SAFETY ASSESSMENT OF PALM KERNEL OIL, PALM KERNEL STEARIN AND PALM KERNEL OLEIN IN MARINE ENVIRONMENT

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ABSTRACT

The acute toxicity of water accommodated fraction (WAF) of crude palm kernel oil (CPKO), crude palm kernel stearin (CPKST) and crude palm kernel olein (CPKOL) to Acartia tonsa *(a marine copepod) and* Skeletonema costatum *(a chain forming marine algae) was determined at three loading rates: 10, 100 and 1000 mg litre^{-1}. WAF methodology was used for the toxicity tests as these palm products are poorly water-soluble. Measurement of the total carbon (TC) of the test medium before the start of the tests confirmed that there were low levels of solubilized material in the WAFs. The mean concentrations of TC in 1000 mg litre^{-1} WAFs prepared from palm kernel oil, palm kernel stearin and palm kernel olein were 4.5, 1.0 and 5.2 mg litre^{-1}, respectively. All the palm products tested were not toxic to* A. tonsa. *Palm kernel oil and palm kernel olein were harmless to* S. costatum *at a loading rate of 10 mg litre^{-1}. They were slightly toxic at 100 mg litre^{-1} and toxic at 1000 mg litre^{-1}. Palm kernel stearin was harmless to* S. costatum *at a loading rate of 100 mg litre^{-1} and only slightly toxic at 1000 mg litre^{-1}.*

Keywords: palm kernel oil, palm kernel stearin, palm kernel olein, *Acartia tonsa*, *Skeletonema costatum*.

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INTRODUCTION

There is general consensus that vegetable oil is generally safe for consumption as food. Large volumes of vegetable oils are shipped throughout the world, subject to various kinds of handling. There may well be spillage, in the worst case scenario, an accident with detrimental effect on the environment. Compounds are classified as pollutants if they cause a detrimental biological effect. This suggests that all compounds, whether considered pollutants or not, can become pollutants if present in a sufficiently large volume, such as those happens in a major spillage.

Palm kernel oil is obtained from the kernel of the oil palm fruit. In its crude form, it is yellowish in colour and is easily bleached to a lighter colour suitable for both edible and inedible applications. The oil has a narrow melting range of 25°C-28°C and a much sharper solid fat content profile (Siew, 2004). The dominant fatty acid is lauric acid, constituting 46% to 51% of it. Fractionation of palm kernel oil gives a liquid (olein) and solid (stearin) fractions. The longer chain (≥C12) saturated acids occur in the stearin while the short chain (C6-C10) and unsaturated acids in the olein. Palm kernel stearin is the product most suitable for making cocoa-butter substitutes. It can be used directly or improved by hydrogenation. Palm kernel olein, on the other hand, is used in imitation dairy products or hydrogenated for confectionery.

Palm kernel oils (PKO) are transported in very large volumes by sea around the world. Malaysia exported 850 649 t of PKO in 2005. The major buyers were the USA, which took 198 093 t (23% of Malaysian exports), European Union (EU) 169 769 t (20%), Japan 61 060 t (7.2%), China 48 050 t (5.6%) and Turkey 40 437 t (MPOB, 2006).

The environmental toxicity of crude palm kernel oil (CPKO), crude palm kernel stearin (CPKST) and

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crude palm kernel olein (CPKOL) was assessed via the marine acute toxicity tests. The marine copepod *Acartia tonsa* and marine algae *Skeletonema costatum* were exposed to water accommodated fractions (WAFs) of these products at 10, 100 and 1000 mg litre^{-1}. The medium preparation and toxicity tests were done by the methods approved by GESAMP (Joint Group of Experts on the Scientific Aspects of Marine Environmental Protection), a United Nation advisory body established by the International Maritime Organization (IMO) and other organizations, and based on the GESAMP protocol for preparation of poorly soluble complex mixtures and ISO testing guidelines.

This paper describes the toxicity tests and discusses the effect loading (EL_{50}) values obtained, *i.e.* the loading rate at which 50% of the exposed *A. tonsa* are immobilized, or at 50% reduction in the algal growth.

MATERIALS AND METHOD

Test Samples

CPKO, palm kernel stearin and palm kernel olein were obtained from a Malaysian palm oil company. On receipt, they were stored in the dark at 21°C until use. The appearance of the samples is shown in *Table 1*.

TABLE 1. APPEARANCE OF SAMPLE

Oil	Appearance at 21°C
Crude palm kernel oil (CPKO)	Yellow, solid
Crude palm kernel stearin (CPKST)	Cream/yellow, solid
Crude palm kernel olein (CPKOL)	Dark yellow, semi-solid

Test Method

Preparation of test medium. The materials were poorly water-soluble; therefore, the WAF method was used to prepare the test media. WAFs were prepared by adding the appropriate amount of test item to a known volume of test medium (sterilized natural seawater for *A. tonsa* and sterile marine algal medium for *S. costatum*) to give loading rates of 10, 100 and 1000 mg litre^{-1}. Some of these products are readily biodegradable, therefore all the glassware were autoclaved at 121°C for 15 min and the seawater UV sterilized before used.

The water used to prepare the algal medium for *A. tonsa* was natural seawater. The water was filtered under vacuum and stored at 4°C in the dark until use. The algal medium for *S. costatum* was prepared by adding nutrient stock solutions to autoclaved seawater to give the final medium with the composition shown in *Table 2*. Nutrient stock solutions were prepared by dissolving Analar grade salts to the concentrations recommended by ISO 10253:1998, water quality - marine algal growth inhibition test with *Skeletonema costatum* and *Phaeodactylum tricornutum*. They were then autoclaved and stored in the dark at 4°C.

TABLE 2. CONCENTRATION OF NUTRIENTS IN THE *S. costatum* CULTURE MEDIUM

Nutrient	Concentration in final medium*
$FeCl_3.6H_2O$	149.0 μg litre^{-1} (Fe)
$MnCl_2.4H_2O$	605.0 μg litre^{-1} (Mn)
$ZnSO_4.7H_2O$	150.0 μg litre^{-1} (Zn)
$CuSO_4.5H_2O$	0.6 μg litre^{-1} (Cu)
$CoCl_2.6H_2O$	1.5 μg litre^{-1} (Co)
H_3BO_3	17.1.0 mg litre^{-1}
Na_2EDTA	15.0 mg litre^{-1}
Thiamine hydrochloride	25.0 μg litre^{-1}
Biotin	0.005 μg litre^{-1}
B_{12}	0.05 μg litre^{-1}
K_3PO_4	3.0 mg litre^{-1}
$NaNO_3$	50.0 mg litre^{-1}
$Na_2SiO_3.5H_2O$	14.9 mg litre^{-1}

Note: * For some nutrients, the final concentrations are only for the metal within the parentheses and not for the whole compound.

The algal medium were transferred into WAF vessels (2 litres conical flasks with a tap fitted close to the base) and gently stirred with a magnetic stirrer while the sample was carefully dispensed to the surface of the media using glass syringes of appropriate sizes (50, 500 or 5000 μl). The volume of sample dispensed was approximately that required to produce the desired loading rate, *i.e.* 10, 100 or 1000 mg litre^{-1}. The actual amount added was determined by weighing the syringe before and after dispensing the sample.

The contents of the WAF vessels were stirred at 300 rpm for 24 hr in a temperature-controlled room set at 20°C ± 2°C. After 24 hr, the stirrers were switched off and the contents left to settle for 2 hr to 3 hr for the undissolved material to separate out. The aqueous phases – the WAFs – were then drawn off via the tap for use in the tests. The control mediums were sterilized seawater (*A. tonsa*) and algal medium (*S. costatum*) stirred the same way as the WAFs.

Test organisms. Toxicity tests were performed using the standard test methods - ISO 14669:1999, water quality – determination of acute lethal toxicity to marine copepods *(Copepoda, Crustacea)* on the marine

copepod, *A. tonsa*, and ISO 10253:1998, water quality - marine algal growth inhibition test with *Skeletonema costatum* and *Phaeodactylum tricornutum* on the marine algae, *S. costatum*.

The *A. tonsa* were obtained from Guernsey Sea Farms, Vale, Guernsey, England, as age-standardized adults, 13 – 15 days old. They were maintained in a temperature-controlled room at 20°C ± 2°C with a 16 hr light and 8 hr dark photoperiod and fed *ad libitum* with mixed marine algae supplied with them.

S. costatum (CCAP 1077 / 5) was obtained from the Scottish Association for Marine Science Research Services Ltd, Dunstaffnage, Oban, Scotland. Prior to the test, 1 ml aliquots from the stock culture were examined under a microscope using a Sedgewick Rafter cell and a healthy culture (well-developed cells, forming chain) selected to provide the initial inoculum to the starter culture. The average chain lengths were determined using a Coulter particle counter (Model ZM). The 1.2 ml^{-1} flask of culture was used to inoculate two starter culture flasks containing 200 ml sterile medium giving a concentration of 1 x 10^4 cells ml^{-1}. The flasks were incubated at 20°C ± 2°C in an incubator shaker (orbital) under constant illumination (9000 lux) for four days.

***Acute toxicity tests on* A. tonsa**. The tests were conducted in 150 ml glass crystallizing dishes containing 100 ml of either the control (seawater) or test solution (WAF). Three dishes per WAF were prepared. Another three dishes containing natural seawater were set up as the controls. Ten *A. tonsa* were put in each dish using a modified glass pipette.

The immobilized *A. tonsa* were recorded and removed from the test vessels after 24 and 48 hr exposure. *A. tonsa* was considered immobile if they failed to respond when touched with the end of a sealed glass pipette. At the end of the tests, a few drops of formalin were added to the test dishes to preserve the remaining *A. tonsa* for subsequent counting.

The tests were performed in a temperature-controlled room set at 20°C ± 2°C, with artificial illumination of 16 hr light and 8 hr dark cycle. The solutions were not renewed or aerated during the tests.

***Acute toxicity tests on* S. costatum.** The tests were conducted in 250 ml Erlenmeyer flasks containing 100 ml WAF, inoculated with sufficient *S. costatum* to give an initial estimated cell concentration of 10^4 cells ml^{-1}. Three flasks were prepared for each WAF and an additional six flasks containing only the algal medium as controls. An additional flask from each WAF and the control were not inoculated to serve as blanks. The data from the blanks were used during particle counting to correct for the particles, other than algae, present in the media. The flasks were stoppered and incubated in an incubator shaker (100 cycles per minute) under constant illumination (7400 - 8550 lux) at 20°C ± 2°C for three days. The particles in each flask were counted at the start of the test and after 24 hr, 48 hr and 72 hr incubation using a particle counter (Coulter Model ZM). At the end of the test, estimations of the mean chain length of *S. costatum* in one flask from each concentration were made by microscopic examination of at least 50 chains in a 1 ml sub sample.

Reference toxicant. To verify the sensitivity of the test organisms, the reference material, 3,5-dichlorophenol (3,5-DCP, CAS No. 591-35-5, Sigma Aldrich Ltd.), was prepared at a single concentration for each test. For the test with *A. tonsa*, 3,5-DCP was prepared at 1.0 mg $litre^{-1}$ in autoclaved seawater, and for *S. costatum* at 1.5 mg $litre^{-1}$ in sterilized algal medium. The solutions were stirred for at least 1 hr prior to testing.

Measurement of total carbon. TC analysis (Shimadzu 5000) was conducted on duplicate aliquots (ca. 10 ml) of all the WAFs at the beginning of the exposure period. The test vessels and analytical sample vials were cleaned thoroughly before use (acid wash) and dried in an oven to minimize contamination.

Treatment of Results

A. tonsa. The range of loading rates within which 50% of the exposed *A. tonsa* were immobilized (EL_{50}) after 24 hr and 48 hr were determined from the data.

S. costatum. The effects of the samples on the algal growth were evaluated using two approaches (ISO 10253, 1998):

- comparison of the areas under the growth curve (a); and
- comparison of the average specific growth rates (μ).

The range of loading rates within which a 50% reduction in algal growth occurred (EL_{50}) after 72 hr was determined on the basis of both the area under the growth curve (termed E_bL_{50}) and their specific growth rate (termed E_rL_{50}).

RESULTS AND DISCUSSION

Behaviour of Test Samples in Water

The WAFs of the samples were assessed by visual inspection on addition of the sample to the media and at the end of the settling period. The results of these evaluations are given in *Table 3*. *Figure 1* shows the sample being stirred in the WAF vessel during WAF preparation.

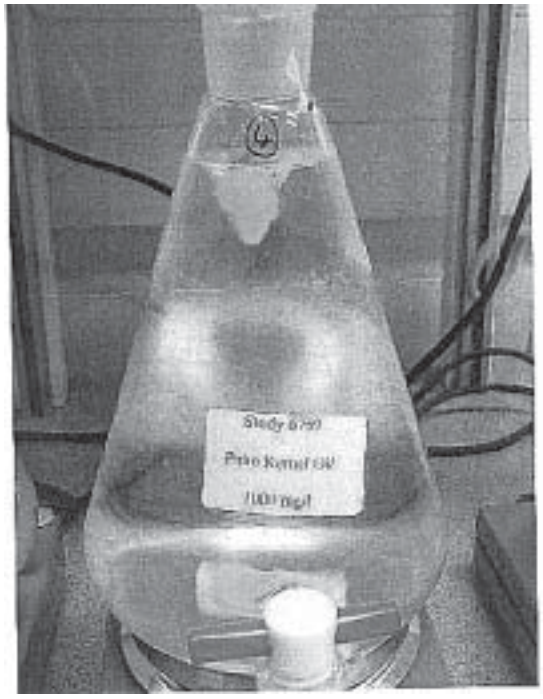

Figure 1. Water accommodated fraction (WAF) of a palm kernel product during stirring.

Chemical Analysis

The TC found in the WAFs of every sample is shown in *Table 4*, it increased with the loading rate. The TC in WAFs prepared for the *A. tonsa* tests was comparable to the TC in WAFs for the *S. costatum* tests.

Toxicity Tests

A. tonsa. The toxicities of the WAFs of CPKO, CPKST and CPKOL at different loading rates are shown in *Table 5* and a summary of the EL_{50} values in *Table 7*.

The immobilization of *A. tonsa* in the control vessels was 11% after 48 hr, which is within the limit of control mortality permitted for a valid test, *i.e.* 15% (ISO 14669, 1999). The percentage of *A. tonsa* immobilized when exposed to 1.0 mg litre^{-1} 3,5-DCP was 58% after 48 hr, which was also within the range of 20% - 80% as stipulated in the standard.

The WAFs prepared from CPKO, CPKST and CPKOL were not toxic to *A. tonsa* with 48 hr EL_{50} values of > 1000 mg litre^{-1}, *i.e.* the highest loading rate tested.

TABLE 3. APPEARANCE OF WATER ACCOMMODATED FRACTIONS (WAFs) OF PALM KERNEL PRODUCTS

Sample	Appearance of WAF (1000 mg litre^{-1} loading rate)	
	At start of stirring	**At end of settling period**
CPKO	Disc shaped solids or lumps spinning in vortex with smaller lumps at a lower level in the flask	Disc shaped solids or 2 large lumps on the surface. Oil also coating the stirring bar.
CPKST	Disc- or cone-shaped solids with smaller lumps at a lower level in the flask	Irregular- or cone-shaped lumps on the surface of the media.
CPKOL	Mass of small globules spinning in the body of the medium or cone-shaped globules in vortex with smaller ones at a lower level in the flask	Large lumps on the surface with a substantial amount coating the stirring bars in both tests.

TABLE 4. CONCENTRATIONS OF TOTAL CARBON IN TEST MEDIUM BEFORE START OF TEST

Sample	Loading rate (mg litre^{-1})	*A. tonsa* medium			*S. costatum* medium		
		TC* (mg litre^{-1})	TC** (mg litre^{-1})	% TC in WAF	TC* (mg litre^{-1})	TC** (mg litre^{-1})	% TC in WAF
Control	-	28.5	-	-	31.0	-	-
CPKO	10	28.1	-0.4	**-1.4**	30.7	-0.3	**-1.0**
	100	29.1	0.6	**2.0**	31.8	0.8	**2.5**
	1 000	33.2	4.7	**14.0**	35.2	4.2	**11.9**
CPKST	10	28.3	-0.2	**-0.7**	30.7	-0.3	**-1.0**
	100	28.6	0.1	**0.3**	30.9	-0.1	**-0.3**
	1 000	29.2	0.7	**2.0**	32.3	1.3	**4.0**
CPKOL	10	28.4	-0.1	**-0.4**	30.8	-0.2	**-0.6**
	100	29.7	1.2	**4.0**	32.4	1.6	**5.0**
	1 000	33.9	5.4	**16.0**	36.1	5.1	**14.1**

Notes: * Mean of three replicates.
** Test minus control.

TABLE 5. IMMOBILIZATION OF *A. tonsa* EXPOSED TO WATER ACCOMMODATED FRACTIONS (WAFs) OF CRUDE PALM KERNEL OIL (CPKO), CRUDE PALM KERNEL STEARIN (CPKST), CRUDE PALM KERNEL OLEIN (CPKOL) AND 3,5-DICHLOROPHENOL (DCP)

Sample	Loading rate (mg litre^{-1})	Number of *A. tonsa* of exposed	Cumulative number (N) and percentage (P) *A. tonsa* immobilized after exposure period			
			24 hr		48 hr	
			N	P	N	P
Control	-	35	1	3	4	11
CPKO	10	35	0	0	6	17
	100	34	1	3	3	9
	1 000	32	2	6	5	16
CPKST	10	33	2	6	3	9
	100	36	1	3	5	14
	1 000	39	5	13	10	26
CPKOL	10	31	1	3	7	23
	100	34	1	3	2	6
	1 000	35	0	0	2	6
3,5-DCP	1	33	4	12	19	58

S. costatum. The toxicities of the palm samples towards *S. costatum* are summarized in *Table 6* while the EL_{50} values are given in *Table 7*. The plots of the mean chain numbers of *S. costatum* against time for each sample and 1.5 mg litre^{-1} 3,5-DCP are shown in *Figures 2* to *5*.

The average chain density in the control vessels increased by a factor of 38 in 72 hr, more than the 16-fold increase in growth required by the test method for a valid test (ISO 10253, 1998), indicating that good growth occurred in the control vessels. The effect of 3,5-DCP on *S. costatum* between 0 and 72 hr reduced the area under the growth curve by 87% and the average specific growth rate by 37% compared to the controls. The reduction in growth rate complies with the OSPAR (1995) guidelines, which state that a 20%-80% reduction in growth should be seen when *S. costatum* is exposed to 1.5 mg litre^{-1} 3,5-DCP.

The WAF prepared from 1000 mg litre^{-1} CPKO completely retarded the growth of *S. costatum* after 72 hr. The WAF from 100 mg litre^{-1} CPKO caused a 83% reduction in the biomass (A, area under the growth curve) of *S. costatum* compared to the controls. An inspection of the specific growth rates (μ) however, indicated that the algae began to recover after 48 hr, as the overall growth rate in 72 hr was

TABLE 6. GROWTH OF *S. costatum* EXPOSED TO WATER ACCOMMODATED FRACTIONS (WAFs) OF CRUDE PALM KERNEL OIL (CPKO), CRUDE PALM KERNEL STEARIN (CPKST), CRUDE PALM KERNEL OLEIN (CPKOL) AND 3,5-DICHLOROPHENOL (DCP)

Sample	Loading rate (mg litre^{-1})	Mean reduction in area under the growth curve (A) relative to unfiltered controls (%)			Mean reduction in average specific growth rate (μ) relative to unfiltered			Mean number of cells per chain controls (%)
		24 hr	48 hr	72 hr	24 hr	48 hr	72 hr	72 hr
Starter culture	-	-	-	**-**	-	-	**-**	3.6
Control	-	-	-	**-**	-	-	**-**	4.3
CPKO	10	18	15	**8.4**	12	5.8	**1.4**	4.4
	100	81	93	**83**	65	81	**28**	4.6
	1 000	80	97	**100**	64	> 100	**> 100**	-
CPKST	10	4.9	10	**9.7**	9.5	8.1	**6.3**	4.3
	100	19	16	**10**	12	6	**1.5**	4.0
	1 000	80	94	**84**	64	92	**28**	4.6
CPKOL	10	26	27	**17**	20	13	**4.2**	4.4
	100	87	97	**93**	76	> 100	**49**	4.7
	1 000	87	98	**100**	76	> 100	**> 100**	-
3,5-DCP	1.5	79	94	**87**	66	90	**37**	4.1

TABLE 7. SUMMARY OF EL_{50} VALUES DETERMINED FROM BOTH TOXICITY TESTS

Sample	Range of loading rates within which EL_{50} values are found (mg litre^{-1})		
	A. tonsa immobilization (48 hr)	Reduction in growth of *S. costatum* compared to controls (%) after 72 hr	
		Area under growth curve (A) (E_bL_{50})	Specific growth rate (μ) (E_rL_{50})
CPKO	> 1 000	10 – 100	100 – 1 000
CPKST	> 1 000	100 – 1 000	> 1 000
CPKOL	> 1 000	10 –100	100 – 1 000

only reduced by 28% compared to the controls. No effects on growth were seen in the WAF prepared from 10 mg litre^{-1} CPKO. This shows that the EL_{50} of CPKO, based on the area under the growth curve (E_bL_{50}), is between 10 and 100 mg litre^{-1}, and the EL_{50}, determined by the specific growth rate (E_rL_{50}), is between 100 and 1000 mg litre^{-1} (*Table 7*).

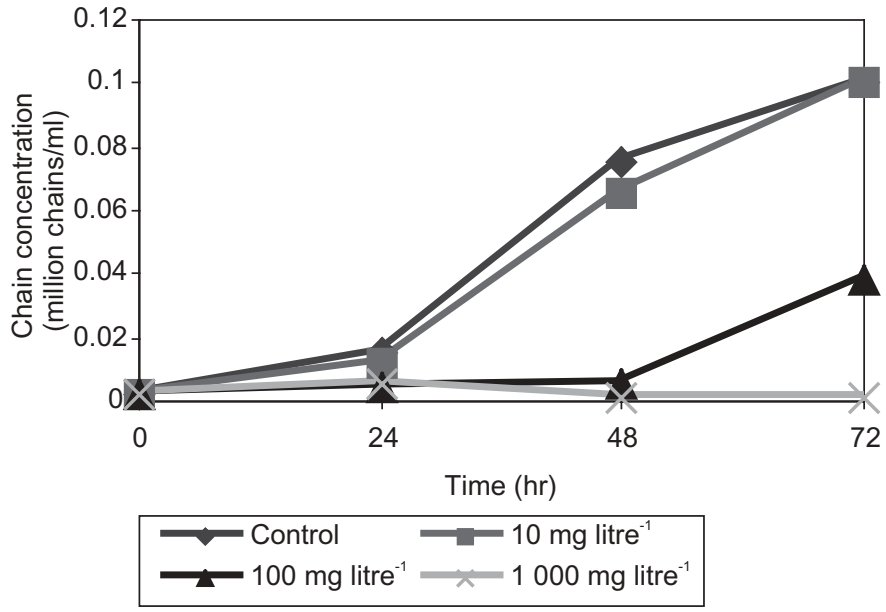

Figure 2. Growth of S. costatum *exposed to crude palm kernel oil (CPKO) over 72 hr.*

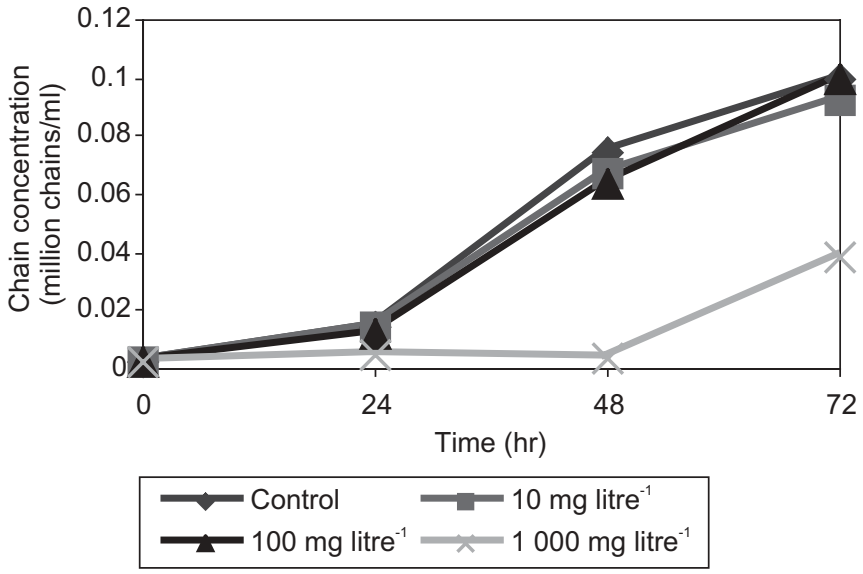

Figure 3. Growth of S. costatum *exposed to crude palm kernel stearin (CPKST) over 72 hr.*

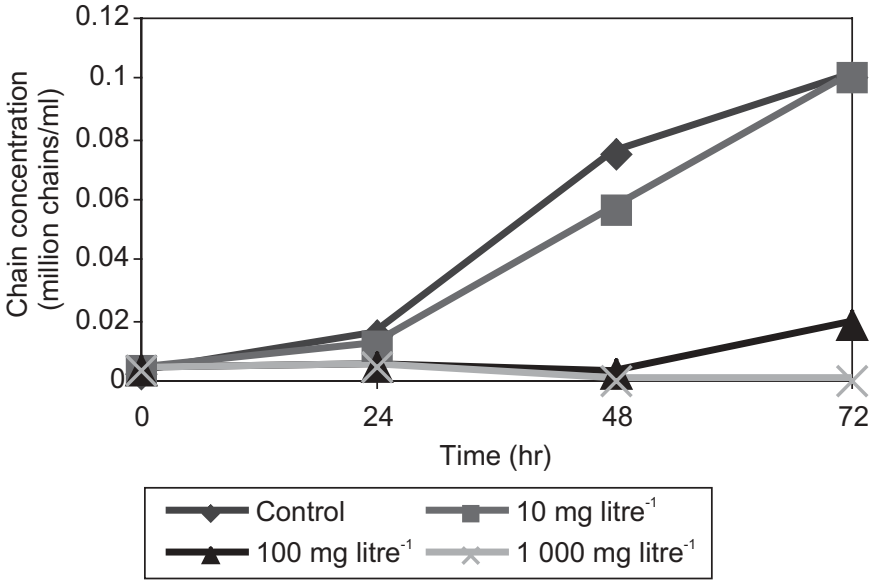

Figure 4. Growth of S. costatum *exposed to crude palm kernel olein (CPKOL) over 72 hr.*

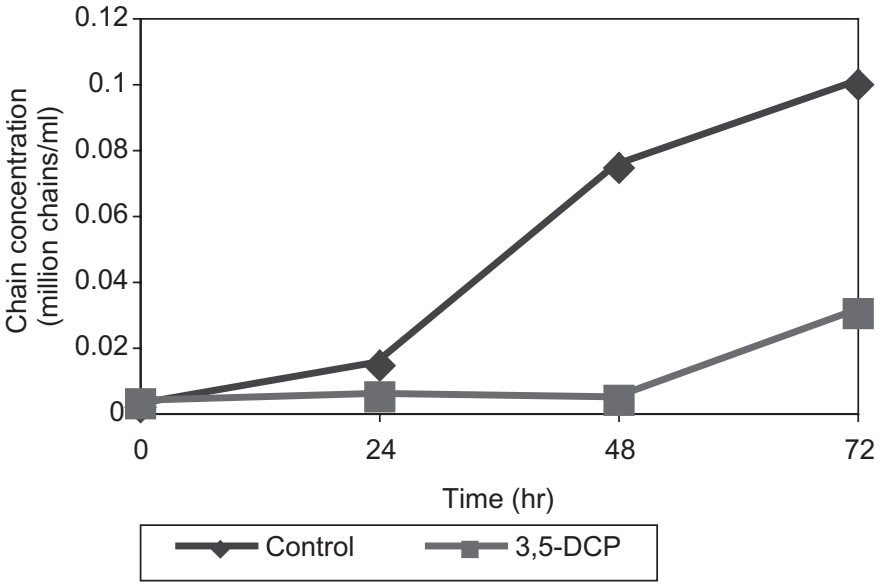

Figure 5. Growth of S. costatum *exposed to reference compound, 3,5-DCP, over 72 hr.*

The WAF from 1000 mg litre^{-1} CPKOL completely retarded the growth of *S. costatum*, based on A and μ, after 72 hr. The WAF of 100 mg litre^{-1} CPKOL caused 93% reduction in A. The overall μ after 72 hr was reduced by 49% compared to the controls. No effects on growth were seen by the WAF prepared from 10 mg litre^{-1} CPKOL. The EL_{50} for CPKOL, based on the area under the growth curve (E_bL_{50}), is between 10 and 100 mg litre^{-1} while the EL_{50} determined *via* the specific growth rate (E_rL_{50}) is between 100 and 1000 mg litre^{-1}.

The WAFs prepared from 1000 mg litre^{-1} CPKST however, did not completely retard the growth but only reduced it by 84% based on A and 28% based on μ after 72 hr. The WAFs from 10 and 100 mg litre^{-1} CPKST were not toxic to *S. costatum* (reduction of < 7%). This means that the EL_{50} values for CPKST, based on the area under the growth curve (E_bL_{50}), is between 100 and 1000 mg litre^{-1} while the EL_{50} based on the specific growth rate (E_rL_{50}) is > 1000 mg litre^{-1}.

CPKST was therefore less toxic to *S. costatum* than CPKO and CPKOL. This may be related to the solubility of the palm kernel products as total carbon analysis of the WAFs indicated that CPKST is only sparingly soluble in water (0.3%-4.0%) compared to CPKO (2.0%-14.0%) and CPKOL (4.0%-16.0%) (bold columns in *Table 4*).

Table 6 also shows that the average number of cells per chain in all the WAFs were similar. This means that, where algal growth occurred, the average chain length was not affected by any of the samples or by the reference compound. The growth of *S. costatum* was completely retarded in 1000 mg litre^{-1} CPKO and CPKOL, therefore no chains were found in the WAFs.

CONCLUSION

The many reported spills of bulk cargoes at sea have resulted in the public's grave concern over the safety of products to the marine organisms and habitat.

The toxicity tests on *A. tonsa* showed that the EL_{50} values for CPKO, CPKST and CPKOL were all > 1000 mg litre^{-1}. None of the palm kernel products prepared as WAFs at loading rates of 10, 100 and 1000 mg litre^{-1} were toxic to *A. tonsa*.

Neither CPKO nor CPKOL was toxic to *S. costatum* at 10 mg litre^{-1}. However, they were slightly toxic at 100 mg litre^{-1} and toxic at 1000 mg litre^{-1}, where algal growth was initially inhibited but recovered after 48 hr. CPKST was not toxic to *S. costatum* at 10 and 100 mg litre^{-1}. It was initially toxic to *S. costatum* at 1000 mg litre^{-1}, but algal growth recovered after 48 hr. CPKST was less toxic to *S. costatum* than CPKO and CPKOL due to its very low solubility in water as measured by total carbon analysis of the WAFs.

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