Journal of Environment and Earth Science
ISSN 2224-3216 (Paper) ISSN 2225-0948 (Online)
Vol.6, No.6, 2016

Acute Toxicity of Palm-Based Methyl Ester Sulphonates (MES) towards Daphnia magna

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Abstract
Palm-based MES is an anionic surfactant derived from palm oil through sulphonation of fatty acid methyl esters. It has good surface-active properties and biodegradability, excellent detergency and is less sensitive to water hardness. It has been used in powder and liquid detergent formulations and other cleaning applications. The evaluation of MES in aquatic ecosystems is vital as they are discharged in large volumes into the environment. An attempt was made to evaluate the acute effect of palm-based MES on Daphnia magna through acute immobilisation studies, OECD 202 (48 hour EC_{50}). MES of various chain lengths were selected for the investigation. Based on the results, the EC_{50} of MES (based on % immobilization) decreased (became more toxic) with increasing carbon chain length of the surfactant. This trend was also observed in many other anionic surfactants. MES is well suited for environmentally friendly detergent due to its good biodegradability and its toxicity will not pose any environmental effect on aquatic organisms. The present study provides relevant data concerning the effects of MES surfactant on freshwater invertebrate, which are useful to establish water quality criteria in a regulatory framework.
Keywords: palm-based surfactant; acute immobilization test; freshwater invertebrate; OECD; REACh

1. Introduction

The need to protect the aquatic and terrestrial biota from uncontrolled releases of pollutants has gradually triggered the development of methods capable of evaluating the adverse effects of chemicals over the past few decades. In order to evaluate the risks and effects of chemicals in the environment, a number of standardized test methods have been developed within the EU (European Chemicals Bureau, 2010) and the US (ASTM, 2011). The objective is to identify and assess any adverse effects that chemicals may have and to estimate relationships between exposure and severity of effects (European Chemicals Bureau, 2010). The OECD (Organisation for Economic Co-operation and Development) for example, has developed a collection of guidelines for testing of chemicals. These guidelines are currently being used by governmental agencies, industries and independent laboratories (OECD, 2011).

Surfactants are used for a variety of purposes but primarily in commercial detergents, personal care and household cleaning products. They were originally made from renewable resources, but today most of them are of petrochemical origin. Still, renewables have not entirely lost their importance, and are in fact regaining popularity due to their sustainability, good cost-performance ratio and environmentally friendliness.

A technology to produce an anionic surfactant, methyl ester sulphonates (MES), from palm oil is available through conversion of the oil to methyl ester, followed by hydrogenation to reduce the unsaturation and then sulphonating the ester to MES. It has good surface-active properties and biodegradability, excellent detergency and is less sensitive to water hardness (Salmiah *et al.*, 1998; Razmah *et al.*, 2004, 2006). MES has been manufactured in large scale all over the world. *Table 1* shows the global MES producers as reported by Zulina (2013).

Table 1. Global MES producers and their production capacity in 2012

Company	Location	Annual capacity (MT)
Lion Eco Chemicals Sdn. Bhd.	Malaysia	25,000
KL Kepong Oleomas Sdn. Bhd.	Malaysia	50,000
Guangzhou Keylink Chemical Co.	China	40,000
Stepan	USA	50,000
USA Huish Detergent Inc.	USA	80,000
Dersa, Bogota	Colombia	15,000
Lion Corporation	Japan	40,000
Zhejiang Zanyu Technology Co. Ltd	China	60,000
Guangzhou Langqi	China	36,000
Shandong Zoupingfuhai	China	30,000
Shandong Jinlun	China	60,000
Jiangsu Haiqing	China	100,000
PT Wilmar Nabati, Gresik	Indonesia	50,000

Source : Zulina (2013)

The use of MES in detergents started in the early 1990s in Japan (Masuda *et al.*, 1994; Masuda, 1995). Huish is producing MES from palm oil and using it in commercial laundry detergents such as Costco Kirkland Brand Select Ultra and Safeway Select Ultra. The Safeway Select Ultra II has the highest level of MES, *i.e.* about 23.5% of the total formulation (Zulina *et al.*, 2006).

Due to their widespread use, surfactants have become common constituents in municipal effluent and river water. Surfactants in surface water have become an environmental concern and, as a consequence, toxicity data on their effects on freshwater and marine life have been gathered since the early 1950s (Lewis, 1992).

The aquatic ecotoxicity of a substance can be measured by many different methods. Basically, aquatic organisms are exposed to the substance in a number of concentrations over a period of time. For example, a method using fish as test species, exposing the fish to the substance over 96 hours and determining an LC_{50}-value, *i.e.* the concentration where 50% of the fish dies (LC=lethal concentration). Alternatively, one can determine sub-lethal effects. An example might be testing for immobility of *Daphnia magna*, where the concentration that causes immobility of 50% of the organisms can be determined in a similar way. In this case, the effect value is called an EC_{50}-value (EC=effect concentration).

One of the most internationally used bioassays for toxicity screening of chemicals is the acute toxicity test with *Daphnia magna* (*D. magna*). *D. magna* is commonly used because of its suitability for laboratory testing such as relatively small, short life cycle, parthenogenetic reproduction, high fecundity, ubiquitous occurrence and cycle, and the fact that they are relatively easy to culture and maintain in the laboratory (Gopi *et al*., 2012). Test protocols for undertaking acute toxicity tests with *D. magna* have been described in scientific literature since 1960s (Persoone *et al*., 2009).

It is important to evaluate the ecotoxicity of MES at various trophic levels since, nowadays, in addition to excellent performance, good economic prospects and sustainability, a product must also fulfil the ecological requirements in order to be accepted worldwide. The ecotoxicity of palm-based MES towards organism at higher trophic levels in the aquatic ecosystem has been studied *via* fish acute toxicity test and has been reported by Razmah and Salmiah (2004) and Razmah *et al.* (2006). This paper will discuss the acute toxicity of palm-based MES towards organism at a lower trophic level, *i.e. D. magna*.

2. Materials and Methods

2.1 Test organism

D. magna primary culture was obtained from the Fisheries Research Institute (FRI), Glami Lemi, Negeri Sembilan, Malaysia. The original stock culture was brought back from Ghent, Belgium. The culture was maintained in the test facility by periodical culturing. The medium was changed twice in a week and was also checked for parameters such as pH, temperature and dissolved oxygen.

At the start of the test, the daphnids used were less than 24 hours old and, to reduce variability, only the third brood progeny was used. ISO 6341 (2012) even imposes that the test organisms should be at least third generation offspring. The daphnids were derived from a healthy stock (*i.e.* showing no signs of stress such as high mortality, presence of males and ephippia, delay in the production of the first brood, discoloured animals, *etc*.). All organisms used for a particular test were controlled to ensure they originated from cultures established from the same stock of daphnids. The stock of *D. magna* was maintained in culture conditions (light, temperature, medium) similar to those to be used in the test.

2.2 Test and reference substances

Test substances were palm-based MES of various chain lengths (C12, C14, C16, C16/18:60/40) produced from palm stearin methyl esters in the Malaysian Palm Oil Board (MPOB)'s MES pilot plant and commercial MES (C16/18:80/20) obtained from MES producer in Malaysia. The active in each sample was more than 80 %.

A simple way to get an estimate of the health and the sensitivity of test organisms is to perform tests on reference chemical. Potassium dichromate ($Cr_2K_2O_7$) used as the control/reference chemical was procured from Merck KGaA, Germany.

2.3 Mineral salts for preparation of holding and dilution water

ISO medium (OECD 202, 2004) was used as holding and dilution water since the daphnids survived in it for the duration of the culturing, acclimation and testing without showing signs of stress. ISO medium was made up by adding specific amounts of mineral salts of analytical grade to deionised water (*Table 2*). All chemicals were purchased from Merck KGaA, Germany.

Table 2. Preparation of ISO medium

Stock solutions (single substance)		To prepare the ISO medium, add the following volumes of stock solutions to 1 L water
Substance	Amount added to 1 L water	
Calcium chloride, $CaCl_2.2H_2O$	11.76 g	25 ml
Magnesium sulphate, $MgSO_4.7H_2O$	4.93 g	25 ml
Sodium bicarbonate, $NaHCO_3$	2.59 g	25 ml
Potassium chloride, KCl	0.23 g	25 ml

Source : OECD 202 (2004)

The pH was maintained in the range of 6-9 while hardness between 140-250 mg L^{-1} (as $CaCO_3$).

2.4 Test method

Acute ecotoxicity tests were conducted to assess the effects of palm-based MES towards *D. magna* and to determine the 48-hour EC_{50} value of MES according to standard method OECD 202 (2004), *Daphnia* sp., Acute Immobilization Test. The young daphnids are exposed to the test substance at a range of concentrations for a period of 48 hours. In acute test, the measured parameter is immobilization, *i.e.* the inability of daphnids to resume swimming within 15 second after gentle agitation. Immobilisation is recorded at 24 hours and 48 hours, and compared with control values.

2.5 Conditions of Exposure

2.5.1 Test solutions

A series of test solutions of the chosen concentrations were prepared by dilution of a stock solution. Stock solutions of palm-based and commercial MES were prepared by dissolving 100 mg of the test substance in 1000 mL of the ISO medium. The tests were carried out without the adjustment of pH (6-9).

2.5.2 Test groups and controls

Test beakers (100 mL glass beakers) were filled with 50 mL of ISO medium and solution of test substance. One ISO medium control series was run in addition to the treatment series.

Range-finding tests were conducted to determine the range of concentrations for the definitive tests. Twenty daphnids, divided into four groups of five daphnids each, were used at each test concentration and for the controls. About 2 mL of test solution was provided for each daphnid (*i.e.* a volume of 10 mL for five daphnids per test beaker). For this purpose, the daphnids were exposed to a series of widely spaced concentrations of the test substance (logarithmic series), *i.e.* 0.0, 0.1, 1.0, 10.0 and 100.0 mg L^{-1}. The daphnids were exposed to each test concentration for 24 hours without any replicates. The test beakers were loosely covered to reduce the loss of water due to evaporation and to avoid the entry of dust into the solutions.

Based on the results obtained from the range-finding test, five test concentrations were used in definitive tests, arranged in a geometric series with a separation factor not exceeding 2.2. The highest concentration tested should results in 100 % immobilisation and the lowest concentration tested should preferably give no observable effect. Ten daphnids were exposed to each test concentration and control for 48 hours. These tests were conducted in triplicates.

2.5.3 Incubation conditions

The temperature of the incubator was set at 20±2 ^{0}C. A 16-hour light and 8-hour dark cycle was used. The test vessels were not aerated and the daphnids were not fed during the test.

2.6 Test Procedures

2.6.1 Sensitivity test

The sensitivity of the assays is intrinsically dependent on the experimental abiotic and biotic factors, which are selected for the culturing and the testing, including the *D. magna* strain, and the type and nutritional value of the algal food. The data on potassium dichromate is an important prerequisite for test laboratories since it is used to validate the test.

An acute immobilisation test was conducted to determine the 48 hours EC_{50} of potassium dichromate. The study was performed with nominal concentrations of 0.0, 0.1, 0.2, 0.4, 0.8 and 1.6 mg L^{-1}. Three replicates were used for each concentration and each containing 5 daphnids (less than 24 hours old) in 50 mL of ISO medium. The test was performed at 20±2 ^{0}C with a photoperiod of 16 hours light and 8 hours dark. During the experiment, daphnids were not fed. In each test beaker, the immobilised daphnids were recorded at 24 hours of exposure.

2.6.2 Acute toxicity test

For each test concentration and control, three replicates were used and each containing 10 daphnids (less than 24 hours old) in 50 mL of ISO medium. Each test beaker was checked for immobilised daphnids at 24 and 48 hours after the beginning of the test. Immobilised daphnids were removed immediately when observed. Not more that 10 % of the daphnids should have been immobilised in the control for the test to be valid. The percent

immobilisation was calculated as follows,

% Immobilisation = (No. of daphnids immobilised / No. of daphnids exposed) ×100

The EC_{50} value is the concentration that immobilised 50 % of the daphnids within a stated exposure period and is calculated *via* probit analysis using the SPSS (Statistical Package for the Social Sciences) program with 95% confidence limits.

2.6.3 Analytical measurements

The dissolved oxygen, pH and temperature were measured daily in each test and control beaker. The dissolved oxygen concentration at the end of the test should be ≥ 3 mg L^{-1} in control and test beakers. The pH should not vary by more than 1.5 units in any one test. In this study, the results of the tests were expressed on the basis of the nominal concentration.

3. Results and Discussion

3.1 Sensitivity test

As emphasized in all guidelines and norms on standard acute toxicity tests, "sensitivity" and "precision" are the two major key factors for the evaluation of credibility of the test results at the intra-laboratory as well as at the inter-laboratory level. A simple way to get an estimate of the health and the sensitivity of test organisms is to perform tests on reference material such as potassium dichromate. As indicated in many publications, reference testing on particular compounds also serves as the best tool for determining the "precision" of toxicity tests, *i.e.* the closeness of agreement between test results.

Sensitivity test was performed on $K_2Cr_2O_7$ for which an acceptability range of 0.6–2.1 mg L^{-1} has been set in standard ISO 6341(2012) for the 24 h EC_{50} of the acute *D. magna* assay. The value obtained in the laboratory (0.83 mg L^{-1}) was within the sensitivity range set by ISO 6341 standard (*Table 3*). This confirmed the assay sensitivity and can be used for testing.

Table 3. Number of immobilized daphnids at different $K_2Cr_2O_7$ concentrations and the EC_{50} values after 24 h observation

Concentration of $K_2Cr_2O_7$ (mg L^{-1})	**No. of immobilized daphnids* (24 h)**	**EC_{50} (mg L^{-1})****
0.0	0	
0.1	0	
0.2	0	0.83
0.4	0	
0.8	8	
1.6	20	

*3 replicates, 5 daphnids /replicate

**With 95% confidence limit, calculated *via* probit analysis

3.2 Ecotoxicity tests

The acute tests have been conducted on palm-based C12, C14, C16 and C16:18 MES produced in MPOB, and commercial MES (C16:18). The highest concentration tested in the range-finding test, *i.e.* 100 mg L^{-1}, showed none of the daphnids were immobilized within 24 hours of test duration when tested on C12 MES. This shows that the 24 h EC_{50} value for this sample is higher than 100 mg L^{-1}, and according to the test method, no further test (definitive test) need to be conducted since the sample can be considered as non-toxic. However, if 10 % immobilization is observed at the end of the test, a full study has to be conducted. For other MES samples, immobilization was observed at various concentrations, therefore definitive test need to be conducted.

In the definitive test, 100 % immobilisation was recorded at the concentrations of 160.0 mg L^{-1} for C14 MES (*Table 4*), and 1.6 mg L^{-1} for C16 MES, C16:18 MES and commercial MES (*Table 5*). The concentration of C14 MES at 40.0 mg L^{-1} and concentration of C16 MES, C16:18 MES and commercial MES at 0.4 mg L^{-1} did not affect the mobility of the *D. magna*. The EC_{50} (48 h) of C14, C16, C16:18 and commercial MES is 77.6 mg L^{-1}, 1.15 mg L^{-1}, 0.77 mg L^{-1} and 0.76 mg L^{-1} , respectively, with 95% confidence limits (*Table 6*).

Table 4. Percent immobilization of daphnids at different C12 and C14 MES concentrations

Concentration* (mg L^{-1})	Immobilization (%) (48 h)
0.0	0
20.0	0
40.0	0
80.0	36.7
160.0	100

*3 replicates, 10 daphnids /replicate

Table 5. Number of immobilized daphnids at different MES concentrations

Concentration (mg L^{-1})	Immobilization (%) (48 h)		
	C16 MES	C16:18 MES	Commercial MES
0.0	0	0	0
0.2	0	0	0
0.4	0	0	13.3
0.8	0	60	53.3
1.6	100	100	100

*3 replicates, 10 daphnids /replicate

Table 6. Data on 48 h EC_{50} (with 95% confidence limits) of MES samples

Result	C12 MES	C14 MES	C16 MES	C16:18 MES	Commercial MES
EC_{50} (mg L^{-1})	> 100.0	77.6	1.15	0.77	0.76
95% confidence limits (mg L^{-1})	-	65.8 – 96.8	0.99 – 1.39	0.68 – 0.91	0.67 – 0.90

The effect of the hydrophobic carbon chain length on the toxicity of MES was also studied. Data from *Table 6* indicated that MES with the shorter chains were less toxic than the longer ones. This trend was also observed in many other surfactants. Generally, the longer the chain, the higher was the toxicity. This observation is in tandem with observation made by other researchers with most anionic surfactants in which, the toxicity increases with the chain length so long as there is sufficient solubility.

Previous ecotoxicity studies conducted using fish as test species (Razmah and Salmiah, 2004) also showed increased in toxicity values of MES with an increased in carbon chain length. The acute toxicity of surfactants towards aquatic organisms is not class compound-specific but material- and structure-specific. For anionic surfactants, the aquatic toxicity depends mainly on the length of the carbon chain in the molecule. A certain dependency of the toxicity on the chain length of the alkyl group has been observed in the homologues of alkyl sulphate and alkylbenzene sulphonates (Schoberl *et al.*, 1988; Potokor, 1992; Fendinger *et al.*, 1994). However, a systematic dependence of the toxicity on the chain length is only recognizable in fully water-soluble compounds.

Palm-based MES however, is not expected to cause environmental concern because its high biodegradability will leave very little residual and therefore is not toxic to water organisms. Previous study on biodegradation (Masuda, 1995) showed that MES samples were mineralized by microorganisms and gradually disappeared in environmental surface water, such as river water. Razmah and Salmiah (2004) found that palm-based MES was readily biodegradable in the OECD 301D Closed Bottle Test with more than 80% degraded in only eight days. The MES exists primarily in the ionized form at environmental pHs. Due to its ionized properties, it can be assumed that bioaccumulation is insignificant.

4. Conclusion

Public concern for the safety of products to the user and environment and for the conservation of natural resources is at an all-time high. Palm-based MES may help to meet the needs for environmental safety. It is a good and inexpensive active ingredient derived from renewable resources, which can be used in detergent formulations *in lieu* of the current petrochemical products. Less MES is needed for the same detergency as the conventional surfactants, thus lowering the organic load in wastes discharged to the environment.

The length of the carbon chain affected the toxicity of palm-based MES where the longer the chain, the higher is the toxicity. However, due to their rapid biodegradation in the environment, palm-based MES will not pose any environmental effect on aquatic organisms. MES is thus well suited for environmentally friendly detergent due to its good biodegradability and toxicity comparable to the current, high volume anionic surfactants, such as LAS and SLS. The time seems ripe for MES to be used in cleaning products to fulfil the social responsibility of the detergents industry to a cleaner and better environment.

5. Acknowledgements

The authors would like to thank the Director-General of MPOB for permission to publish this paper and Mr Saiful Nizam Shamsudin for his technical assistance.

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