during

Table 1
Properties and tested nominal concentration range of surfactants studied

Chemicals/chemical acronyms	Type of surfactant	Molecular weight ($g\ mol^{-1}$)	CAS No.	Water solubility[a]	Nominal concentration range ($mg\ l^{-1}$)
Dodecylbenzene sulfonate/LAS	Anionic	348.48	25155-30-0	5–10 $g\ l^{-1}$	0.01–50
Lauryl sulfate/SDS	Anionic	288.4	151-21-3	100 $g\ l^{-1}$	0.01–100
Perfluorooctane sulfonate/PFOS	Anionic	538.22	2795-39-3	680 $mg\ l^{-1}$	10–200
Pentadecafluorooctanoic acid/PFOA	Anionic	431.10	3825-26-1	3.4 $g\ l^{-1}$	100–750
Benzethonium chloride/Hyamine 1622	Cationic	448.1	121-54-0	44.8 $g\ l^{-1}$	0.05–50
Cetyltrimethylammonium bromide/CTAB	Cationic	364.46	57-09-0	15 $g\ l^{-1}$	0.05–50
4-nonylphenol/NP	Nonionic	220.35	104-40-5	$4.9 \pm 0.4\ mg\ l^{-1}$	0.15–1
octylphenol ethoxylate/Triton X-100	Nonionic	624	9002-93-1	Miscible	1–100

[a] MSDS from different manufacturers.

the entire 96-h experimental period. The organisms without detectable movement were considered dead and removed from the test solution.

2.4. Treatments for oxidative stress and ChE inhibition studies

The test concentration of each surfactant for examining the effects of enzyme activities was chosen according to the results of acute toxicity of the present study as well as considerations for environmental relevance. The highest test concentration for each surfactant was selected based on the highest concentration causing no mortality from 48 h acute toxicity studies. In all cases, two independent experiments were conducted for each surfactant and each treatment group was performed in triplicate at each experiment. Ten animals (body length $= 0.9 \pm 0.1$ cm) per treatment group were exposed to each surfactant at three different concentrations or dechlorinated tap water as a control group for 48 h. Antioxidant enzyme and ChE activity measurements were conducted from the same experiments whereas lipid peroxidation measurements were made in separate experiments.

2.5. Antioxidant enzyme activity measurements

At the end of each experiment, the medium was removed and the planarians were rinsed gently with distilled water at least three times. Then whole-body homogenates in 500 μl of 0.1 M sodium phosphate buffer (pH 7.5) containing 0.1 mM PMSF were immediately prepared and centrifuged at 12000 g for 30 min at 4 °C. The supernatants were collected and used for the assays of catalase (CAT), superoxide dismutase (SOD) and ChE. CAT activity was measured using Aebi's method (Aebi, 1984). If brief, the reaction mixture contained 50 μl of enzyme extract in 950 μl of 0.05 M potassium phosphate buffer (pH 7.0) with 30 mM H_2O_2. The reaction was performed at room temperature (25 ± 1 °C) for 90 s and CAT activity was determined from the rate of H_2O_2 decrease in absorbance at 240 nm using extinction coefficient 39.4 $M^{-1}\ cm^{-1}$. Each assay was done in duplicate and the average of the two values was used in the final data analysis.

The SOD activity assay was based on the inhibition of nitroblue tetrazolium (NBT) reduction using the method of Beauchamp and Fridovich (1971). The reaction mixture contained 50 mM sodium phosphate buffer (pH 7.8), 0.075 mM NBT, 13 mM L-methionine, 0.1 mM EDTA and 0.002 mM riboflavin with a series of samples ranging from 5 to 30 μg of the enzyme extract. The mixtures were illuminated by fluorescent lamps (light intensity was 5450 lux) for 20 min at 25 °C. Identical solutions held in the dark served as blanks. One unit of SOD was defined as the amount of enzyme that produced a 50% inhibition of NBT reduction under assay conditions. Each assay was done in duplicate and the average of the two values was used in the final data analysis.

2.6. ChE activity measurement

ChE present in planarian were characterized by measuring ChE inhibition after *in vitro* incubation of planarian homogenates for 30 min in the presence of specific inhibitors. Three inhibitors were used, including tetraisopropyl pyrophosphoramide (iso-OMPA), 1,5-bis(4-allydimethylammoniumphenyl)pentan-3-one dibromide (BW284C51) and eserine sulfate. ChE activity was assayed immediately after incubation with inhibitors in a concentration range from 10^{-3} to 10^{-8} M from three experiments. Each experiment was a pool of 100 planarians and measured in duplicate.

Measurements of ChE activity were performed using the colorimetric method of Ellman (Ellman et al., 1961) with acetylthiocholine iodide as substrate and dithiobisnitrobenzoate (DTNB) as reagent at room temperature (25 ± 1 °C). In brief, the reaction mixture contained 100 μl of enzyme extract in 1400 μl of 0.05 M sodium phosphate buffer (pH 8.0) in the presence of 0.033 mM DTNB. The reaction was triggered by addition of 10 μl of 75 mM acetylthiocholine iodide to the sample mixture. The rate of increase in optical density of the reaction medium was measured using a Hitachi UV/VIS spectrophotometer at 412 nm for 120 s. Each assay was done in duplicate and the average of the two values was used in the final data analysis. Because no selective inhibitor was used in this enzymes assay, the activities measured are referred as ChE.

2.7. Lipid peroxidation assay

Lipid peroxidation was assayed using a modified procedure of Buege and Aust (1978). Planarians were homogenized in 300 μl of 0.15 M KCl with 0.02% BHT immediately. An aliquot of 100 μl homogenate were added to 500 μl of 0.8 % thiobarbituric acid (TBA) in 24% trichloracetic acid (TCA). The mixture was centrifuged at 12000*g* for 30 min at 4 °C. Supernatants were transferred to new eppendorf tubes, heated at 95 °C for 30 min in the boiling water bath, then cooled in an ice bath and centrifuged at 12000*g* for 5 min. The absorbance of the supernatant was recorded at 532 nm corrected for nonspecific turbidity by subtracting the absorbance at 600 nm. The degree of lipid peroxidation was expressed as concentration of thiobarbituric acid-reactive substances (TBARS) participating in the reaction per milligram of tissue protein. Each assay was done in duplicate and the average of the two values was used in the final data analysis.

2.8. Protein measurement

Total protein concentration of the homogenate was measured using Bradford's method (Bradford, 1976). A standard curve was constructed for the protein concentration of 1–12.5 μg ml^{-1} of bovine serum albumin. All protein measurements were performed in triplicate and the average of the three values is reported for tissue protein contents of each sample.

2.9. Data analysis

The nominal concentrations that were lethal to 50% of the organisms (LC_{50}) for each chemical at 24, 48, 72 or 96 h were calculated using trimmed Spearman–Karber analysis with trimmed Spearman–Karber Program (version 1.5) obtained from Environmental Monitoring Systems Laboratory (USEPA, Cincinnati, Ohio). The LOAEL values (the lowest concentration producing animal mortality significantly different from the controls) and NOAEL (the highest concentration producing no mortality significantly different from the controls) were determined by Dunnett's multiple comparison procedure using the Minitab Statistical Program (version 13.2).

For comparison reasons, data were expressed as ratio of change from the respective control value (taken as 1) for ChE activities from different surfactant treatments, and were the mean ± SD of six samples from two separate experiments. Although data are presented graphically as the ratio of control enzyme activities in the absence of treatments, all statistical evaluations were performed on absolute, nonreferenced data using Minitab Statistical Program (version 13.2). Data were first tested for normality with a Kolmogorov–Smirnoff test and for equality of variance with a Bartlett's test. Because some date did not meet assumptions of normality or homogeneity of variance, all statistical comparisons for enzyme activities or lipid peroxidation were performed by nonparametric Kruskal–Wallis test. If a significant result was found, Mann–Whitney U test was used to determine which treatment groups were significantly different from the controls. In all cases, $P = 0.05$ was accepted to indicate statistical significance.

3. Results

3.1. Acute toxicity

There were considerable variations in LC_{50} values of eight surfactants to planarians. The 48-h LC_{50} values for planarians ranged from 0.36 to 536 mg l^{-1}, revealing a 1489-fold difference (Table 2). The toxicity of descending order according to 48-h LC_{50} was as follows: SDS > NP > LAS > Hyamine 1622 > CTAB > Triton X-100 > PFOS > PFOA. The 96-h LC_{50} values for planarians ranged from 0.36 to 458 mg l^{-1}, indicating a 1272-fold difference (Table 2). The toxicity of descending order according to 96-h LC_{50} was as follows: SDS > CTAB > NP > LAS > Hyamine 1622 > Triton X-100 > PFOS > PFOA. For SDS, all mortalities occurred within the first 24 h of exposure and the 24-h LC_{50} was the same as the 96-h LC_{50}. For other surfactants, most mortalities occurred during the first 72 h of exposure, except for CTAB (Table 2). Among surfactants tested, SDS always had the highest acute toxicity while PFOA had the lowest toxicity to planarian at each exposure period.

The 48- and 96-h NOAEL and LOAEL values of mortalities of different surfactants for planarians in this study were summarized in Table 3. There were no difference in the values of the NOAEL and LOAEL values of SDS, NP and CTAB between 48 h and 96 h of exposure. Following 48 h of exposure, the NOAEL and LOAEL of mortality was in the order of SDS > NP > LAS > Hyamine 1622 > CTAB > Triton X-100 > PFOS > PFOA. Following 96 h of exposure, the LOAEL of toxicity in descending order was SDS = Hyamine 1622 = CTAB > NP > LAS > Triton X-100 > PFOS > PFOA, and the NOAEL was in the decreasing toxic order of SDS > NP = Hyamine 1622 = CTAB > LAS > Triton X-100 > PFOS > PFOA.

3.2. Antioxidant enzyme activities

There were significant increases in CAT activities on planarians exposed to LAS at 0.5 or 1 mg l^{-1} and to PFOS at 5 or 10 mg l^{-1} (Table 4). On the other hand, there were no significant changes in SOD activities exposed to any surfactant used in the present study (Table 4).

3.3. ChE characterization

Esterine sulfate, an inhibitor of ChEs but not of other esterases, had a strong effect on planarian ChE to less than 10% of inhibition at concentration equal or higher than 10^{-6} M (Fig. 1). It confirmed that ChE activity measurement used acetylthiocholine iodide as substrate was mainly

Table 2
Nominal concentrations of LC_{50} (mg l^{-1}) from 24 to 96 h for *Dugesia japonica* exposed to different surfactants

Chemicals[a]	LC_{50} (mg l^{-1}) at nominal concentrations			
	24 h	48 h	72 h	96 h
LAS	6.83 (5.95–7.84)[b]	1.79 (1.68–1.90)	1.51 (1.44–1.58)	1.45 (1.38–1.52)
SDS	0.36 (0.32–0.39)	0.36 (0.32–0.39)	0.36 (0.32–0.39)	0.36 (0.32–0.39)
PFOS	53 (49–56)	25 (24–27)	19 (18–19)	17 (16–18)
PFOA	548 (544–552)	536 (528–544)	519 (509–530)	458 (427–491)
Hyamine 1622	7.58 (7.01–8.19)	2.34 (2.20–2.50)	2.24 (2.12–2.35)	2.04 (1.85–2.26)
CTAB	3.16 (2.94–3.42)	2.83 (2.50–3.20)	1.63 (1.27–2.09)	0.45 (0.34–0.60)
NP	>1	0.87 (0.83–0.92)	0.85 (0.81–0.89)	0.85 (0.81–0.89)
Triton X-100	11.8 (11.6–12.1)	9.4 (9.1–9.6)	8.8 (8.6–8.9)	8.5 (8.4–8.7)

[a] Actual chemical names refer to Table 1.
[b] Values in parentheses are 95% confidence intervals.

Table 3
Nominal concentrations of NOAEL (mg l^{-1}) and LOAEL (mg l^{-1}) according to mortality determined using Dunnett's test for *Dugesia japonica* exposed to surfactants after 48- and 96-h exposure

Chemicals[a]	48 h		96 h	
	LOAEL (mg l^{-1})	NOAEL (mg l^{-1})	LOAEL (mg l^{-1})	NOAEL (mg l^{-1})
LAS	2	1.5	1.5	1
SDS	0.5	0.25	0.5	0.25
PFOS	20	18	18	12
PFOA	500	450	450	400
Hyamine 1622	5	1	1	0.25
CTAB	1	0.25	1	0.25
NP	0.9	0.75	0.9	0.75
Triton X-100	10	7.5	7.5	5

[a] Actual chemical names refer to Table 1.

due to ChE activity and not to other types of esterases. BW284C51, a specific inhibitor of AChE, inhibited planarian ChE to less than 40% at concentrations equal to or higher than 10^{-4} M (Fig. 1). In addition, iso-OMPA, a specific BChE inhibitor, did not significantly inhibit ChE activity in all concentrations tested, with a maximum of inhibition of 53% at 10^{-3} M. These data indicated that AChE and BChE were both presented in planarian ChE, whereas AChE contribution to the total ChE activity in planarian was greater than BChE contribution.

3.4. ChE activity

Inhibition of ChE activities was found in planarians exposed to Hyamine 1622 at all concentrations tested in this study (Fig. 2). In addition, there were significant decreases in ChE activities in planarians exposed to PFOA at 50 or 100 mg l^{-1}, to PFOS at 10 mg l^{-1}, and to NP at 0.5 mg l^{-1} (Fig. 2). On the other hand, a significant increase in ChE activities was observed in planarians exposed to Triton X-100 at 5 mg l^{-1}.

3.5. Lipid peroxidation

Effects of lipid peroxidation were not observed on planarians exposed to any surfactant tested at current concentrations used (data not shown).

Table 4
Antioxidant activities (mean ± SD; $n = 6$) in planarian exposed to different surfactants after 48-h treatment

Surfactant[a]	Nominal concentration (mg l^{-1})	CAT activity (H_2O_2 μ mol protein mg^{-1} min^{-1})	SOD activity (U protein mg^{-1} min^{-1})
LAS	0	4.62 ± 1.62	1.3 ± 0.8
	0.1	4.76 ± 1.25	1.4 ± 0.6
	0.5	8.49 ± 2.11*	1.4 ± 0.5
	1	10.45 ± 2.95*	1.8 ± 0.5
SDS	0	11.51 ± 1.73	1.4 ± 0.6
	0.01	9.17 ± 1.80	0.8 ± 0.3
	0.05	10.41 ± 1.05	1.1 ± 0.2
	0.1	10.73 ± 1.07	0.9 ± 0.4
PFOS	0	11.51 ± 1.73	1.5 ± 0.6
	1	13.39 ± 1.99	1.2 ± 0.3
	5	14.85 ± 1.22*	0.8 ± 0.3
	10	15.33 ± 1.44*	1.5 ± 0.7
PFOA	0	5.43 ± 1.54	1.6 ± 1.0
	10	4.40 ± 0.84	1.4 ± 0.4
	50	6.50 ± 2.91	1.4 ± 0.5
	100	6.45 ± 2.16	1.4 ± 0.6
Hyamine 1622	0	5.43 ± 1.54	1.6 ± 1.0
	0.1	6.07 ± 1.04	1.1 ± 0.4
	0.5	6.25 ± 1.71	1.3 ± 0.4
	1	7.14 ± 1.40	1.0 ± 0.4
CTAB	0	5.62 ± 1.24	1.7 ± 0.9
	0.05	5.02 ± 1.53	1.2 ± 0.7
	0.1	5.07 ± 1.56	1.1 ± 0.3
	0.5	7.40 ± 2.30	1.2 ± 0.3
NP	0	5.27 ± 0.74	1.7 ± 0.9
	0.05	4.33 ± 1.29	1.2 ± 0.7
	0.1	4.85 ± 1.79	1.1 ± 0.3
	0.5	5.20 ± 1.23	1.2 ± 0.3
TritionX-100	0	4.62 ± 1.62	1.3 ± 0.8
	0.5	3.74 ± 0.40	0.9 ± 0.4
	1	4.01 ± 0.88	1.2 ± 0.3
	5	5.19 ± 1.56	0.9 ± 0.4

[a] Actual chemical names refer to Table 1.
* A significant difference from the respective controls at $P < 0.05$.

4. Discussion

The differences in acute toxicity among eight surfactants to planarians were at least in the range of three orders of magnitudes. In general, the literature indicated cationic surfactants were more toxic to aquatic organisms than

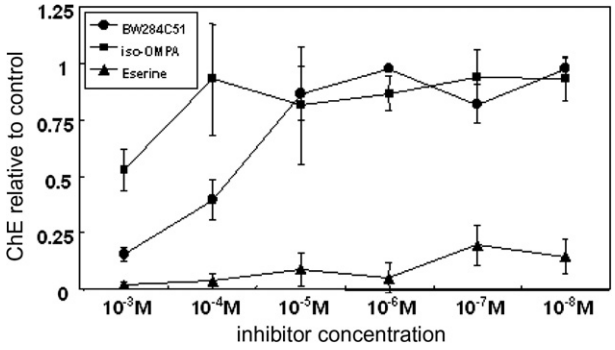

Fig. 1. *In vitro* effects of different inhibitors on ChE activities of planarian after 30 min of incubation with different concentrations of inhibitors. Each symbol was 4 represented data (mean ± SD) from three individual experiments using pools of 100 planarians.

anionic and/or nonionic surfactants (Lewis and Suprenant, 1983; Singh et al., 2002). However, both the most and the least toxic surfactants are anionic surfactants in the present study. In fact, the toxicity order associated with surfactant types could be dependent on an artifact of the limited number of surfactants tested rather than a real difference in relative toxicity of surfactant types (Warne and Schifko, 1999). Therefore, it might explain why no clear relationship exists between surfactant types and acute toxicity in planarians. Moreover, *Daphnia magna* has been suggested to be the most sensitive species to surfactants (Lewis and Suprenant, 1983; Sandbacka et al., 2000; Cserháti et al., 2002). Data on surfactant toxicity obtained from the 48-h planarian toxicity test in this study were compared with those from 48-h toxicity tests with *D. magna* in the literature. Based on these limited data (Table 5), *D. japonica* appears similar to *D. magna* in sensitivity to the surfactants tested.

The results of this study clearly show that the acute toxicity of SDS was considerably higher than that of other surfactants tested in *D. japonica*. By searching SDS ecotoxicity data on the PAN Pesticides Database (http://www.pesticideinfo.org/Search_Chemicals.jsp), newly fertilized embryos of horse clam (*Tresus capax*) was the most sensitive species to SDS with a 48-h LC_{50} of 0.36 mg l^{-1}. The 48-h LC_{50} of 0.36 mg l^{-1} for planarian obtained in this study also indicate that freshwater planarians have highly sensitivity to SDS among aquatic organisms tested in the literature. However, no effects were observed on planarian ChE or antioxidant enzyme activities at a range of 0.01–0.1 mg l^{-1} of SDS. In fact, SDS was found to significantly inhibit the AChE activity of *Mytilus galloprovincialis* haemolymph at 50 mg l^{-1} or higher under *in vitro* condition (Guilhermino et al., 1998), or depress significantly the AChE activity of *D. magna in vivo* at concentration equal to or higher than 11.9 mg l^{-1} (Guilhermino et al., 2000a). On the other hand, no effects were found on CAT activity in the fish liver of *Lateolabrax japonicus* or AChE activity in the brain of *L. japonicus* exposed to 1 mg l^{-1} of SDS after 12 or 18 days of treatment (Wu et al., 2005). No apparent changes of planarian CAT or ChE activities

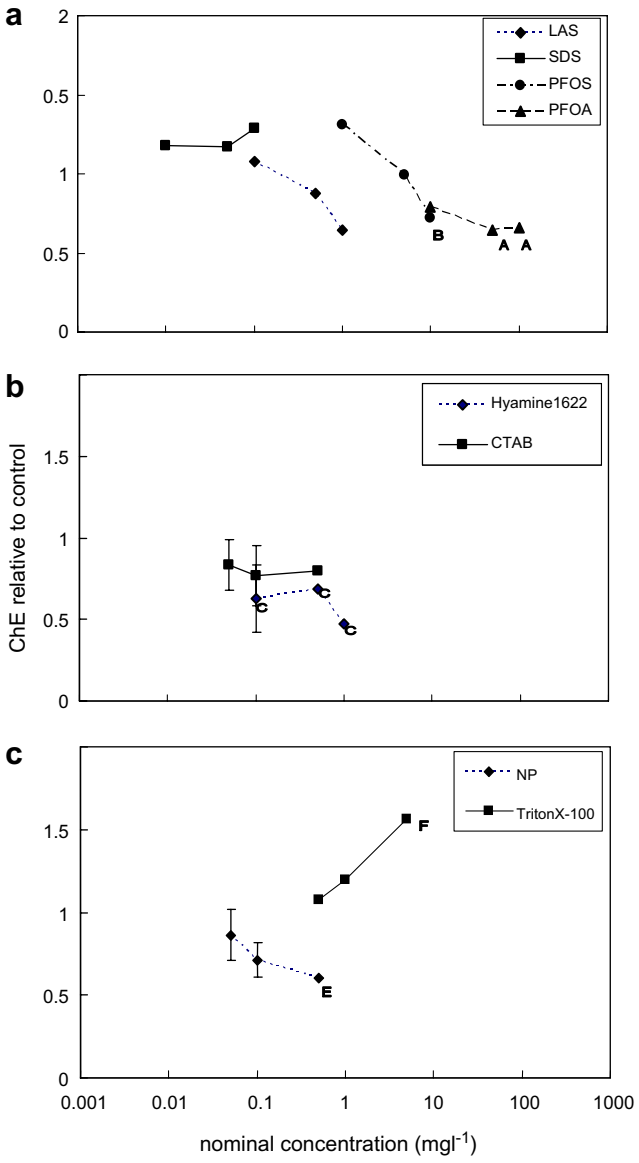

Fig. 2. ChE activities in planarian exposed to (a) anionic surfactants, (b) cationic 8 surfactants, and (c) nonionic surfactants after 48-h treatment. Values are expressed 9 as mean change from the respective control values (taken equal to 1), $n = 6$. Each character is represented significantly different from the respective control group ($P < 0.05$) for each surfactant treatment.

exposed to SDS might be due to the very low concentrations (0.01–0.1 mg l^{-1}) of SDS used in the present study.

It is well established that ChE inhibition can be used as a useful biomarker for organophosphate and carbamate pesticides both *in vivo* (Day and Scott, 1990) and *in vitro* (Hamers et al., 2000). However, several studies published in the last decade have demonstrated the possible effects of surfactants on ChE activity (Guilhermino et al., 1998, 2000a). In this study, both ionic and nonionic surfactants caused an inhibition of ChE activities. For example, a cationic surfactant, Hyamine 1622, significantly depressed ChE activities in planaria at nominal concentrations from

Table 5
Comparisons of 48-h LC_{50} (mg l^{-1}) for *Dugesia japonica* and *Daphnia magna* exposed to different surfactants under static condition

Surfactant[a]	48-h LC_{50} (mg l^{-1}) of *Dugesia japonica* from this study	48-h LC_{50} (mg l^{-1}) of *Daphnia magna* from the literature	Sources
LAS	1.79	9.55	Guilhermino et al. (2000b)
SDS	0.36	6.2–9	LeBlanc (1982)
PFOS	25	130	Boudreau et al. (2003)
PFOA	536	268	Boudreau (2003)
Hyamine 1622	2.34	–	–
CTAB	2.83	0.05	Knops et al. (2001)
NP	0.87	0.19	Comber et al. (1993)
Triton X-100	9.4	–	–

[a] Actual chemical names refer to Table 1.

0.1 to 1 mg l^{-1}, and an anionic surfactant, PFOA, also significantly reduced planarian ChE activities at nominal concentrations of 50 and 100 mg l^{-1}. On the other hand, one nonionic surfactant, NP, significantly decreased ChE activities in planaria at nominal concentration of 0.5 mg l^{-1} while the other nonionic surfactant, Triton X-100, significantly increased planarian ChE activities at 5 mg l^{-1}. This result is in agreement with a previous report showing that some alkylphenolic compounds, including NP, could significantly inhibit AChE activity of rat neuronal cell line PC12 (Talorete1 et al., 2001). The immediate result of AChE inhibition is an increase in acetylcholine which may result in a decrease in cholinergic receptor number, which is a compensatory response to an acetylcholine buildup. Interestingly, Jones et al. (1998) did report that NP exposure could cause a decrease in brain muscarinic cholinergic receptors in three species of trout. However, an inhibition of ChE activity by NP *in vivo* system has not been published.

Inhibition of ChE can be mediated through the binding of the inhibitor to the catalytic site or peripheral anionic site of the enzyme (Bourne et al., 2003). In contrast to the substrate acetylcholine and to inhibitors such as organophosphate and carbamate pesticides, the chemical structure of surfactants examined in this study are lack of the molecular interaction potential of the ester group to bind in the catalytic site of the enzyme. However, surfactants might change ChE activity by binding to the anionic site of the enzyme or by changing allosteric interaction of enzyme (Cserháti, 1995; Marcel et al., 2000). In addition, it has been suggested that some surfactants could change enzyme activity by modifying the soluble AChE conformation after interaction with surfactant micelles (Guilhermino et al., 1998). In fact, molecular mechanisms for the inhibition or enhancement of ChE activity by different surfactants remain unclear and merit further studies.

5. Conclusion

In the present study, *D. japonica* showed a similar sensitivity to surfactants compared with *D. magna*, but revealed a distinctly different pattern of sensitivity to different surfactant types. The sensitivity of this organism to different types of surfactants serves as an ideal bioindicator of aquatic toxicity. It has been suggested that various environmental pollutants may cause oxidative stress in aquatic organisms (Livingstone, 2003). Only two anionic surfactants, LAS and PFOS, significantly increased planarian CAT activities in this study. On the other hand, there were no effects observed in planarian SOD activities or tissue concentrations of lipid peroxidation product exposed to eight surfactants at current concentrations used in this study. This result suggested that oxidative stress posed by eight surfactants might be negligible in planarians under the current test conditions. To the best of my knowledge, this is the first report showing the ChE activity inhibition by NP, PFOS and PFOA in aquatic animals *in vivo*. This result also supports the hypothesis that diverse classes of environmental pollutants can inhibit ChE activity in various organisms (Payne et al., 1996; Guilhermino et al., 1998, 2000a). Further mechanistic studies are needed to define how surfactants directly or indirectly change ChE activities at molecular basis. NP, PFOS and PFOA are ubiquitous environmental pollutants in the aquatic environment, therefore, the implication of ChE inhibition of NP, PFOS and PFOA on neurological and behavioral effects on aquatic animals warrants further investigation.

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