Importance of Genotoxicity Studies on Methyl Ester Sulfonates for Regulatory Compliance

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INTRODUCTION

Oleochemicals are derived from natural plants such as palm oil and other vegetable oils. There are five basic oleochemicals, namely fatty acids, fatty alcohols, fatty methyl esters, fatty amines and glycerol (Ong *et al*., 1989). Palm-based oleochemicals have a diverse range of applications, including as surfactants, personal care products, soaps, detergents and food additives. Surfactants are the largest market segment, alongside personal care and home care products (Grandview Research, 2014). One of the main surfactants is methyl ester sulphonates (MES).

MES are anionic surfactants derived from palm oil (Chemithon, 2008; Salmiah *et al.,* 1998). MES hold higher prospects in future use, considering it is an alternative anionic surfactant to petrochemical-based substances such as alkyl benzene sulfonates (LAS), fatty acid sulfates (FAS) and alkyl sulfates (AS) (Ismail *et al.,* 2002). The cost of producing MES is reasonable and their environmental friendly characteristics drive the industry's interest in producing them commercially (Zulina *et al.,* 2006; Razmah and Salmiah, 2004). The higher biodegradability properties of MES (94%-95%) allow the industry to formulate a product which has low surfactant content with high stability detergents and less energy consumption (20%) when used as a detergent (Zoller, 2009). MES are well-known for having superior detergency, water hardness tolerance, rapid biodegradability and low production cost (Martinez *et al.,* 2010). These have also created enormous opportunities for palm-based MES to be developed and emerge into the industry as they also hold the key to a sustainable future (Siwayanan *et al.,* 2014).

A recent analysis of the oleochemical market by Zion Market Research resulted in a forecast that global oleochemicals are expected to reach USD 26.8 billion in 2022, and are anticipated to grow at a compound annual growth rate (CAGR) of 8.2% over the next seven years (*Figure 1*) (Zion Market Research, 2017). Grandview Research reported that the fatty acids market of the Asia Pacific, particularly used in soaps and detergents, was valued at USD 1799.1 million in 2015, and is estimated to grow at CAGR of 4.9% from 2016 to 2024. Besides being the largest consumer, the Asia Pacific region is also the leading producer of oleochemicals and accounted for over 40% of the total volume in 2015 (Grandview Research, 2016). The 40% share in global MES volume was consumed by the household detergents and personal care products markets (Market Research Store, 2015).

Malaysia is one of the major global producers of palm oil which has become the top national commodity. In earlier times, the industry mainly concentrated on upstream activities such as germination of oil palm seeds production, management of seedlings at nursery, cultivating palms for the production of fruit bunches in plantations, processing and harvesting fresh fruit bunches (FFB) in mills for crude palm oil (CPO) and palm kernel oil (Guan *et al*., 2013). In response to the government's new strategies for increased industrialisation, CPO refineries were established in the 1970s, and helped in the transition of the Malaysian economy from commodity-based to manufacturing-based (MIDA, 2013). The country began to produce a wide range of palm oil products, ranging from edible oils to various raw materials for oleochemical production.

Government support through the introduction of the Economic Transformation Programme (ETP) helped to boost market growth in the expansion of the downstream sectors by producing high value oleochemical derivatives

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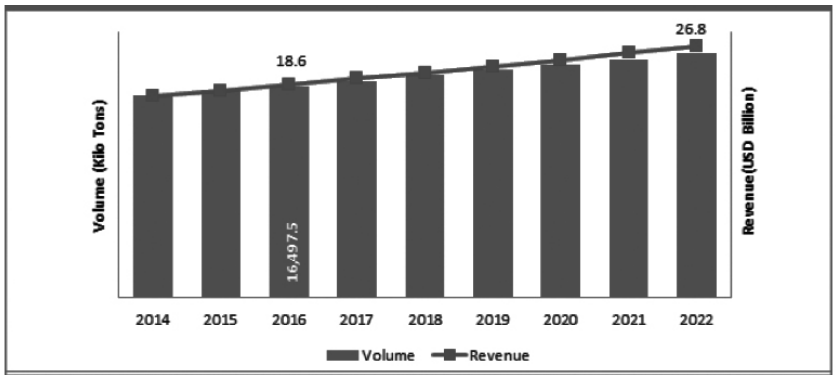

Source: Zion Market Research (2017).

Figure 1. Projected growth of global oleochemical markets from 2014-2022.

focusing on six key products, *i.e.* agrochemicals, surfactants, bio-lubricants, bio-polyols, glycerol derivatives and bio-based chemicals. The implementation of ETP gave the oil palm industry a new focus when it was identified as one of the 12 National Key Economic Areas (NKEA) to drive the nation's economy while giving focus to the sustainable development of the oil palm industry. It is a comprehensive effort to empower Malaysia towards becoming a high-income nation by 2020, highlighting a 10-year economic roadmap (Choo, 2012). The drive to increase downstream capacity while improving upstream productivity, and to move into the high value products segment, is crucial to ensure that Malaysia remains at the forefront of global palm oil production and exports as well as of transformation into a high income nation (ETP, 2010).

Oleochemical products from the palm oil industry contribute significant growth to the economy of Malaysia. According to the Malaysian Investment Development Agency (MIDA), Malaysia is now one of the top producers of oleochemicals in the world and the largest oleochemical sector due to her diverse chemical industry (MIDA, 2013). More than 15 oleochemical plants, owned by global players such as IOI, Emery and Kuala Lumpur Kepong (KLK), produce raw material for the manufacturing industries, including the food, pharmaceuticals, cosmetics, and detergent sectors. Based on MPOB figures, exports of oleochemicals amounted to 2.76 million tonnes, with EU being the major importer for oleochemicals at 0.53 million tonnes or 19.2% of the total oleochemical exports, followed by China, USA and Japan. Methyl ester was the second major oleochemicals exported in 2016 amounting to 17% of the total oleochemicals exported, alongside fatty acids, soap noodles and glycerine (MPOB, 2016).

The growing importance of biodiesel as an alternative to petroleum-based fuels has been the major driving factor in the growth of the oleochemicals industry. Biodiesel or saturated palm methyl esters can be obtained from a renewable feedstock with widespread availability, which includes CPO (Grandview Research, 2016).

Malaysia has been an exporter of biodiesel to EU which is the major export destination accounting for 83.5% of the total biodiesel exports (MPOB, 2016). For biofuel consumption, only a small amount of biodiesel can be used for blending while allowing unmodified vehicles to function satisfactorily. 5% refers to a blend of 5% palm methyl ester and 95% petroleum-based diesel (Mohamadi *et al.,* 2016). Besides, the saturated methyl esters can be diversified and utilised as feedstock for MES by the sulfonation process. Due to the small local market, there will be a quantity of MES in surplus which needs to be exported. Hence, it is important to have a complete dossier of MES, including toxicity data, to meet global regulations such as EU REACh, China REACh, K-REACh and others.

REGISTRATION, EVALUATION, AUTHORISATION AND RESTRICTION OF CHEMICAL SUBSTANCES (REACH) REGULATIONS

The European chemicals legislation REACh requires industry to register and provide information on substances they produce or import into Europe at or above 1 t yr^{-1} in amount (European Commission EC, 2006). The physicochemical and toxicological information on the substances, and the subsequent hazard and risk assessment submitted to the European Chemicals Agency (ECHA) by registrants, form the basis for how chemicals are regulated and managed within the European Union (EU) (Ingre-Khans *et al.*, 2016). REACh registration includes three different stages: registration, evaluation, authorisation and/or restrictions. ECHA acts as a central point in the REACh system by managing and coordinating detailed evaluation of chemicals identified, besides maintaining the public database on hazard information. The agency serves to confirm compliance of the registration dossiers or proposals with the regulation to ensure that the chemical safety assessments provided are accurate and conclusive to avoid any uncertainties (ECHA, 2008).

REACh registration guidelines state that chemicals produced or imported in quantities of ≥ 1 t yr^{-1} (per manufacturer/importer) must be registered in a central database. Unregistered substances may not be manufactured or

imported into the European Union in amounts > 1 t yr^{-1} (Rudén and Hansson, 2010). *Table 1* shows the minimum level of information for a technical dossier submission for the REACh registration process, which includes genetic toxicology data. This is applicable to substances manufactured in EU or imported in quantities of ≥ 10 t yr^{-1}. Additional toxicity data are necessary to complete the registration procedure, including data on *in vivo* skin and eye irritation, acute mammalian toxicity and *in vitro* cytogenicity test using mammalian cells. It is necessary to carry out an *in vitro* gene mutation study using mammalian cells subsequently if there is a negative result in the mutagenicity testing. Nonetheless, further *in vivo* mutagenicity studies shall be conducted if a positive result is obtained in any of the tests (Rudén and Hansson, 2010).

It has been the major goal of the REACh legislation to make the use of chemicals, both in Europe and worldwide, safer by evaluating the toxicity data of existing chemicals. The REACh system aims to provide toxicity data of chemicals to regulators within a very short time-frame, in order to register, evaluate and limit exposure to the most hazardous ones (Ingre-Khans *et al.*, 2016). Establishment of toxicity data is crucial in ensuring the accuracy of the hazard risk assessments following the standard test method of the Organisation for Economic Cooperation and Development (OECD) guidelines and compliance with the International Conference on Harmonisation (ICH) regulations (Krishna and Gopalakrishnan, 2016).

GENETIC TOXICOLOGY

Genetic toxicology is a study that identifies agents which are toxic to the hereditary material of living systems, namely, deoxyribonucleic acid (DNA) (Krishna and Gopalakrishnan, 2016). The types of DNA damage measured range from reversible to irreversible damage involving structural and numerical chromosome changes, and are the result of interaction with hazardous compounds which regulate the conformity of the genome (Mateuca *et al.*, 2006). Genotoxicity testing forms an essential component of safety and hazard evaluations of all substances, ranging from pharmaceuticals, industrial chemicals, pesticides, biocides, food additives, cosmetics ingredients to veterinary drugs. These assessments are applicable for regulatory purposes and international legislations in that they assess the potential risk of carcinogenicity and heritable mutations, and are aimed at protecting human and animal health (Corvi and Madia, 2017). A stepwise approach in genotoxic hazard assessments towards human health is applied by the basic test of *in vitro* assays, followed by confirmation with *in vivo* testing (Corvi and Madia, 2017). The standard *in vitro* and *in vivo* tests which are commonly used for genotoxicity testing under OECD guidelines are shown in *Table 2*.

The recent banning of the use of *in vivo* assays for genotoxic assessment of cosmetic ingredients (EU, 2003) and for the broad chemical evaluation programme (REACh) in EU has caused the industries to shift to alternative testing strategies for toxicology. The 7th amendment to the EU Cosmetics Directive testing ban states that *in vivo* follow-up genotoxicity is prohibited for cosmetic ingredients (SCCS, 2015; EC, 2009), while for industrial chemicals and biocidal products, appropriate *in vivo* follow-up testing is required only if there is a positive outcome in one or more of the *in vitro* genotoxicity tests (EU, 2012; EC, 2008). *In vitro* methods for toxicological assessment of chemicals and pharmaceuticals become increasingly important, as there is total reliance on results validation because they demonstrate high throughput and relevant predictive value (Krishna and Gopalakrishnan, 2016; Seager *et al.*, 2014).

In vitro methods typically involve cytogenetic damage evaluation in either primary cells or mammalian cell lines (Zelazna *et al.*, 2011). The diverse nature of the mechanism involved in genotoxicity causes it to be impractical to rely

TABLE 1. REACH DATA REQUIREMENTS ON TOXICITY

Category	t yr^{-1}				
	< 1	≥ 1	≥ 10	≥ 100	≥ 1000
Chronic toxicity and carcinogenicity[a]	No	No	No	No	(Yes?)
Reproductive toxicity (one generation)[a]	No	No	No	(Yes?)	(Yes?)
Sub-chronic (90 days)[a]	No	No	No	(Yes?)	(Yes?)
Screening for reproductive toxicity	No	No	(Yes?)	(Yes?)	(Yes?)
Sub-acute (28 days)[a]	No	No	(Yes?)	Yes	Yes
Acute toxicity second route[a]	No	No	Yes	Yes	Yes
Skin + eye irritation (*in vivo*)[a]	No	No	Yes	Yes	Yes
Additional mutagenicity tests (*in vitro*)	No	No	Yes	Yes	Yes
Acute toxicity oral route[a]	No	(No?)	Yes	Yes	Yes
Mutagenicity (*in vitro*)	No	(No?)	Yes	Yes	Yes
Skin sensitisation[a]	No	(No?)	Yes	Yes	Yes
Skin + eye irritation (*in vitro*)	No	(No?)	Yes	Yes	Yes

Note: (No?): Testing can be triggered according to certain criteria.
(Yes?): Testing can be waived according to certain criteria.
[a]: The test has a direct use for classification purposes.

TABLE 2. STANDARD *in vitro* AND *in vivo* GENETIC TOXICITY TESTS BASED ON OECD GUIDELINES

In vivo		*In vitro*	
Test name	**OECD Test no.**	**Test name**	**OECD Test no.**
Mammalian erythrocyte micronucleus test	TG 474	Bacterial reverse mutation assay	TG 471
Mammalian bone marrow chromosomal aberration test	TG 475	Mammalian chromosomal aberration test	TG 473
Transgenic rodent somatic and germ cell gene mutation assay	TG 488	Mammalian cell gene mutation tests i) HRPT ii) Mouse lymphoma assay (MLA)	TG 476 TG 490
Mammalian alkaline comet assay	TG 489	Mammalian cell micronucleus test	TG 487

TABLE 3. A COMPARISON OF THE SENSITIVITY AND SPECIFICITY OF STANDARD *in vitro* ASSAYS FOR THE ASSESSMENT OF GENOTOXICITY

Assay	**Sensitivity (%)**	**Specificity (%)**	**References**
Bacterial reverse mutation (Ames test)	58.8 49.4	73.9 80.3	Kirkland *et al.* (2005) Matthews *et al.* (2006)
Gene mutation test mouse lymphoma assay (MLA), or Thymidine kinase (TK)	73.1 62.8	39.0 44.2	Kirkland *et al.* (2005) Matthews *et al.* (2006)
Chromosome aberration test (CA)	65.6 55.3	44.9 63.3	Kirkland *et al.* (2005) Matthews *et al.* (2006)
Micronucleus test (MN)	78.7 87.3 89.2	30.8 23.1 55.0	Kirkland *et al.* (2005) Matthews *et al.* (2006) Corvi *et al.* (2008)
Ames + MN	85.9	121.0	Kirkland *et al.* (2005)
Ames + CA	75.3	34.6	Kirkland *et al.* (2005)
Ames + MLA + MN	90.7	5.0	Kirkland *et al.* (2005)
Ames + MLA + CA	84.7	22.9	Kirkland *et al.* (2005)

on one single test for detecting all classes of genotoxic carcinogens (Pfuhler *et al.*, 2010). International guidelines comprising those of REACh, ICH and the Scientific Committee on Consumer Safety (SCCS) recommend the use of more than one test for comprehensive assessments of genotoxicity which cover three major endpoints, *i.e.* gene, chromosome and genome mutations (Eastmond *et al.*, 2009; ICH 2008).

An optimal number of core battery tests with a total of three *in vitro* assays, comprising induction of gene mutations in bacterial and mammalian cells, in combination with either *in vitro* chromosome aberration or micronucleus test has been suggested by different regulation guidelines to be the basic testing requirements for genotoxicity (Corvi and Madia, 2017). However, a previous study conducted by Kirkland *et al.* (2005) has shown that although a combination of more than two tests increases sensitivity, it further decreases the specificity of what the tests measure. *Table 3* shows a comparison of sensitivity and specificity of combinations of the *in vitro* genotoxicity assays available.

In an effort to reduce, replace and refine (3R) the use of animals in genotoxicity testing, the European Union Reference Laboratory for Alternatives to Animal Testing (EURL ECVAM) has come out with strategic plans to enhance *in vitro* testing performance in genotoxicity testing (ECVAM, 2013). It is recommended that a core battery test consisting of the combination of Ames and micronucleus tests be used as a basis to cover the three genetic endpoints. Apparently, the core battery has been accepted in current guidelines for risk and hazard assessment of chemical substances and cosmetic ingredients as well as hazard assessment of foods and animal feeds (SCCS, 2015; COM, 2011; EFSA, 2011).

TOXICITY OF SURFACTANTS

The toxicity of anionic surfactants on human health has been explored extensively, covering dermal toxicity, oral toxicity, genotoxicity and carcinogenicity. Environmental aspects, such as ecotoxicity towards soil, the microbial world and aquatic systems, have been included as well (Razmah *et al*., 2016; Chaturvedi and Kumar 2010; Schweigert *et al.,* 2000). A review was conducted by Rebello *et al.* (2014) on surfactant toxicity of sodium dodecyl sulfate (SDS) and linear alkyl benzene sulfonate on human health and the environment (soil, microbial world, aquatic systems). In addition, a study on the toxicological properties of alkyl sulfates, primary alkane sulfonates and α-olefin sulfonates on human health was conducted under the high production volume chemicals programme of OECD. The different types of sulfonates showed negative results in genotoxicity assays (Wibbertmann *et al.*, 2011). *Table 4* shows several types of toxicity studies conducted on different types of anionic surfactants.

TABLE 4. TOXICITY STUDIES ON DIFFERENT TYPES OF ANIONIC SURFACTANTS

Type of anionic surfactant	Type of studies	Organism	Source
Alkyl sulfates, primary alkane sulfonates, α-olefin sulfonates,	Dermal toxicity, oral toxicity, genotoxicity, reproductive toxicity, carcinogenicity	Rats, rabbits, humans	Wibbertmann *et al.* (2011)
	Ecotoxicity	Fish, aquatic plants	Könnecker *et al.* (2011)
Alkyl-sulfonate	Genotoxicity, carcinogenicity	Mice, humans	Snodin and Teasdale (2015)
SDS	Dermal toxicity	Humans	Romanelli *et al.* (2004)
LABS	Ecotoxicity	Microbes, aquatic systems	Ivankovic *et al.* (2009)
MES	Ecotoxicity	Fish: Tilapia	Siwayanan *et al.* (2014)
			Razmah *et al.* (2016)

To date, there are limited or no available data on the safety (including genotoxicity) of MES towards human health. Safety studies on MES have been carried out mainly on ecotoxicity and effects on the environment (Razmah *et al.,* 2016). Therefore, genotoxicity assessment of MES via the recommended Ames and micronucleus tests needs be carried out to determine any genotoxic effect resulting from repeated exposure of this substance to humans.

CONCLUSION

Oleochemicals from the palm oil industry have significant economic advantages in adding value to the country's commodity. Demand for oleochemical products from the local market as well as regional markets, especially for palm-based methyl ester sulfonates, is increasing. Therefore, the compilation of genotoxicological safety data for MES will provide a complete MES dossier to comply with the requirements of global safety and health regulations, and will subsequently grant their future export to world markets.

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