# ACUTE ECOTOXICITY (48-hr $EC_{50}$) ASSESSMENT OF PALM-BASED METHYL ESTER SULPHONATES (MES) TOWARDS *Daphnia magna*

**RAZMAH, G*; SITI AFIDA, I*; NOORAZAH ZOLKARNAIN* and HAZIMAH, A H***

**ABSTRACT**

*Palm-based methyl ester sulphonates (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 (48 hr $EC_{50}$). MES of various chain lengths were selected for the investigation. Based on the results, the $EC_{50}$ of MES (based on % immobilisation) 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 environmental-friendly detergent due to its good biodegradability and its toxicity which 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 immobilisation test, freshwater invertebrate, OECD, REACh.

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## 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 standardised test methods have been developed within the European Union (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 Organisation for Economic Co-operation and Development (OECD) 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,

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

and are in fact regaining popularity due to their sustainability, good cost-performance ratio and environmental-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 also well-known for its high tolerance against calcium ion where its sensitivity to water hardness is small relative to that of linear alkyl benzene sulphonates (LAS) (Salmiah *et al.*, 1998; Hama, 2002; Smulders *et al.*, 2002; Razmah and Salmiah, 2004; Razmah *et al.*, 2006). MES has been manufactured in large scale all over the world. *Table 1* shows the global MES producers as reported by Zulina (2013).

The use of MES in detergents started in the early 1990s in Japan (Masuda *et al.*, 1994; Masuda, 1995). Huish Detergent Inc. is producing MES from palm oil and using it in commercial laundry detergents. One of these commercial laundry detergents has the highest level of MES, *i.e.* about 23.5% of the total formulation (Zulina *et al.*, 2006).

Due to their widespread applications, surfactants used in the domestic and industrial domains are directly discharged into waterways and have become common constituents in municipal effluent and river water. This practice may pose environmental problems in the waterways including toxicity of the surfactants to microorganisms and fish, foaming, euthrophication and reduction of oxygen transfer into the water that can hinder the self-purification process (Hashim *et al.*, 1992). Therefore, the environmental safety assessment of surfactants and related products should primarily focus on the aquatic ecosystem. 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, in a method using fish as test species, the fish are exposed to the substance over 96 hr and the $LC_{50}$-value, *i.e.* the concentration where 50% of the fish dies (LC = lethal concentration), is determined. 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 *D. magna*. This freshwater crustacean 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

**TABLE 1. GLOBAL METHYL ESTER SULPHONATES (MES) PRODUCERS AND THEIR PRODUCTION CAPACITY IN 2012**

| Company | Location | Annual capacity (t) |
|---|---|---|
| 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).

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 article will discuss the acute toxicity of palm-based MES towards organism at a lower trophic level, *i.e. D. magna.*

## MATERIALS AND METHOD

### Test Organism

*Daphnia 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 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 hr 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 that 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.

### 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) MES pilot plant. Commercial MES (C16/18:80/20) was obtained from MES producer in Malaysia. The active matter in each sample was more than 80%.

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

### 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.

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

### Test Method

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

### Conditions of Exposure

***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

**TABLE 2. PREPARATION OF ISO MEDIUM**

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

Source: OECD 202 (2004).

medium. The tests were carried out without the adjustment of pH (6-9).

***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 litre$^{-1}$. The daphnids were exposed to each test concentration for 24 hr 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 hr. These tests were conducted in triplicates.

***Incubation conditions.*** The temperature of the incubator was set at 20 ± 2°C. A 16-hr light and 8-hr dark cycle was used. The test beakers were not aerated and the daphnids were not fed during the test.

**Test Procedures**

***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 hr $EC_{50}$ 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 litre$^{-1}$. Three replicates were used for each concentration and each containing five daphnids (less than 24 hr old) in 50 ml of ISO medium. The test was performed at 20±2°C with a photoperiod of 16 hr light and 8 hr dark. During the experiment, daphnids were not fed. In each test beaker, the immobilised daphnids were recorded at 24 hr of exposure.

***Acute toxicity test.*** For each test concentration and control, three replicates were used and each contained 10 daphnids (less than 24 hr old) in 50 ml of ISO medium. Each test beaker was checked for immobilised daphnids at 24 and 48 hr 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 with 95% confidence limits.

**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 litre$^{-1}$ 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.

## RESULTS AND DISCUSSION

**Sensitivity Test**

As emphasised 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 levels. 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 litre$^{-1}$ has been set in standard ISO 6341(2012) for the 24 hr $EC_{50}$ of the acute *D. magna* assay. The value obtained in the laboratory (0.83 mg litre$^{-1}$) was within the sensitivity range set by ISO 6341 standard (*Table 3*). This confirmed the assay sensitivity and that it could be used for testing.

**TABLE 3. NUMBER OF IMMOBILISED DAPHNIDS AT DIFFERENT $K_2CR_2O_7$ CONCENTRATIONS AND THE $EC_{50}$ VALUES AFTER 24 hr OBSERVATION**

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

Note: * Three replicates, five daphnids per replicate. **With 95% confidence limit.

## Ecotoxicity Tests

The acute tests were 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 litre$^{-1}$, showed none of the daphnids were immobilised within 24 hr of test duration when tested on C12 MES. This shows that the 24 hr $EC_{50}$ value for this sample is higher than 100 mg litre$^{-1}$, 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% immobilisation is observed at the end of the test, a full study has to be conducted. For other MES samples, immobilisation was observed at various concentrations, therefore definitive tests needed to be conducted.

In the definitive test, 100% immobilisation was recorded at the concentrations of 160.0 mg litre$^{-1}$ for C14 MES (*Table 4*), and 1.6 mg litre$^{-1}$ for C16 MES, C16:18 MES and commercial MES (*Table 5*). The concentration of C14 MES at 40.0 mg litre$^{-1}$ and 0.4 mg litre$^{-1}$ for C16 MES, C16:18 MES and commercial MES did not affect the mobility of the *D. magna*. The $EC_{50}$ (48 hr) of C14, C16, C16:18 and commercial MES is 77.6 mg litre$^{-1}$, 1.15 mg litre$^{-1}$, 0.77 mg litre$^{-1}$ and 0.76 mg litre$^{-1}$ , respectively, with 95% confidence limits (*Table 6*). The $EC_{50}$ value is calculated via probit analysis using statistical software SPSS (Statistical Package for the Social Science).

The effect of the hydrophobic carbon chain length on the toxicity of MES was also studied. Data from *Table 6* indicated that MES of shorter carbon chains were less toxic than MES with longer carbon chains. Generally, the longer the carbon chain, the higher was the toxicity. This observation is in tandem with the 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 an increase in toxicity values of MES with increase in carbon chain length. The acute toxicity of surfactants towards aquatic organisms is not class compound-specific but more to 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).

**TABLE 4. PERCENT IMMOBILISATION OF DAPHNIDS AT DIFFERENT CONCENTRATIONS OF C14 METHYL ESTER SULPHONATES (MES)**

| Concentration* (mg litre$^{-1}$) | Immobilisation (%)** (48 hr) |
|---|---|
| 0.0 | 0 |
| 20.0 | 0 |
| 40.0 | 0 |
| 80.0 | 36.7 |
| 160.0 | 100 |

Note: * Three replicates, 10 daphnids per replicate. ** Average value of three replicates.

**TABLE 5. NUMBER OF IMMOBILISED DAPHNIDS AT DIFFERENT METHYL ESTER SULPHONATES (MES) CONCENTRATIONS**

| Concentration (mg litre$^{-1}$) | Immobilisation (%)** (48 hr) | | |
|---|---|---|---|
| | 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 |

Note: * Three replicates, 10 daphnids per replicate. ** Average value of three replicates.

**TABLE 6. DATA ON 48 hr $EC_{50}$ (with 95% confidence limits) OF METHYL ESTER SULPHONATES (MES) SAMPLES**

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

However, a systematic dependence of the toxicity on the chain length is only recognisable in fully water-soluble compounds.

Palm-based MES 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 mineralised 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 ionised form at environmental pH. Due to its ionised properties, it can be assumed that bioaccumulation is insignificant.

## CONCLUSION

Public concern for the safety of products to the users 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 24 hr $EC_{50}$ value for C12 MES is higher than 100 mg litre$^{-1}$, *i.e.* the highest concentration tested in the range-finding test, and according to the test method, the sample can be considered as non-toxic. The $EC_{50}$ (48 hr) of C14, C16, C16:18 and commercial MES is 77.6 mg litre$^{-1}$, 1.15 mg litre$^{-1}$, 0.77 mg litre$^{-1}$ and 0.76 mg litre$^{-1}$, respectively. The results indicated that MES of shorter carbon chains were less toxic than MES with longer carbon chains.

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 environmental-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 detergent industry to a cleaner and better environment.

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