

A COMPARATIVE STUDY OF THE ECOTOXICITY OF PALM-BASED METHYL ESTER SULPHONATES (MES) TO TILAPIA AND *Daphnia magna*

RAZMAH, G*; SITI AFIDA, I*; ZULINA, A M*; NOORAZAH, Z* and HAZIMAH, A H*

ABSTRACT

Methyl ester sulphonates (MES) is a surfactant derived from plant resources, suitable as active ingredient in detergents. MES possesses good surface-active properties, good detergency and tolerant to water hardness. In this study, the ecotoxicity of MES was evaluated through the 48 hr Daphnia magna immobilisation test and the 96 hr fish acute toxicity test with Tilapia. MES samples with different alkyl chain lengths (C14, C16 and C16:18) produced by the Malaysian Palm Oil Board (MPOB) and commercial MES (C16:18) were tested. Results from all tests indicated that Daphnia was more sensitive to toxic effects from MES than was Tilapia. There is also significant difference in ecotoxicity responses for palm-based MES of various chain lengths regardless of the species tested. The ecotoxicity increased as the hydrophobicity of the MES increased due to increase of alkyl chain length. However, less than 30% of MES is used in detergent products and will not pose environmental effects on aquatic organisms. MES is therefore suitable for environmental compatible cleaning products in view of its ecotoxicity that is on par to the widely used anionic surfactants, such as linear alkylbenzene sulphonate (LAS). The use of MES in cleaning products may help the industry to fulfil its social responsibility to a cleaner and better environment.

Keywords: palm-based surfactant, acute immobilization test, freshwater invertebrate, fish; OECD; REACh.

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INTRODUCTION

A wide range of products and applications used by consumers and industry today contain surface-active agents, or shortly surfactants. Their use ranges from primary production processes to enhancing the quality of finished products. Therefore, surfactants are present in products such as motor oils, pharmaceuticals, cosmetics, detergents, and also in electronic printing, magnetic recording,

biotechnology, microelectronics and viral research (Rosen and Kunjappu, 2012). About 56% of the overall demand for surfactants in 2014 accounted for cleaners and detergents for household segments (Ceresana, 2015).

Surfactants contributed a global turnover of 33.2 billion USD in 2014. Ceresana, a market research institute, predicted the annual revenues of surfactants to increase by 2.5% per annum to around 40.4 billion USD until 2022. The growth of the global surfactants market within 2015 to 2020 is estimated at a CAGR of 5.3% by volume and 5.5% by value. In 2014, the global market, in terms of volume, is dominated by the anionic surfactants segment with 7.7 million tonnes and followed by non-anionic

* Malaysian Palm Oil Board,
6 Persiaran Institusi, Bandar Baru Bangi, 43000 Kajang,
Selangor, Malaysia.
E-mail: razmah@mpob.gov.my

surfactants with 6.3 million tonnes. Emerging markets especially in China, India and Brazil, as a result of new investments and projects, are predicted to boost the growth of surfactant market in the future (Marketsandmarkets.com, 2015).

Surfactants can be produced from both petrochemical (synthetic) and oleochemical (renewable) raw materials. The petrochemical feedstock is mainly derived from crude oil and converted to different surfactant intermediates, whereas oleochemical feedstock is commonly derived from plant oils (palm and coconut), plant carbohydrates (sorbitol, sucrose and glucose) and animal fats (tallow) (Holmberg *et al.*, 2003; Rust and Wildes, 2008).

Methyl ester sulphonates (MES) are anionic surfactants derived from palm oil (Masuda *et al.*, 1993; Salmiah *et al.*, 1998; Chemithon, 2008) and their utilisation as a surface active agent offers several advantages including renewability, biodegradability, dispersion ability, detergency in hard water, high calcium tolerance and an ability to maintain enzyme activity (Matheson, 1996). The use of MES in commercial detergents started in the early 1990s in Japan (Masuda, 1995).

The technology to produce MES involves converting palm oil to methyl ester, followed by hydrogenation and later sulphonation of the ester. The feedstock used to produce MES in Malaysian Palm Oil Board (MPOB) is palm stearin (Zulina *et al.*, 2006). Large companies, such as Lion Corporation (Japan), Stepan (USA) and Huish Detergent Inc. (USA), have been producing and using MES in their commercial cleaning products.

Zion Research (2014), in its latest report, highlighted the global market value and volume of MES was at 0.57 billion USD and 0.46 million tonnes, respectively. The market volume between 2015 and 2020 is projected to expand at CAGR of 18.6% to reach 1.58 billion USD by 2020 (Empowered News, 2015).

In 2014, Europe consumed over 40% of the MES market volume, followed by North America and Asia Pacific. It is projected that Asia Pacific will become the most rapid emergent market for MES in the future (Empowered News, 2015). According to Frost and Sullivan (2008), the market for MES is steadily growing, and more effort is being put to intensify its market potential by building product awareness. MES's exceptional potential to be used as environmental-friendly surfactant in detergents is seen as the driver for its growth. Its usage in this manner is beginning to be tapped due to the current demand for green and biodegradable raw materials in the surfactants market and this will eventually lead to potential high demand for MES.

Previously, the development of MES was stalled due to the lack of production facilities. Today, MES is gaining heightened interest as a result

of its numerous benefits. MES outcores linear alkylbenzene sulphonates (LAS), a petroleum-based surfactant and currently the workhorse in detergent industry, in many areas. These include high purity, high activity levels, the absence of any volatile organic compounds, low di-salt content, gentleness on the skin and suitability for both liquid and powder detergents. In 2014, more than 40% share in global MES volume was consumed by the household detergents market. Personal care market was the second largest user for MES and this area is projected to show the highest market growth for MES in the future (Empowered News, 2015).

There is an increase in environmental concern over the use of chemicals in large volume as these chemicals may pollute the environment depending on their exposure period, persistence in the environment, and the behaviour of the affected organisms (Cunningham and Saigo, 1990). One of these chemicals is surfactant, where due to its widespread use, can be a potential toxicant when a large quantity enters the environment. Aquatic toxicity and aquatic behaviour are of major importance for surfactants since they are mainly released into the environment via wastewaters (Hrenovic and Ivankovic, 2007).

Aquatic toxicity, or ecotoxicity, is the study of the effects of a material on aquatic organisms at various trophic levels, from subcellular through individual organisms to communities and ecosystems. It is evaluated through the response of the affected aquatic organisms to the material (APHA, 1980). Ecotoxicity tests have become an essential element in the holistic evaluation of aquatic pollution. Nowadays, the chemical and physical tests are no longer sufficient to assess the potential effects of the material on aquatic organisms. Information associating chemical structure and ecotoxicity are important in explaining the effect of surfactants on aquatic and other organisms. Knowing which physico-chemical properties of a surfactant influence its toxicity could point to surfactants with minimal biological effects. Synthesis of new surfactants could also be guided by these findings.

There are different techniques to measure aquatic toxicity, both standard and screening, and the endpoint is often a LC₅₀ (50% lethal concentration) or an EC₅₀ (50% effect concentration). As the knowledge about surfactants' toxicity grew, different methods to test were developed, some of which were standardised by the Organisation for Economic Co-operation and Development (OECD), for example acute toxicity test on algae, on *Daphnia magna* and on fish (OECD, 1992).

This article is aimed at extending the knowledge, particularly regarding the effects of palm-based MES, upon non-target freshwater invertebrates and fish. Thus, in the present study *D. magna* and Tilapia were used to test the acute effects of palm-

based MES, with the goal of comparing the acute toxicity of MES to organisms in two different trophic levels, leading to a discussion of the importance of evaluating the ecotoxicity behaviour of palm-based products in the aquatic environment and to demonstrate the environmental-friendliness of this surfactant.

MATERIALS AND METHODS

Materials

Palm-based MES (C14, C16 and C16/18:60/40) were manufactured using palm methyl esters in MPOB's MES plant. The palm stearin methyl ester feedstock is a typical sulphonation feedstock for use in laundry detergent formulations (Chemithon, 2008). Commercial palm-based MES (C16/18:60/40) was obtained from a local MES supplier and used for comparison. All samples contained more than 80% active content.

Methods

Fish acute toxicity test (OECD 203, 1992). The OECD 203 is a widely used test to evaluate the acute toxic effects of materials to aquatic organisms in short-term exposure and static conditions. The test method recommends seven freshwater fish to be used for testing, but, only *Cyprinos carpio* could survive in tropical freshwater.

Due to difficulty in obtaining this species locally, *Oreochromis niloticus*, or commonly known as Tilapia, a local freshwater fish, was used instead as it fulfilled most of the criteria for test species (APHA, 1980; OECD 203, 1992; Razmah and Salmiah, 2004; Razmah *et al.*, 2006), *i.e.* ready availability throughout the year, easily maintained, convenience for testing, commonly presence throughout the country and previous experience has confirmed its suitability for ecotoxicity testing as per the *OECD 203 Guidelines for Testing of Chemicals* (1992).

The fishes, (2 to 5 cm), supplied by a local fish supplier, were first acclimatised to the test conditions for a week. They were fed daily with commercial fish food. A day before the test, feeding was stopped and the fish were not fed during the bioassay.

The fish toxicity tests started with range-finding test (24 hr) and then followed by definitive test (96 hr). The range-finding test used concentrations in logarithmic series while the definitive test tested concentrations in geometric series. All concentrations were analysed in duplicate, including the control (without test substance), which was tested simultaneously (OECD 203, 1992; Razmah *et al.*, 2006).

The acute toxicity of a substance to fish is expressed as the concentration that kills half of

the fish exposed during the test, or LC₅₀. Fish are considered dead if there is no visible movement and if touching of the caudal peduncle produced no reaction. The dissolved oxygen, temperature and pH of the water, and the number of dead fish were recorded at 24 hr, 48 hr, 72 hr and 96 hr. The LC₅₀ value is calculated via probit analysis with 95% confidence limits using SPSS (Statistical Package for the Social Sciences) software.

Daphnia sp., acute immobilisation test (OECD 202, 2004). OECD 202 test was conducted to assess the effects of palm-based MES towards *Daphnia magna* (*D. magna*), a freshwater water flea which is one trophic level lower than fish. *D. magna* has proven to be a simple and sensitive test species for predictive toxicity studies (Sandbacka *et al.*, 2000). The young *daphnids* were exposed to various concentrations of the test substance for 48 hr and the immobilisation, *i.e.* the inability of *daphnids* to resume swimming within 15 s after gentle agitation, were determined after 24 hr and 48 hr, and compared with control values.

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 *daphnids* used in the test were less than 24 hr old and only the third brood progeny was used to reduce variability. ISO 6341 (2012) even imposes that the test organisms should be at least third generation offspring and 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.*). The *daphnids* used in a test were controlled to ensure that they were produced from cultures established from the same stock of *D. magna*. The stock of *D. magna* was maintained in the laboratory with conditions (light, temperature, medium) similar to those used in the test.

As with OECD 203, range-finding tests (24 hr) were initially conducted and based on the results obtained, a series of concentrations with a separation factor of less than 2.2 was used in definitive tests (48 hr). The highest concentration tested should result in total immobilisation of the *daphnids* while the lowest concentration tested should give no observable effect. These tests were conducted in triplicates (OECD 202, 2004) and each test beaker was observed at 24 hr and 48 hr. Immobilised *daphnids* were removed immediately when observed. The EC₅₀ value is calculated via probit analysis with 95% confidence limits using SPSS.

Analytical measurements. The pH, temperature and dissolved oxygen were determined daily in each

test and control solutions. The dissolved oxygen concentration in control and test beakers at the end of the test should be more than 3 mg litre⁻¹ while the pH did not vary by ≥ 1.5 units in any one test. In this study, the results were expressed based on the nominal concentration.

RESULTS AND DISCUSSION

The percentage of motionless *D. magna* or dead Tilapia in the control was determined within the test period to check the validity of the tests. According to test guidelines OECD 202 and 203, if the immobilisation of *D. magna* or the mortality of Tilapia exceeded 10%, or the dissolved oxygen was less than 3 mg litre⁻¹ in control and test solutions at the end of the test, or the variation in pH exceeded 1.5 units, the study would be considered invalid. However, these circumstances did not occur in both experimental set-ups. At the end of the tests, the immobilisation or mortality in the controls never exceeded 10%, while the dissolved oxygen and pH also were within the stipulated range as required in the OECD guidelines. Thus, the toxicity tests were considered valid.

The acute tests were conducted on palm-based C14, C16 and C16:18 MES produced in MPOB, and commercial MES (C16:18). The ecotoxicity values for the four surfactants toward Tilapia and *D. magna* are exhibited in Table 1. The sensitivity of the local fish Tilapia was found comparable to those of the standard species normally used in ecotoxicity test (Razmah and Salmiah, 2004). There were insignificant differences in the ecotoxicities of the surfactants when tested on the local fish and the recommended fishes.

Table 1 shows that the LC₅₀ and EC₅₀ values of different chain lengths of MES to the same organism differed up to 10-fold for both Tilapia and *D. magna*. *D. magna* was more affected by ecotoxicity effects of MES than was Tilapia. The results exhibit significant differences in toxicity responses between structurally related palm-based MES irrespective

of the test species. The toxicity increased as the hydrophobicity of the MES increased due to increase in alkyl chain length. According to Boeije *et al.* (2006), the ecotoxicity of surfactants normally increases logarithmically with a linear increase in alkyl chain length.

The acute toxicity effects of palm-based and commercial C16:18 MES on *D. magna* and Tilapia were similar and the most toxic compared to the other MES samples. Previous ecotoxicity studies conducted using fish as test species (Razmah and Salmiah, 2004; Masuda *et al.*, 1994) also showed an increase in toxicity values of MES with increase in carbon chain length. As observed by other researchers (Schoberl *et al.*, 1988; Potokor, 1992; Fendinger *et al.*, 1994), the possible reason for this is the greater interaction of the longer chain homologues with cell membranes (Ivankovic and Hrenovic, 2010). However, the reliance of the toxicity on the alkyl chain length is only applicable for compounds that are fully soluble in water. This may be due to lower bioavailability of longer chain homologues, as they have lower solubility (Garcia *et al.*, 2001).

Although palm-based MES seemed to be moderately to highly toxic to aquatic organisms, their effects in the environment are harmless. Since only 10% - 30% of MES is used in detergent products, equivalent to other commercial surfactants, palm-based MES will not cause environmental alarm. MES occurs mainly in the ionised form at environmental pH and as a result, it can be assumed that bioaccumulation is insignificant. The dilution in aquatic environment will cause the local predicted environmental concentration of MES to be very low (Razmah *et al.*, 2006). In addition, risk assessment study conducted by Miura (2007) found that MES as 'low risk level' or of 'no concern' to the environment.

CONCLUSION

Considerable amounts of detergents used in the domestic and industrial domains are directly discharged into waterways. These may cause problems in the aquatic environment including toxicity of the surfactants to aquatic organisms, foaming, eutrophication, *etc.* Therefore, the surfactants used and released in large quantity into the environment are vital to be as innocuous as possible for the environment with low ecotoxicity and easily biodegraded.

Palm-based MES is a good and sustainable surfactant to be used in detergent formulations as an alternative to surfactants derived from petrochemicals. The results demonstrated considerable variation in ecotoxicity responses within structurally related palm-based MES regardless of the species tested. *D. magna* was found to be more sensitive to toxic effects from MES than

TABLE 1. ACUTE ECOTOXICITIES OF MES ON *D. magna* AND TILAPIA

Result***	C14 MES	C16 MES	16:18 MES	Commercial C16:18 MES
<i>D. magna</i> , EC ₅₀ (mg litre ⁻¹)*	77.6	1.15	0.77	0.76
Tilapia, LC ₅₀ (mg litre ⁻¹)**	22.6	12.6	1.41	1.41

Note: *Three replicates, 10 *daphnids*/concentration.

**Two replicates, 10 fish/concentration.

*** With 95% confidence limit.

MES – methyl ester sulphonate.

Tilapia due to size of the test species. The toxicity increased as the alkyl chain length and resultant hydrophobicity of the MES increases. This trend was also observed in many other anionic surfactants. However, since less than 30% of MES is used in detergent products, palm-based MES will not pose environmental effects on aquatic organisms.

Hence, the use of palm-based MES in environmental-friendly detergent may help the detergent industry to fulfil its social responsibility to a cleaner and better environment. MES has good biodegradability and its ecotoxicity is better or comparable to the commercial, high volume anionic surfactants, such as LAS and sodium lauryl sulphate. The use of palm-based MES will help to stimulate Malaysia's agricultural economies and lessen the dependence on imported petroleum-based surfactants.

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