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PARTITION COEFFICIENT, WATER SOLUBILITY AND AQUATIC TOXICITY OF SHORT-CHAIN PALM FATTY ACIDS

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ABSTRACT

The octanol/water partition coefficient (P_{ow}) *and water solubility* (S_w) *are important physico-chemical properties for an environmental risk assessment of a chemical substance, as well as ecotoxicity. It can be an indication of the extent to which the chemical might bioaccumulate in aquatic organisms. The log* P_{ow} *of palm-based fatty acids was estimated using high performance liquid chromatography (HPLC) method, while the* S_w *value was determined using linear correlation between* S_w *and log* P_{ow} *of reference compounds. The ecotoxicity of palm-based fatty acids was measured using test method OECD 203, Fish Acute Toxicity with common carp (*Cyprinos carpio*) as the test species. The short-chained C6 to C10 palm fatty acids were chosen for this study because they are generally toxic and can cause irritation to human and may have an adverse effect towards aquatic organisms. The log* P_{ow} *values showed that both C6 and C8 fatty acids had low affinity towards aquatic organisms, but C10 fatty acid showed medium affinity towards these organisms. As for water solubility, C6 fatty acid had high affinity towards water, while both C8 and C10 fatty acids showed medium affinity towards water. The* LC_{50} *value of C6, C8 and C10 palm FAs are 7 mg litre*$^{-1}$*, 7 mg litre*$^{-1}$*and 20 mg litre*$^{-1}$*, respectively. It can be concluded that, the higher the carbon chain number of the fatty acid, the higher is the partition coefficient value, the water solubility decreases. The palm-based fatty acid can be considered as moderately toxic to aquatic organisms. These data can be used by the palm-based oleochemical manufacturers for product registration, preparation of product dossier or for marketing purposes.*

Keywords: HPLC, physico-chemical properties, aquatic environment, bioaccumulation.

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INTRODUCTION

The most important and widely used properties of a substance for an environmental exposure assessment are biodegradability, ecotoxicity, aqueous solubility and the octanol / water partition coefficient. Partition coefficient (P_{ow}) describes the distribution of a solute in coexisting organic and aqueous phases that are needed in a large variety of applications (Hansch and Leo, 1979). The P_{ow} plays an important role in the early stages of an environmental exposure assessment of a chemical. It gives an indication whether a chemical might bioaccumulate in aquatic organisms (Cairns *et al.*, 1978). It also correlates well with the water solubility and other parameters predicting biological, biochemical and toxic effects,

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such as bioaccumulation and bioconcentration (Lyman *et al*., 1982).

The water solubility (S_w) of an organic compound is the highest equilibrium concentration it can achieve as a dissolved portion in aqueous solution. If the compound's concentration in the water phase has reached the maximum value of its water solubility, the solution is saturated with respect to this particular compound. In the saturated state, the solute is in equilibrium with its pure compound phase. A further increase in the amount of the compound beyond its water solubility level will lead to the formation of this pure compound phase as a separate phase (Van Leeuwen *et al*., 2007).

The S_w of organic compounds is an important indication of their mobility in the environment. Increasing S_w often implies decreasing hydrophobicity in terms of P_{ow}. High S_w indicates that the compound has a low tendency for sorption into suspended or particulate organic matter and thus shows a low tendency to bioconcentrate in aquatic organisms. The classes of affinity of chemicals for the different environmental compartments in relation to the P_{ow} and S_w value are shown in *Table 1*.

The assessment of ecotoxicity for the classification of chemicals and environmental risk assessments is typically by following the *OECD Guidelines 201, 202* and *203* (OECD, 1984), which assess the environmental impact of a substance in an aqueous environment by studying its impact on algae, daphnia and fish, respectively. These are standard species which represent the aquatic compartment. The toxicity data from freshwater and marine species are considered equivalent, although this is not true for all substances. The United States Fish and Wildlife Services has classified chemical substances in a few rating scheme to the aquatic toxicity. The rating scheme are summarised in *Table 2.*

Fatty acids (FA) are widely used in household cleaning products, cosmetics, lubricants and other miscellaneous industrial applications and coatings. FA are obtained from oils and fats including palm oil, by hydrolysis under high pressure and temperature. Fractional distillation of the mixture separates the shorter chain FA (≤ C14) from the longer one (> C14). Depending on the distillation conditions, FA of different grades and purities can be produced.

The release of FA into the environment during manufacturing, processing, distribution, use or disposal can have an adverse impact on both natural and man-modified ecosystems and their components, and also on living organisms. These short chain FA had been shown to cause one or more of the following effects, *i.e*., corrosion, skin/eye irritation, or foul smell. Kabara (1979) showed that caprylic acid (C6) and capric acid (C10) were classified as corrosive, and a study done by Vershueren (1996) showed that the longer the carbon chain length of FA (C6 to C10), the higher was the toxicity.

The physico-chemical data for short-chained palm FA (C6, C8 and C10) are required for

TABLE 1. CLASSES OF AFFINITY OF CHEMICALS FOR THE DIFFERENT ENVIRONMENTAL COMPARTMENTS IN RELATION TO THE PARTITION COEFFICIENT AND WATER SOLUBILITY

Affinity	Water	Aquatic organisms
	Solubility (S_w), g litre^{-1}	Partition coefficient, Log P_{ow}
High	>1.0	> 5
Medium	0.001 - 1.0	3 - 5
Low	< 0.001	< 3

Source: Vighi and Calamari (1993).

TABLE 2. RATING SCHEME USED BY THE UNITED STATES FISH AND WILDLIFE SERVICES FOR AQUATIC TOXICITY

Rating	LC_{50} (mg litre^{-1})
Super toxic	< 0.01
Extremely toxic	0.01 – 0.1
Highly toxic	0.1 – 1.0
Moderately toxic	1.0 – 10.0
Slightly toxic	10 – 100.0

Source: Drozd (1991).

registration under REACh (Registration, Evaluation, Authorisation and Restriction of Chemicals) regulation. Guidance on Information Requirements and Chemical Safety Assessment issued by the European Chemical Agency (ECHA) provides how to assess whether or not a substance is persistent (P), bioaccumulative (B) and toxic (T), PBT or very persistent and very bioaccumulative (vPvB) and states that a substance with octanol-water partitioning coefficient, Log $P_{ow} \leq 4.5$ is assigned as 'not B and not vB'.

Preparation of material safety data sheet (MSDS), or product dossier, generally requires inclusion of the water solubility and partition coefficient data and the acute aquatic toxicity data for identification of chemical substances. This will help to understand their hazards so that these substances can be classified and labelled with suitable risks and safety phrases, and packaged in an appropriate manner. For those who are working with chemicals, it is important that they know how to recognise those chemicals that are hazardous, how to reduce exposure, and ultimately how to reduce any risks to humans and the environments.

The purpose of this study is to determine the partition coefficient and water solubility of short-chained palm-based FA and to assess the effects of these FA in aquatic environments.

MATERIALS AND METHODS

Materials

Commercial C6, C8 and C10 palm-based FA with purity between 95% to 99% were obtained from the local oleochemical manufacturers. The number of samples received from the manufactures were two samples of C6, five samples of C8 and five samples of C10 FA. Reference compounds used included hexanoic acid, octanoic acid, decanoic acid, dodecanoic acid, tetradecanoic acid and hexadecanoic acid purchased from Sigma Aldrich, USA with purity between 98% to 99%. The HPLC-grade methanol was purchased from Fisher Scientific, USA.

Methods

Determination of partition coefficient using high performance liquid chromatography (HPLC) method. The HPLC method enables partition coefficient of a substance to be estimated in the log P_{ow} range between 0 and 6 (OECD 117, 2004). The partition coefficient was established based on correlation between the HPLC retention times against the existing measured log P_{ow} values for reference compounds. Basically, the reference compounds used should preferably be structurally similar to those compounds being studied (Fujisawa and Masuhara, 1981).

The analyses were carried out using a JASCO HPLC PU1580 (Japan) instrument equipped with an auto-sampler. The injection volume was set at 20 μl. Chromatographic separation was performed on a Merck (Germany) Lichrosphere 100 Reverse Phase C8 (particle size 5 μm) column (length 250 mm x i.d 4 mm). The flow rate of the mobile phase, methanol (MeOH): water (90:10 v/v), was set at 0.50 ml min^{-1} and the column temperature was set at 54°C. The analysis run time was 20 min.

For every test, formamide (unretained compound) and reference compounds (with known P_{ow}), *i.e.* hexanoic acid, octanoic acid, decanoic acid, dodecanoic acid, tetradecanoic acid and hexadecanoic acid, were run together with the palm-based FA (unknown P_{ow}). The plot of log P_{ow} against log K (capacity factor) was built and the log P_{ow} value for palm-based FA were determined using this linear correlation.

Determination of water solubility. For similar classes of compounds, excellent linear relationship was found between aqueous solubility (S_w) and partition coefficient (log P_{ow}) as described by the equation of $\log P_{ow} = a \log (1/S_w) + b$ (Schwarzenbach *et al*., 2004). Therefore, by measuring the partition coefficient of a compound, its aqueous solubility can be determined or *vice versa*. The plot of log P_{ow} against log $1/S_w$ was established, and was used to calculate the S_w of the palm-based FA.

All data storage and handling, calculations of retention time and simple statistical calculations were performed using Microsoft Window Excel.

Acute toxicity test. Aquatic toxicity is the toxicity of a substance towards aquatic organisms. It is assessed by the response of the aquatic organisms to the substance (APHA, 1980). The test used was for determination of acute toxicity of a substance towards fish in static conditions based on the *OECD Guidelines for Testing of Chemicals* (OECD 203, 1992), *i.e.* OECD 203 Fish, Acute Toxicity Test. The freshwater fish, common carp (*Cyprinos carpio*), was used as the test organism.

The fish (2-5 cm) were obtained from a local fish supplier. They were acclimatised for two weeks to test the conditions and fed daily with commercial dry fish food until the day before the test. The fish were not fed during the bioassay and were exposed to the test substance at four concentrations (in geometric series) up to a final concentration of 100 mg litre^{-1} for 96 hr. The mortalities were recorded at 24, 48, 72 and 96 hr, and the concentration that killed 50% of the fish, or the LC_{50}, was determined using SPSS statistic.

RESULTS AND DISCUSSION

Correlation Graph

In order to correlate the HPLC retention time of a compound with its P_{ow}, a correlation graph was established using six reference compounds. The HPLC retention time (tR) for each reference compound is shown in *Table 3*. Formamide was used to determine the dead time (t_o), *i.e.* the average time a solvent molecule required to pass through the column. The data from *Table 3* were then used to establish the correlation graph of log P_{ow} *vs.* log K (*Figure 1*).

Partition Coefficient

The log P_{ow} of C6, C8 and C10 palm FA were calculated from the correlation graph of log P_{ow} *vs.* log K (*Figure 1*), which was plotted using the reference compounds. The results are shown in *Table 4*.

As soon as a chemical substance is introduced into the environment, it will move from its point of entry to its final destination, that is the environmental compartments at which it has more affinity. The log P_{ow} of commercial C6 FA was between 1.98 to 2.20. Based on *Table 1* (Vighi and Calamari, 1993), C6 FA has low affinity to accumulate in living organisms (log P_{ow} <3), either directly or via food chains. Therefore, this compound will not affect the aquatic organisms if it enters the aquatic environment.

The log P_{ow} for commercial C8 and C10 FA were in the range of 3.17 to 3.25 and 4.28 to 4.40, respectively. These FA have medium affinity towards aquatic biota, *i.e.* there is a tendency to bioaccumulate in aquatic organisms such as fish and aquatic plants. According to the *Guidance on Information Requirements and Chemical Safety Assessment* issued by ECHA using REACh registration, a substance with log $P_{ow} \leq 4.5$ is assigned as 'not bioaccumulative and not very bioaccumulative'.

As for the bio-concentration factor (BCF), the BCF value for the C10 FA is 66, calculated for octanoic acid, using a measured log P_{ow} of 3.05 and a regression equation log BCF = log P_{ow} - 1.23 (Mackay, 1982). According to screening criteria for bioaccumulation stipulated in the ECHA's *Guidance on Information Requirements and Chemical Safety*

TABLE 3. RETENTION TIME OF REFERENCE COMPOUNDS

Reference compound	Log P_{ow} *	tR ± SD (min) (n=6) (RSD%)	Log K K= (tR – t_o)/t_o
Formamide (unretained compound)	-	4.65 ± 0.16 (3.33)	-
Hexanoic acid	1.92	5.61 ± 0.08 (1.46)	-0.69
Octanoic acid	3.05	6.13 **± 0.09 (1.48)**	-0.49
Decanoic acid	4.09	6.90 **± 0.10 (1.45)**	-0.32
Dodecanoic acid	4.60	8.04 **± 0.09 (1.14)**	-0.14
Tetradecanoic acid	6.11	9.59 **± 0.14 (1.43)**	0.026
Hexadecanoic acid	7.17	11.88 **± 0.27 (2.41)**	0.192

Note: SD - standard deviation.
RSD - relative standard deviation.

Source: * Felicia *et al.* (2009).

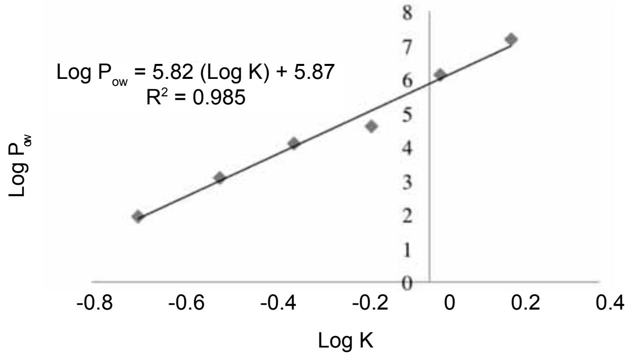

Figure 1. Graph of log P_{ow} vs. *log K for reference samples.*

TABLE 4. DATA ON RETENTION TIME (tR) AND LOG P_{ow} OF PALM-BASED FATTY ACIDS

Fatty acid	tR* (min) ± SD**	Log P_{ow}	Affinity toward aquatic organisms
C6	5.65 ± 0.01 to 5.74 ± 0.01	1.98 to 2.20	Low
C8	6.25 ± 0.01 to 6.30 ± 0.02	3.17 to 3.25	Medium
C10	7.13 ± 0.01 to 7.25 ± 0.01	4.28 to 4.40	Medium

Note: * Average of four replicates.
** Standard deviation.

Assessment - Chapter R.11: PBT Assessment, this C10 FA is considered unlikely to be bioaccumulative (BCF value < 2000) or very bioaccumulative (BCF value < 5000) in aquatic organisms.

Water Solubility

The plot of log P_{ow} against log ($1/S_w$) of reference compounds has been established (*Figure 2*) based on the data from Felicia *et al.* (2009) (*Table 5*). This plot was then used to calculate the aqueous solubility of commercial palm-based FA. The results are shown in *Table 6*.

The water solubility of C6 FA is 6.75 to 12.5 g litre^{-1}. Based on *Table 1*, this FA showed high affinity towards water compartments. This C6 FA, if discharged into the aquatic environments, will not pose adverse effect towards aquatic organisms. Whereas, if it is discharged on the soil, it will remain there until it is in contact with water and get transported elsewhere during any water movements (Vighi and Calamari, 1993).

The solubility of C8 and C10 FA ranged from 0.35 g litre^{-1} to 0.44 g litre^{-1} and 0.01 g litre^{-1} to 0.02 g litre^{-1}, respectively and showed medium affinity toward water compartments. Accidental spillage and disposal of C8 and C10 FA to aquatic environments can cause ecological problems where they may disrupt the movement and oxygen consumption of aquatic organisms. It can be concluded that, the higher the carbon chain length of FA, the lesser it can dissolve in water.

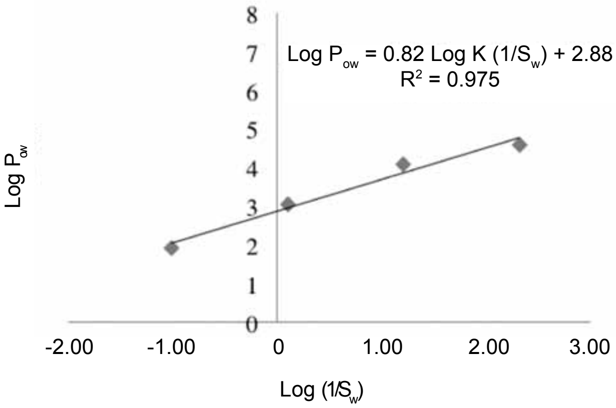

Figure 2. Graph log P_{ow} vs. *log ($1/S_w$) for reference samples.*

TABLE 5. PARTITION COEFFICIENT AND WATER SOLUBILITY FOR REFERENCE COMPOUNDS

	log P_{ow} *	Solubility (g litre^{-1}) *
Hexanoic acid	1.92	10.26
Octanoic acid	3.05	0.788
Decanoic acid	4.09	0.062
Dodecanoic acid	4.60	0.0048

Source: *Felicia *et al.* (2009).

TABLE 6. PARTITION COEFFICIENT AND WATER SOLUBILITY OF COMMERCIAL PALM-BASED FATTY ACIDS

Fatty acid	Log P_{ow}	Solubility (g litre^{-1})	Affinity towards water
C6	1.98 to 2.20	6.75 to 12.5	High
C8	3.17 to 3.25	0.35 to 0.44	Medium
C10	4.28 to 4.40	0.01 to 0.02	Medium

TABLE 7. EFFECT OF FATTY ACIDS ON AQUATIC ORGANISMS

Fatty acid	pH	Dissolved oxygen (mg litre^{-1})	Ecological effects 96 hr LC_{50} (mg litre^{-1})
C6	6.50	~8.3	7
C8	7.20	~8.3	7
C10	7.15	~8.3	20

Acute Toxicity Test

The toxicity of palm-based C6, C8 and C10 FA was determined using common carp (*Cyprinos carpio*) as the test species. The ecotoxicity values for these FA are shown in *Table 7*. The LC_{50} values for palm-based C6, C8 and C10 FAs are 7.0 mg litre^{-1}, 7.0 mg litre^{-1} and 20 mg litre^{-1}, respectively. According to *Table 2*, the results showed that these substances are considered moderately toxic to aquatic organisms (LC_{50} = 1-10 mg litre^{-1}). The higher LC_{50} (lower toxicity) of C10 FA may be due to its lower solubility in water. Materials which are poorly soluble in water present special problems since low solubility masks the toxicity dependence on chain length. Safe and environmental-friendly methods must be considered for disposal or handling all of these substances in order to minimise the impact on environment.

CONCLUSION

The partition coefficient value of C6, C8 and C10 palm FA are between 1.98 to 2.20, 3.17 to 3.25 and 4.28 to 4.40, respectively, while the water solubility value for these compounds are in the range 6.75 to 12.5 g litre^{-1}, 0.35 to 0.44 g litre^{-1} and 0.01 to 0.02 g litre^{-1}, respectively. The LC_{50} value of C6, C8 and C10 palm FA are 7 mg litre^{-1}, 7 mg litre^{-1} and 20 mg litre^{-1}, respectively. These values show that as the carbon chain length of FA increases, the log P_{ow} value also increases, the solubility in water decreases. These substances are moderately toxic to aquatic organisms. Higher P_{ow} values indicate that these fatty acids are less soluble in water. These data may be used by the local FA manufacturers for preparation of product dossier, product registration or for marketing purposes.

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