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Acute Toxicity of Alkylpolyglucosides to *Vibrio fischeri*, *Daphnia magna* and Microalgae: A Comparative Study

E. Jurado · M. Fernández-Serrano · J. Núñez Olea · M. Lechuga · J. L. Jiménez · F. Ríos

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Abstract In this paper, toxicity values of alkylpolyglucosides have been determined by applying the 24-h immobilization test with *Daphnia magna*, the LumiStox® 300 test which employs the luminescent bacteria *Photobacterium phosphoreum* and the test with *Selenastrum capricornutum*. Three alkylpolyglucosides with different alkyl chain and degree of polymerisation have been tested. For all tests, the results indicated that *Vibrio fischeri* was more sensitive to toxic effects from alkylpolyglucosides than was *D. magna* or *S. capricornutum*. The results demonstrate considerable variation in toxicity responses within structurally related glucose-based surfactants regardless of the species tested. The toxicity increased as the critical micelle concentration decreased, and as the alkyl chain length and resultant hydrophobicity increased.

Keywords Alkylpolyglucosides · Non-ionic surfactants · Aquatic toxicity · Risk assessment

Alkylpolyglucosides (APGs) are prepared on the basis of renewable raw materials, namely (starch/sugar) and fatty alcohols (vegetable oils). They belong to the group of non-ionic surfactants and can be described in terms of an acetal structure (Fig. 1), with R being fatty-alcohol radicals of 8–16 carbons atoms and DP the average number of glucose units per alkyl radical, ranging between 1 and 2. The most favourable property of APGs is excellent skin compatibility under typical use conditions, leading to extensive use as a sole source and/co-surfactant in formulations for consumer products (Messinger et al. 2007). Their application properties make them useful surfactants for laundry and dishwashing detergents, cleaning products, cosmetic preparations and food technology. Thus, they have the potential for wide distribution in the aquatic environment. The environmental-risk assessment of surfactants is fundamental, given that they are high-volume chemicals, which are broadly disposed of in riverine environments as components of treated wastewaters (Belanger et al. 2000).

Surfactants are one of the most important components in laundry and household cleaning products, comprising from 15% to 40% of the total detergent formulation (Scheibel 2004). After use, residual surfactants and their degradation products are discharged to sewage treatment plants or directly to surface waters, then dispersed into different environmental compartments. Due to their widespread use and high consumption, surfactants and their degradation products have been detected at various concentrations in surface waters, sediments and sludge-amended soils (Ying 2005). The surfactants are required to be as inocuous as possible for the environment: low toxicity and easy biodegradation. The aspect of environmental impact of chemicals is governed mainly by their ecotoxicity, which is relatively high in the case of surfactants because of their surface activity and the resulting action against biological membranes (Steber et al. 1995). Surfactant ecotoxicity typically increases logarithmically with a linear increase in alkyl chain length (Boeije et al. 2006).

Many types of bioassays, in which the test organism includes representatives from micro-organisms, plants, invertebrates and fish, are available to establish the toxicity levels of compounds for aquatic organisms, but many of

E. Jurado · M. Fernández-Serrano (✉) · J. Núñez Olea · M. Lechuga · J. L. Jiménez · F. Ríos
Department of Chemical Engineering, Faculty of Sciences, University of Granada, Campus Fuentenueva s/n, 18071 Granada, Spain
e-mail: mferse@ugr.es

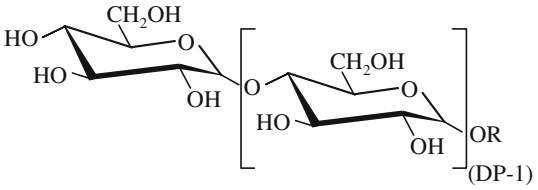

Fig. 1 Structure of alkylpolyglucosides

these tests are also time-consuming and not routinely applicable because they are not simple to use, expensive and without reliable analytical strategies. Moreover, the use of higher organisms as test species may also be ethically undesirable. There is a need to replace acute toxicity tests on fish with more effective assays. Although several bioassays using microorganisms have been described, most of the bacterial screening tests have been based on luminiscence measurements, because in this way they are rapid, reproducible, and simple to use, they cause no ethical problems, and they are cost-effective (Farré et al. 2001). The characteristics of speed, reliability, and normalization of the toxicity results by bioassays with luminescent bacteria make them ideal for gathering data on toxicity, which can be compared and statistically studied for establishing correlations between toxicity and the chemical structure and/or different properties of the compounds assayed. Assays using luminescent bacteria are gaining wide acceptance for the quick and simple determination of the toxicity of chemical compounds in surface water and wastewater, as well as in extracts of solid matrices. This explains the fact that, together with the *Daphnia* assay, these are listed as approved "bioassays" to characterize toxic and dangerous wastes. *Daphnia magna* has proven to be a sensitive and simple laboratory model for predictive toxicity studies (Sandbacka et al. 2000).

The present study was conducted to advance our understanding of the relationships between toxicity and structural parameters in the field of surfactants, applying the ecotoxicity assay with luminescent bacteria, *D. magna,* and microalgae to different alkylpolyglucosides. The toxicity study with different organisms is also useful to identify the organism most sensitive to the toxic effects, so that limits for surfactants in surfaces waters can be established. On the other hand, the structure–toxicity relationship is useful to choose the most suitable surfactant in the design of detergent formulas.

Materials and Methods

The surfactants used in this study are the commercial alkylpolyglucoside Glucopone 650 EC (APG 650), Glucopone 600 CS UP (APG 600) and Glucopone 215 CS UP (APG 215) supplied by Kao Corporation S.A. (Tokyo, Japan). Table 1 shows the average degree of polymerization (DP), the number of C atoms in the alkyl chain (R), the mean percentages of main homologs, the critical micelle concentration (CMC) and the percentage of water in the surfactants. The rest of the reagents used were grade chemical quality and supplied by Panreac.

Three toxicity tests were undertaken: the LumiStox® 300 test which employs the luminescent bacterium *Photobacterium phosphoreum*, the 24-h inmobilization test with *D. magna* (freshwater crustacea), and the 72-h algal growth inhibition test with *Selenastrum capricornutum*.

In the first one, measurements were taken with the measuring system LumiStox® 300, which consists of an instrument for measuring bioluminescence and an incubation unit according to the UNE–EN ISO 11348-2 guideline (UNE-EN ISO 11348-2). The toxicity measurement is based on the luminous intensity of marine bacteria of the strain *Vibrio fisheri* NRRL-B-11177 after a certain exposure time to a toxic substance. The luminescent bacteria, dehydrated and frozen at −18°C, were reactivated with the suspension supplied by Dr. Lange. The assay conditions were pH 7.0 and a NaCl concentration of 2%. Duplicate measurements were taken for incubation times of 15 and 30 min. The toxicity value was measured as EC_{50} or EC_{20}, which are, respectively, the surfactant concentrations that inhibit 50 and 20% after 15 and 30 min of exposure. When necessary, the sample was filtered prior to the assay.

Acute toxicity tests with *D. magna* were performed in Standard Reference Water (SRW) according to the UNE–EN ISO 6341 guideline (UNE-EN ISO 6341). The tests were performed in 100 mL polystyrene vessels, with 50 mL of SRW in each one. Twenty neonates (<24 h) were transferred to vessels containing different concentrations of the test chemical, and the vessels were closed with a polyethylene cap. The neonates were separated from adults every day. There was no feeding and no aeration during the tests and the tests were run at 20 ± 1°C. Immobility was determined visually after 24 h. For each alkylpolyglucoside, controls and at least five concentrations were used for the determination of the mobility inhibition of 50% of the *Daphnia* population (IC_{50}).

A 72-h algal growth-inhibition test with the microalga *S. capricornutum* was administered according to the OECD 201 guideline (OECD 1984). The procedure consists of filling culture vials with appropriate volumes of nutrient medium and solutions of the surfactant being tested. At the beginning of the test, inocula of algae were added to the test and control vials, and were kept under stable and predetermined incubation conditions.

Inocula were cultivated at 25 ± 1°C and constant uniform illumination (8000 lux). After 24, 48, and 72 h the algal density was determined to establish whether growth

Table 1 Description and properties of the surfactants employed in the tests

Commercial name	Structure (Bravo et al. 2005)	Mean percentages of main homologs (Bravo et al. 2005)	CMC, g/L (30°C)	% Water
GCP 215	R: 8–10; DP: 1.42	C_8 (47.1), C_{10} (36.5), C_{12} (9.9)	1.012	37.0
GCP 600	R: 12–14; DP: 1.59	C_8 (17.2), C_{10} (20.0), C_{12} (17.6) C_{14} (31.4) C_{18} (4.3)	0.050	46.6
GCP 650	R: 8–14; DP: 1.35	C_8 (33.5), C_{10} (25.7), C_{12} (12.7) C_{14} (23.3) C_{18} (3.7)	0.153	50.4

had been inhibited or stimulated with respect to control. Cell density was estimated by measuring optical density of the culture at 670 nm.

For all the tests, the surfactant concentration and one control were performed in triplicate for each organism tested.

Results and Discussion

Nominal and actual concentrations of the surfactant in the toxicity tests are shown in Table 2. The surfactant concentration in the aquatic bioassays at the beginning and at the end of the tests have been measured using the anthrone analysis (Buschmann et al. 1995). The aim of these measurements is to ensure that the test organisms do not use the surfactants as sources of carbon and that adsorption to glassware, adsorption to the test organism, and the biodegradation of test materials could be disregarded during the test period. Measured concentrations of surfactants agreed closely with nominal concentrations in the test with *V. fischeri* (Table 2). The greatest deviation between nominal and measured concentrations was with GCP 600, where a difference of 34% occurred in the test with microalgae at time 0. Nominal values were used to calculate EC_{50} and IC_{50} values.

For the LumiStox® system, the initial values of luminous intensity measured were corrected by a factor that takes into account the natural decrease in luminous intensity, even in the absence of the toxic sample:

$$\mathrm{fk} = \frac{I_t(0)}{I_0(0)} \tag{1}$$

with $I_0(0)$ and $I_t(0)$ being the readings of luminous intensity in the well containing concentration 0 at time 0 and t.

The percentage of inhibition (inhibitory effect) was calculated by the expression:

$$H_t = \frac{(I_{0t}(c) - I_t(c))}{I_{0t}(c)} 100 \tag{2}$$

where

$$I_{0t}(c) = \overline{\mathrm{fk}}\, I_0(c) \tag{3}$$

with $\overline{\mathrm{fk}}$ being the average correction factor of the control samples, and $I_0(c)$ and $I_t(c)$ being readings of light intensity in the well containing concentration c at time 0 and t.

The Gamma function, the ratio between the light intensity lost by the bacterial solution and that remaining after exposure to the toxic sample, can be determined by the equation:

$$\Gamma_t = \frac{\overline{H}_t}{100 - \overline{H}_t} = \frac{f_k \cdot I_0(c) - I_t(c)}{I_t(c)} \tag{4}$$

From the results, a linear relationship can be deduced between the function Γ and the concentration of the surfactant used, in the following form:

Table 2 Nominal and actual concentrations of surfactants in toxicity tests

Surfactant	*Vibrio fischeri*	*Daphnia magna*	*S. capricornutum*
Nominal test concentrations in mg/L			
GCP 215	50	200	1,500
GCP 600	50	100	100
GCP 650	50	60	100
Actual test concentrations (95% CI) in mg/L			
GCP 215	t = 0; c = 49 (±1.8)	t = 0; c = 170 (±3.8)	t = 0; c = 1,140 (±21)
	t = 30 min; c = 51(±0.9)	t = 24 h; c = 173 (±1.9)	t = 72 h; c = 1,160 (±115)
GCP 600	t = 0; c = 51(±1.3)	t = 0; c = 92 (±8.3)	t = 0; c = 66 (±4.7)
	t = 30 min; c = 52(±2.1)	t = 24 h; c = 83 (±2.9)	t = 72 h; c = 77(±0.9)
GCP 650	t = 0; c = 50 (±0.9)	t = 0; c = 52 (±2.3)	t = 0; c = 107 (±1.8)
	t = 30 min; c = 51(±1.7)	t = 24 h; c = 52 (±1.1)	t = 72 h; c = 103 (±2.3)

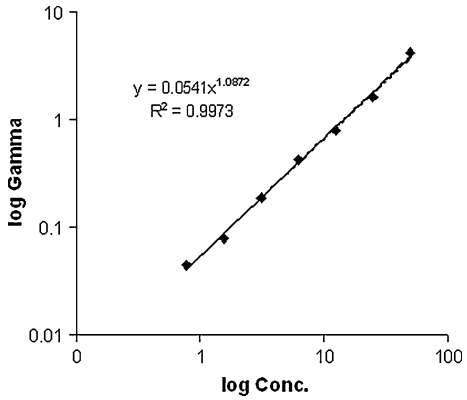

Fig. 2 Linear relationship between the function Γ and concentration according to Eq. 5

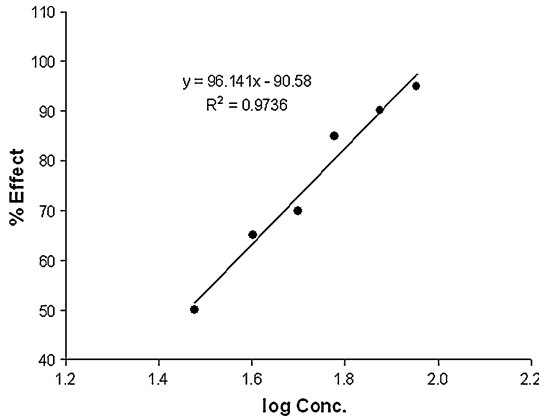

Fig. 3 Linear relationship between the % effect and concentration for the tests with *Daphnia magna*

$$\log(c) = b \cdot \log(\Gamma) + \log(a) \quad (5)$$

An example of the linear relationship is provided for GCP 650 (Fig. 2). The values of EC_{20} and EC_{50}, expressed as mg/L, are the concentrations of surfactant that inhibit 20 and 50%, and are calculated, giving Γ values of 0.25 and 1, respectively. Table 3 shows the results for the different surfactants, in decreasing order of toxicity, for incubation times of 15 and 30 min.

The 24 h IC_{50} values for the tests with *D. magna* were calculated using linear-regression analysis after transformation of dose–response curves by logarithmic transformation of the concentrations (Fig. 3). Table 4 shows (in decreasing order of toxicity) the 24-h IC_{50} values for the tests with *D. magna,* for the different surfactants tested. The smallest concentrations assayed that immobilized all *D. magna*, and highest concentrations assayed that immobilized no *D. magna,* are also shown in Table 4.

EC_{50} values for the tests with microalgae were calculated using linear regression analysis based on the dose-response curves (Fig. 4). Table 5 shows (in decreasing order of toxicity) the EC_{50} values for the tests with microalgae, for the different surfactants tested.

The results presented in Tables 3, 4, 5 and Fig. 5 show that *V. fischeri* was more sensitive to toxic effects from alkylpolyglucosides than either *D. magna* and *S. capricornutum*. Similar results are found in the literature. For example, García et al. (1997) conducted toxicity tests with both *D. magna* and luminescent bacteria for three alkylpolyglucosides (with an alkyl chain range of C_8–C_{16} and degree of polymerization 1.3–1.4) and found that the luminescent bacteria was more sensitive than *Daphnia* to the toxic effects of APGs. However, literature also shows contradictory results. Li and Schröeder (Li and Schröeder 2000) found that *D. magna* was more sensitive than *V. fischeri* to the toxic effects from surfactants, although these authors show global results for alkylpolyglucosides and do not indicate the test conditions. Our results also indicate that although *V. fischeri* is the most sensitive organism, it is the least discriminative. Therefore, a battery of toxicity tests including species such as *D. magna* and *S. capricornutum* may be desirable to evaluate relative toxicities amongst different surfactants.

Among the group of surfactants with this alkyl chain length, alkylpolyglucosides have favourable aquatic toxicity to fish, bacteria, algae, etc. (Willing et al. 2004). Nonylphenol ethoxylate (NPEO) and linear alkylbenzene sulphonate (LAS) showed higher toxicity values than alkylpolyglucosides using *D. magna* and *V. fischeri* (Lewis, 1991), although more recently researchers have indicated that these environmentally friendly labels are slightly toxic, but more toxic than betains, alkylether carboxylates, and even fatty acid polyglycol amines (Li and Schröeder 2000). Karpińska-Smulikowska and Moskal (2004) tested non-ionic phosphoorganic surfactants with *D. magna* and *Paramaecium caudatum*. The 48-h EC_{50} values were 30–760 mg/L for *P. caudatum* and 14–133 mg/L for

Table 3 Acute toxicity data (95% CI) for alkylpolyglucosides for the tests with *Vibrio fischeri* (values of EC_{50} and EC_{20} in mg/L)

Surfactant	EC_{20} (15 min)	EC_{50} (15 min)	EC_{20} (30 min)	EC_{50} (30 min)
GCP 650	3.9 (±0.2)	14 (±0.8)	4 (±0.7)	14 (±0.6)
GCP 600	7.5 (±0.8)	13 (±0.7)	7.3 (±1)	20 (±0.7)
GCP 215	10 (±1)	29 (±0.9)	6.2 (±0.9)	25 (±0.4)

Table 4 Acute toxicity data (95% CI) for alkylpolyglucosides for the tests with *Daphnia magna* (values of IC_{50} in mg/L)

Surfactant	IC_{50}	Smallest concentration that immobilizes all *Daphnia magna* (mg/L)	Highest concentration that immobilizes no *Daphnia magna* (mg/L)
GCP 650	29 (±2.4)	85	10
GCP 600	14 (±1.3)	65	5
GCP 215	111 (±1.2)	192	70

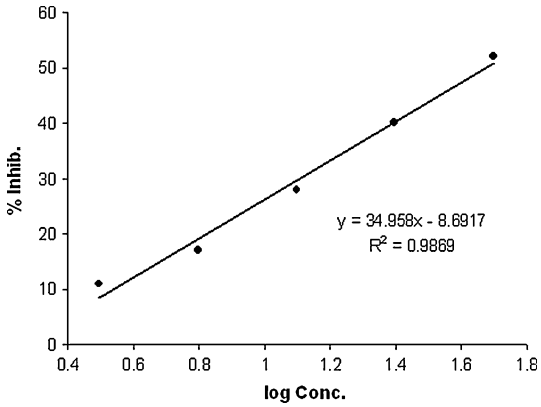

Fig. 4 Linear relationship between the % inhibition and concentration for the tests with *Selenastrum capricornutum*

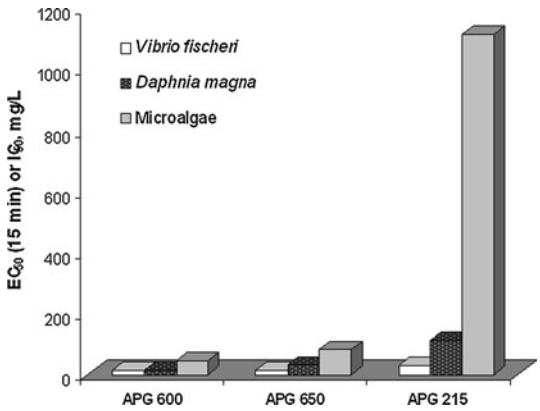

Fig. 5 Determination of alkylpolyglucoside toxicity using *Daphnia magna, Vibrio fischeri*, and *Selenastrum capricornutum*

D. magna, depending on the kind of compound. For alcohol ethoxylates, acute (48 h) tests with *D. magna* showed toxicities values from 0.4 to 0.7 µmol/L (Morrall et al. 2003).

Toxicity (i.e., 15-min EC_{50} values were between 14 and 29 mg/L for the tests with *V. fischeri* (Table 3). The most toxic was the alkylpolyglucoside GCP 600 with the greater alkyl chain length (more hydrophobic), and the least toxic was GCP 215 with the shortest alkyl chain. Similar trends were observed for the differences in toxicities between the short and longer alkyl chain GCPs in the tests with *D. magna* and *S. capricornutum* (Tables 4, 5; Fig. 5), where IC_{50} values of 14 and 111 mg/L were obtained for *D. magna* with GCP 600 and GCP 215, respectively; and EC_{50} values of 81 and 1,110 mg/L for *S. capricornutum* with GCP 650 and GCP 215, respectively. These results agree with those of Morrall et al. (2003) and García et al. (1997), who found that the more hydrophobic alkylpolyglucosides—those with the longest carbon chains for those tested between C_9 and C_{16}—were more toxic toward *D. magna* and *V. fischeri*. The30-min EC_{50} values ranged from 7 to 16 mg/L in those studies. Other studies have also shown that homologues of alkylpolyglucosides with longer alkyl chains present higher ecotoxicity values (Steber et al. 1995; Uppgård et al. 2000).

Table 5 Acute toxicity data (95% CI) for alkylpolyglucosides for the tests with *Selenastrum capricornutum* (values of EC_{50} in mg/L)

Surfactant	EC_{50}
GCP 600	46 (±7.5)
GCP 650	81 (±7)
GCP 215	1,110 (±28)

Our tested materials are located in the region of solubility in water, considering previous studies by Jurado et al. (2008) in a binary system containing GPC 650 and water. A micellar solution first appeared at a surfactant concentration of 55 wt%. Similar behaviour was reported for GCP 215 and GCP 600 (65 wt% and 54 wt%, respectively), Bravo et al. 2007. So, we can state that toxicity is below the limit of solubility for the materials tested.

Apparent reduced toxicity would be observed if the EC_{50} ocurred above the critical micelle concentration of the surfactants. However, available CMC values indicate that observed toxicity values of the APGs occurs below the corresponding CMC of the test materials, while similar findings have been reported with linear alkylbenzene sulphonates and ester sulphonates (Hodges et al. 2006).

From the results, we conclude that *V. fischeri* is the most sensitive of the three species tested. For all three test species, GCP 215 was found to be the least toxic surfactant. GCP 600 was slightly more toxic than GCP 650 for *D. magna*, while the reverse was true for *V. fischeri.* The latter two surfactants were 1.3 and 2.6 times more toxic than GCP 215 for *V. fischeri*. Of the three types of toxicity tests, the test with *S. capricornutum* was the least sensitive for all three alkylpolyglucosides.

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