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TOXICITY TESTING OF OIL DISPERSANT ON *PENAEUS MONODON*

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ABSTRACT

Toxic effects of the oil dispersants Corexit 9527 and BP 1100-X were studied in Thailand. Static acute bioassay with standard reference toxicant (DSS), was carried out on tiger prawn (*Penaeus monodon*) at post larval stage (28-30 days old). Oxygen level (>3 ppm), pH, and temperature were maintained as constant as possible throughout the tests. Probit analysis was applied to determine the 24, 48 and 96 hrs LC 50 at 95% confidence limit. Corexit 9527 was more toxic, and led to delayed moulting as compared to BP 1100-X.

INTRODUCTION

Non persistent oils are gasoline, naphtha, kerosene and diesel which rapidly evaporate from the sea surface. Persistent oils are crude and refined residual oils which disperse slowly and usually require a clean-up action. The rate of oil dispersion largely depends upon the nature of the oil and the condition of the sea. Wave and turbulence often break the oil slicks into small lumps and droplets. Oil droplets exist in suspension while small lumps remain at the surface and coalesce with other droplets to reform a slick. The prevention of spill is, of course, the first consideration. But accidental oil spills occur, and they are usually of greatest concern since these often give rise to conspicuous acute pollution. The immediate treatment of the oil slick is crucial and often involves an oil dispersant to reduce the risk of damage to marine life, public amenities and shorelines.

The selection of a proper component of oil dispersant is extremely important (Canevari, 1969; Dodd, 1974). The surface-active agent must possess a molecular structure which is oleophil (affinity for oil) and hydrophil (affinity for water) at each pole of the molecule. The function of the chemical oil dispersant is not to destroy the oil. Its primary purpose is to increase the surface area of an oil slick by breaking it up into small globules (Oda, 1969). Therefore, application of chemical dispersants is considered to be the most efficient and rapid method to remove oil spills from the surface. Chemical dispersants can reduce oil-water interfacing tension to very low value. Hence, it only takes a small amount of mixing energy to increase the surface area and break the slick into droplets. Dispersants also perform a secondary role by preventing coalescence of oil droplets so they become more susceptible to biodegradation. However, the dispersant itself has some level of toxicity, although the toxicity of modern dispersants is far less than earlier formulations. Generally, dispersants result in increased hydrocarbon concentration in the water column and may lead to biological damage. Smith (1968) reported that damage caused by the Torrey Canyon wrecking in 1967 was due to highly toxic dispersants used in large quantities on particularly sensitive areas which finally led to extensive ecological damage. But the effects of dispersants differ considerably between location with different environmental conditions (Murphy, 1969).

In Thailand the effects on marine life of acute and chronic use of oil dispersants are still inadequately known. The present bioassay of oil dispersants on tiger prawn, *Penaeus monodon* attempts to determine the toxicity of the two oil dispersants Corexit 9527 and BP 1100-X which are widely used. Short-term lethal testing appears to be a suitable method to compare toxicities. The purpose of this bioassay toxicity testing is not only to elucidate the use of least toxic oil dispersant but also to highlight safety applications to enable the dispersant users themselves to judge the suitability of the available dispersants.

MATERIALS AND METHODS

Static acute toxicity tests were carried out according to Sprague (1969) and Buikema *et al.,* (1982).

Two types of dispersants, Corexit 9527 and BP 1100-x were applied. Dodecy 1 Sodium Sulphate (DSS) was used as reference toxicant. The tests were conducted at Phuket Marine Biological Center on tiger prawn (*P. monodon*) at post larval stage, about 28-30 days old (P 28-30 stage). Wet weight and length of the tested shrimps were 160-205 mg and 2.8-3.2 cm, respectively.

The test animals were acclimated and continuously fed in well aerated tanks for about one week prior to the test. They starved 24 hours before start of each experiment. Aeration and food were not given during the test.

Two control tests and 3 replicates were conducted for each test which encompassed 10 shrimps placed individually in 10 small plastic baskets (7x7x14 cm) in order to prevent cannibalism during moulting.

Dissolved oxygen, temperature and pH were recorded in each tank at the beginning of the test and every 24 hours. Fresh medium was added when the level of oxygen dropped below 3 ppm. The frequency of observations followed a logarithmic series of 0.5, 1, 1.5, 2.5, 4, 6, 10, 12, 24, 36, 48, 60, 72, 84 and 96 hrs. The number, weight and length of dead shrimps and moults were recorded at each observation.

Eight concentrations of each dispersants and reference toxicant DSS were tested according to results from a pilot study.

Data treatment was according to Litchfield and Wilcoxon (1949). The LC 50 for 24, 48 and 96 hrs were calculated by the probit method (95% confidence limit). Chi-square values were determined for the best fit of regressions of percentage mortality at specific observation time (probit scale) against dispersant concentrations (log scale). The slope function (S) was calculated.

RESULTS

The number of dead P28-30 stage shrimp larvae (*P. monodon*) exposed to two oil dispersants depended upon the concentration of the chemicals and duration of exposure as shown in Table 1. After 96 hours exposure the toxic threshold concentration LC 50 was relatively low and it appeared to be lower in Corexit 9527 than in BP 1100-X (Table 2). This conclusion was tested. Though the results varied somewhat, the values were within the acceptable range and thus clearly supported the conclusion (Tables 3 & 4).

Table 1. The % cumulative death of *P. monodon* as a function of time and concentration.

Chemical	Conc.	% Cumulative death		
	(ppm)	24 hr	48 hrs	96 hr
Corexit 9527	2	-	-	26.6
	4	10.0	16.66	50.0
	7	10.0	43.33	73.3
	12	13.3	36.66	80.0
	20	13.3	46.68	86.6
	30	40.0	80.00	-
	42	50.0	85.00	-
BP 1100-X.	30	-	-	10.0
	50	-	6.66	13.3
	80	-	13.33	30.0
	1,30	10.0	20.00	43.3
	2,00	23.3	26.66	53.3
	3,20	30.0	36.66	66.6
	4,40	30.0	70.00	-
	6,00	50.0	95.00	-
DSS	5,00	3.3	10.00	13.3
	6,00	13.3	16.66	20.0
	7,00	20.0	26.66	33.3
	8,00	30.0	36.66	53.3
	9,00	30.0	40.00	60.0
	10,00	36.6	43.33	63.3
	11,00	46.6	63.33	-
	12,00	80.0	86.66	-

Daily temperature in air and media showed no significant difference when tested ($P<0.05$). Similarly, very small variations were recorded in pH values of the media. Dissolved oxygen dropped after the first day, but the levels remained around 3 ppm. Analyses of replicates of Corexit 9527, BP 1100-X, and DSS showed that the pH and dissolved oxygen varied from 7.81-8.03 and 3.04-4.04 ppm, respectively, while the controls ranged from 8.00-8.11 pH units, and 5.08-5.15 ppm dissolved oxygen (Table 5).

Table 2. Summary of 24, 48 and 96 hrs LC50 (ppm)

	Corexit 9527	BP 1100 - X	DSS
24 hrs			
LC50 (ppm)	470	5600	10600
$(Chi)^2$	0.66	2.13	6.40
95% CL	355 - 623	4351 - 7207	9711 - 11570
S	3.92	3.39	1.58
48 hrs			
LC 50 (ppm)	160	3600	9600
$(Chi)^2$	7.04	3.14	5.96
95% CL	120 - 213	2818 - 4598	8795 - 10479
S	4	3.27	1.58
96 hrs			
LC 50 (ppm)	42	1600	8200
$(Chi)^2$	0.91	6.43	1.02
95% CL	32 - 55	1253 - 2044	7595 - 8851
S	3.69	3.27	1.49

In the control tanks, the number of moults ranged from 35-50% of total shrimps throughout the 96 hrs test (Table 6). The number of moults were considered non-significantly different when compared to the dispersant (45% for Corexit 9527, 35% for BP 1100-X and 50% for DSS). The total number of moults was lowest in BP 1100-X (18%).

Table 3. The % cumulative death of *P. monodon* as a function of time and concentration, in the confirm test.

Chemical	Conc. (ppm)	% Cumulative death 24 hrs	48 hr	96 hr
Corexit 9527	5	5	2	6
	20	45	6	9
	40	55	7	10
BP 1100-X	1,30	5	1	5
	3,50	35	5	7
	6,00	45	7	9
DSS	8,00	10	4	7
	10,00	25	5	7
	11,00	40	5	8

In Corexit 9527 (49.0%) and DSS (42.6%), no significant differences were observed (P>0.05). The number of live. moulting shrimps during the test was 11.36% for Corexit 9527, 11.82% for BP 1100-X, and 22.5% for DSS.

Mortality of moulting shrimps was higher during exposure to Corexit 9527 (66.93%) than to BP 1100-X (14.42%). In contrast, lower mortality occurred without moulting in shrimps exposed to Corexit 9527 (33.07%) compared to BP 1100-X (85.58%). The reference toxicant (DSS) showed a rather constant percentage of dead shrimps (40-60%).

Table 4. Summary of 24, 48, and 96 hrs LC 50 (ppm) confirm test.

	Corexit 9527	BP 1100-X	DSS
24 hrs			
LC 50 (ppm)	380	5800	11700
$(Chi)^2$	1.21	0.55	0.10
95% CL	270 - 535	4590 - 7328	10949 - 12503
S	3.88	2.52	1.30
48 hrs			
LC 50 (ppm)	135	3500	9500
$(Chi)^2$	0.32	0.11	0.04
95% CL	95 - 191	606 - 4701	7204 - 12528
S	3.97	2.59	3.26
96 hrs			
LC 50 (ppm)	41	1500	5800
$(Chi)^2$	0.66	1.07	0.37
95% CL	29 - 58	1118 - 2013	4776 - 7043
S	3.05	3.20	2.16

Weight and length of shrimps did not show any relationship to the two dispersants (Table 6). However, at the same frequency of depressed moults, Corexit 9527 required only 10-420 ppm while BP 1100-X required 300-6000 ppm during the 24, 48, and 96 hrs experiments. The chi-square and slope function of the probit line showed that Corexit 9527 was more toxic (S-value around 3.9) than the BP 1100-X which had a S-value around 3.20 (Tables 2 & 4). For Corexit 9527, the highest value of S (3.92) was obtained with the 24 hrs test, and the smallest value (3.69) with the 96 hrs test. The S values of BP 1100-X were rather constant around 3.0 for various test durations. The S value of DSS was very constant around 1.5 throughout the tests. The value of LC 50 of reference toxicant (DSS) at 24, 48, and 96 hrs were 10600, 9600, and 8200 ppm, respectively. They were higher than those of Corexit

9527 and BP 1100-X. The test was repeated on a new batch of *P. monodon.* The LC 50 values at 24, 48, and 96 hrs, were 11700, 9500 and 5800 ppm. The two sets of values were not significantly different (P>0.05).

Table 5. Daily temperature (^{0}C), pH, and disolved oxygen (DO) during the test.

Dispersant	Corexit 9527				BP 1100 - X				DSS			
	Temperature		pH	DO	Temperature		pH	DO	Temperature		pH	DO
Day	Room	Water		ppm	Room	Water		ppm	Room	Water		ppm
1	29.5	27.0	8.2-8.4	6.04±0.45	30.0	28.0	8.2-8.4	6.04±0.45	27.0	26.5	8.1-8.3	6.39±0.59
2	29.5	28.0	7.4-8.1	3.06±0.89	28.0	27.0	7.4-8.0	3.04±0.91	27.0	26.5	7.9-8.2	2.76±1.05
3	30.0	28.0	7.7-8.1	2.71±1.05	28.0	27.0	7.8-8.1	2.79±1.12	28.0	26.5	7.9-8.1	3.57±1.76
4	30.0	28.5	7.2-7.7	3.03±0.84	27.5	27.0	7.2-7.8	3.11±0.87	28.5	26.5	7.8-8.0	2.92±0.71

Table 6. Summary of moulting and dead record throughout the experiment (96 hrs) for Corexit 9527, BP 1100-X, and DSS.

Dispersants / Condition	Corexit 9527	BP 1100-X	DSS
Total Shrimps in control	20	20	20
Moulting noted	9 (45%)	7 (35%)	10 (50%)
Total shrimps in test tanks	220	220	240
Total moulting noted	108 (49.09%)	41(18.63%)	101 (42.48%)
Moulted alive shrimps	25 (11.36%)	26 (11.82%)	54 (22.5%)
Number of dead animals in test tanks	124	104	116
Number of dead animals found with moulted skin	83 (66.93%)	15 (14.42%)	47 (40.52%)
Number of dead animals found without moulted skin	41 (33.07%)	89 (85.58%)	69 (59.48%)
Length of animals	3.2 ± 0.474	3.07 ± 0.249	2.81 ± 0.250
Weight of animals	205.05 ± 103.06	184.42 ± 46.66	160 ± 45.53

DISCUSSION

The toxicity test showed that the lowest 24, 48, and 96 hrs LC 50 were found when using Corexit 9527 (470, 160, and 42 ppm, respectively). It reflects that Corexit 9527 is more toxic than BP 1100-X. Similar results have been reported by Bussarawit and Chantrapornsyl (1989) and National Research Council (anonymous, 1989). The value of 48 hrs LC 50 was close to the mean value of 24 and 96 hrs. Shelton (1969) suggested that the 48hrs LC 50 static test was the most useful tool to make a rapid comparison of the acute toxicities of a wide range of oil dispersants.

A number of bioassay studies have been carried out on the effect of Corexit 9527, and BP 1100-X (National Research Council, anonymous, 1989), but the results seem to be incomparable because of different temperatures and test organisms. Previous tests were conducted under temperate zone conditions while the present tests were conducted under tropical conditions. Many studies show that dispersants become less toxic when lowering the temperature (Wells, 1984; Ordzie and Garofalo, 1981).

The UNEP/EAS Project 2.2 (1987) showed that the present dispersants also were tested for toxicity on *Tilapia* spp. in Indonesia, Malaysia, Philippines and Thailand. It was concluded that Corexit 9527 was toxic (48 hrs LC 50 = 80 ppm) while BP 1100-X showed moderate toxicity (48 hrs LC = 960 ppm) according to the toxicity rating (Table 7). All data confirm that Corexit 9527 is more toxic than BP 1100-X on many kinds of organisms under various conditions.

Table 7. Toxicity Rating of Oil Dispersants for the brown shrimp *Crangon crangon* in the U.K., according to Jeffery and Nichols (1973).

48 h LC 50 ($\mu l\ l^{-1}$)	Description	Rating
> 10,000	Practically Non-toxic	1
10,000-1,000	Slightly Toxic	2
1,000-100	Moderately Toxic	3
100-10	Toxic	4
<10	Very Toxic	5

The toxic levels of dispersants in the present study show that the toxicity on *P. monodon* was more severe than found for other species under temperate conditions. It may be considered a result of the higher temperature associated with increased uptake rate of chemicals due to increased activity of the organism at higher temperature.

Survival during moulting was higher in shrimps exposed to reference toxicant (DSS) than to Corexit 9527 and BP 1100-X. This reflects the lower toxicity of DSS compared to the other two dispersants. The numbers of moulted dead shrimps were significantly higher when exposed to Corexit 9527 (66.9%) than those exposed to BP 1100-X (14.4%). This indicates that the freshly moulted shrimps were more susceptible to Corexit 9527 than to BP 1100-X and DSS. Considering the percentage of total moulting noted throughout the experiment, the results also show a delayed moulting effect by BP 1100-X. In addition, it can also imply that the growth (indicated by moults) of *P. monodon* is more affected by Corexit 9527 than by BP 1100-X.

The Canadian Environmental Protection Service's Report recommended Dodecyl Sodium Sulphate (DSS) as a reference toxicant (EPS, anonymous, 1973). Reference toxicants are materials used to relate the toxic response of a test group at one time and place, to the toxic response of another test group of the same species at another time and place (Lee, 1980). LaRoche *et al.,* (1970) suggested that the use of a reference toxicant is desirable as a link between the findings of different investigators, as an internal standard to compare the relative toxicities of substances, and as a measurement of the con-dition of test organisms from different stocks.

The room temperature of 25.58 ± 1.16°C during the test was in accordance with Sprague (1973) who recommended that temperatures should not vary more than ± 1°C. Thus, temperature variations during the tests were not considered to have influenced the results.

Axiak and Abel (1991) and LaRoche *et al.,* (1970) proposed that aeration should be conducted when dissolved oxygen dropped below 3 mg/1. However, in this study, the level of dissolved oxygen was maintained by renewing the test media throughout the test since aeration may degrade or remove some fractions of oil dispersants, particularly volatile components. Aeration was not recommended in the UNEP/EAS Project 2.2 (1987) for the above reason. Renewal of the test media every 24 hours was suggested instead.

The tiger prawn, *P. monodon* was selected as test animal because of its economical importance. In addition, its behaviour, life cycle and distribution is known. The young stage of this shrimp is considered to be one of the most sensitive species to the xenobiotic stress. In Thailand, *P. monodon* is mostly farmed in estuarine areas which are the most susceptible to oil pollution and the use of oil dispersants. It is generally a standard criterion that mortality in the control test should not exceed 10% of total number of test animals (Sprague, 1973). In the present test, no death in control tanks occurred. Furthermore, the results showed that *P. monodon* could be use as test animal for this purpose. Accordingly, it was accepted that the mortality occurred was attributed to the toxicity of oil dispersants.

Litchfield and Wilcoxon (1949) considered LC 50 values a useful tool to judge between toxic threshold levels. However, there is no generally accepted method of deciding what fraction of LC 50 may constitute the safe concentration. Jones (1964) suggested that one tenth of a 48 hrs LC 50 should be permitted.

Toxicity curves are actually very useful as an overall view of what is happening in the test (Sprague, 1973). The goodness of fit of the probit line is estimated by the minimum value of Chi-square. In the present study the results of the actual and confirmed tests at 48 hrs showed no significant heterogeneity and the line had a good fit. It is suggested that the 48 hrs LC 50 of the confirmation test should give a suitable concentration (minimum value) for consideration.

The slope function (S) of the line is as important as the LC 50. The change of S will provide clues about the action of the toxicant. Wilson (1974) suggested that in oil dispersant toxicity tests, changes in S are often indicating loss of toxin occurring during the experiment caused, *e.g.*, by evaporation. S-values varied slightly in the present study and tended to decrease with increased exposure time. Different slopes of the probit line indicated a higher S-value of Corexit 9527, than of BP 1100-X. Ball (1967) suggested that concentrations which should be considered safe, partly depended on the S value of the probit line.

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REFERENCES

anonymous. 1973. Environmental Protection Service (EPS) 1973. Guidelines on the use and acceptability of oil spill dispersants. - Report, EPS 1-EE-73-1, Environment Canada, Ottawa, Canada, 54 pp.

anonymous. 1989. National Research Council 1989. Using Oil Spill Dispersants on the Sea. - National Academy Press. Washington, D.C. 335 pp.

Axiak, V. and P.D. Abel. 1991. Ecotoxicology and the Marine Environment. Elles Horwood Ltd. 268 pp.

Ball, I.R. 1967. The relative susceptibility of some species of freshwater fish poisons I. Ammonia. -Water Research **1**: 767-775

Buikema Jr., A.L., B.R. Niederlehner and J.Cairns Jr. 1982. Biological monitoring: part IV Toxicity testing.- Water Research **16**: 239-262.

Bussarawit, N. and S. Chantrapornsyl 1989. Toxicity of dispersants affected of *Tilapia nilotica. In:* - Proceeding of the 27th Kasetsart University Conference Fisheries Section. National Inland Fisheries Institute. January 30-February 1, 1989.

Canevari, G.P. 1969. General dispersant theory. - Proceeding Joint Conference on Prevention and Control of oil Spills. Washington, D.C. API 177-177.

Dodd, E.N. 1974. Oil and dispersants: Chemical considerations. *In*: L.R. Beynon and E.B. Cowell (*Eds*.). Ecological Aspects of Toxicity Testing of Oil and Dispersants, Barking; Applied Science Publishers Ltd. p. 3-10.

Jeffery, P.G. and J.A. Nichols. 1973. Dispersants for oil spill clean-up operations at sea, on coastal waters and beaches. Stevenage: Warren Spring Laboratory, Department of Trade and Industry, L.R. 193 (OP), 12 pp.

Jones, J.R.E. 1964. Fish and River Pollution. Butterworth, London. 203 pp.

LaRoche, G., R. Eisler, and C.M. Tarzwell. 1970 Bioassay procedure for oil dispersant toxicity evaluation.- Journal of the Water Pollution Control Federation **42**: 1982-1989.

Lee, D.R. 1980. Reference toxicants in quality control of aquatic bioassays. *In*: A.L. Buikema Jr., and J. Cairns Jr. (*Eds*.). - Aquatic Invertebrate Bioassays, American Society for Testing and Materials, Philadelphia, P.A. p. 188-189.

Litchfield Jr, J.T. and F.Wilcoxon. 1949. A simplified method of evaluating dose-effect experiments. - Journal of Pharmacology and Experimental Therapeutics **96**: 99-113.

Murphy, T.A. 1969. Evaluation of the effectiveness of oil dispersing chemicals. *In*: - Proceeding of Joint Conference on Prevention and Control of Oil Spills. p. 199-208.

Oda, A. 1969. Laboratory evaluation of chemical oil dispersants. *In*: - Proceeding of Joint Conference on Prevention and Control of Oil Spills. p. 193-197

Ordzie, C.J. and G.C. Garofalo. 1981. Lethal and sublethal effects of short term acute doses of Kuwait crude oil and a dispersant Corexit 9527 on bay scallops *Argopecten irradians* and two predators at different temperatures.- Marine Environmental Research **5**(3): 195-210,

Shelton, R.G.J. 1969. Dispersant toxicity test procedures. Proceeding Joint Conference on Prevention and Control of Oil Spills. Washington, D.C. API. p. 187-191.

Smith, J.E. 1968. Torrey Canyon Pollution and Marine Life. New York, Cam bridge University Press. 196 pp.

Sprague, J.B. 1969. Measurement of pollutant toxicity to fish: I Bioassay methods for acute toxicity. -Water Research **3**: 793-821.

— 1973. The ABC's of pollutant bioassay using fish. *In*: - Biological Method for the Assessment of Waster Quality, ASTM STP 528, American Society for Testing and Materials. p. 6-30.

UNEP/EAS Project 2.2 1987. Revised protocol for the bioassay of oil dispersants. - Fisheries Research Institute, Malaysia. 99 pp.

Wells, P.G. 1984. The toxicity of oil spill dispersants to marine organisms. *In* : T.E. Allen (*Ed.*) A current perspective in oil spill chemical dispersants: Research, experience, and recommendations. STP 840, Philadelphia, ASTM: 177-202.

Wilson, K. W. 1974. Toxicity tests for ranking oils and oil dispersants. *In*: L.R. Beynon and E.B. Cowell, (*Eds.*) Ecological Aspects of Toxicity Testing of Oil and Oil Dispersants. Barking, Applied Science Publishers : p. 11-22.