# THE TOXICITY of GAS OIL and DISPERSANTS on the BARNACLE *BALANUS AMPHITRITE AMPHITRITE* DARWIN (CRUSTACEA: CIRRIPEDIA) from SHATT AL–ARAB RIVER, IRAQ [+]

# سمية زيت الغاز والمشتتات اتجاه البرنقيل *BALANUS AMPHITRITE AMPHITRITE* (DARWIN) (صنف: القشريات، رتبة: السربيديا) المتواجد في نهر شط العرب، العراق

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**Abstract:**

**The toxicity of gas oil and BP1100 dispersant to the larvae (nauplii) and adult barnacle *Balanus amphitrite amphitrite* was studied. The percentage of non motility and dead individuals of the nauplii and adults of the barnacle was directly proportional with the concentration and exposure time of the chemicals. The adults and nauplii of *B. amphitrite amphitrite* had a similar affectability to the BP 1100. The nauplii killed at lower concentration than the adults. Cirral activity of the adults barnacle was reduced when exposed to BP 1100.**

**A comparison of the relative toxicity of the gas oil and three dispersants (BP1100, BP1100X and Corexit8666) to adult barnacle was also performed. The BP1100 was the most toxic dispersant, while BP1100X was the next most toxic dispersant. The remaining dispersant (Corexit8666) was relatively less toxic to the barnacle tested, while the gas oil was the least toxic.**

**المستخلص:**

**تم دراسة سمية زيت الغاز والمشتت الكيمياوي بي بي 1100 اتجاه الطور اليرقي (نوبليس) والطور البالغ للبرنقيل *B. amphitrite amphitrite*، وجد بان النسبة المئوية للأفراد غير المتحركة والنسبة المئوية للوفيات للطور اليرقي وللطور البالغ تتناسب تناسبا طرديا مع التركيز المستخدم وفترة التعرض للمركبين الكيمياويين. كان للطور البالغ وللطور اليرقي للبرنقيل نفس قابلية التأثر بالمشتت الكيمياوي بي بي 1100 ولكن الطور اليرقي يقتل بتركيز أوطأ من الطور البالغ. أظهرت الدراسة بان فعالية الذوءابات للطور البالغ تختزل بشدة عند تعرض البرنقيل إلى المشتت الكيمياوي بي بي 1100.**

**كما تضمنت الدراسة الحالية مقارنة السمية النسبية لزيت الغاز وثلاثة مشتتات كيمياوية (بي بي 1100 وبي بي 1100 اكس والكوريكست 8666) اتجاه البرنقيل البالغ. كان المشتت الكيمياوي بي بي 1100 أكثر المشتتات**

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**الكيمياوية سمية اتجاه البرنقيل يليه المشتت الكيمياوي بي بي 1100 اكس. في حين كان المشتت الكيمياوي الكوريكست 8666 اقل المشتتات الكيمياوية سمية نسبيا اتجاه البرنقيل، وكان زيت الغاز اقلها سمية.**

## Introduction:

The toxic effects of hydrocarbons and their derivatives on intertidal organisms and their planktonic larvae have been investigated [1, 2]. The toxicity of these chemicals is generally high and has a deleterious effects on marine life [3, 2].
Shatt Al–Arab river, the area of the concern in this paper, is the most important river in the south of Iraq. It is formed from confluence of Tigris and Euphrates rivers. Shatt Al–Arab river flows in a southeasterly direction downstream of Al–Fao and discharges into the Arabian Gulf. It is pours about $5x10^9m^3$ nutrient rich water into the Arabian Gulf each year [4]. The river is about 200km long with an average width of 500m and depth ranging from 8–15m [5]. In the last few years, It has suffered a number of oil spills resulting from groundings and collisions of vessels approaching or leaving its water [6, 7]. Such spills have been generally treated with various oil spill removers, but there have been very little researches undertaken on the effects of such chemicals on river life [8]. The common river barnacle *Balanus amphitrite amphitrite* Darwin is thus ideal candidates on which such studies can be initiated. Therefore, the present study investigates, the toxic effects of gas oil and BP 1100 dispersant on the naupliar larvae and adult of *B. amphitrite amphitrite* and, the comparative toxicity of gas oil and three dispersants (BP1100, BP100X and Corexit8666) on the barnacle.

## Materials and Methods:

### *Chemicals*:
The oils (gas oil-specific gravity @ 15.6°C maximum= 0.8398, viscosity CST @ 37.8°C maximum=6 and sulpher %weight maximum=0.5 and Basrah regular crude oil-medium-API gravity between 28–34) were supplied by Iraqi South Oil Company, Basrah, Iraq. They were transferred to laboratory by dark glass bottle closed tightly and kept in a cold and dark place prior to use. BP1100 and BP1100X dispersants (both non–soluble) were supplied from BP Trading Ltd., Great Britain, and Corexit8666 dispersant (non-soluble) from Esso chemicals. Carbon tetrachloride ($CCl_4$), n-hexane and methylene chloride were supplied from Burdick and Jackson laboratories, Inc. Sodium sulphate was supplied by Supelco SA. It was extracted with methylene chloride for 36h in a soxhlet. Following clean up by extraction, It was dried in an oven at 130˚C for about 24h and deactivated with deionized water at the recommended percentage prior to use.

### *Collection and acclimation of test animals*:
Specimens of adult barnacle *B. amphitrite amphitrite* with a shell diameter of between 2–8mm (which were gauged to possess ripe naupliar masses) were collected during low tides from clean location of Shatt Al-Arab river from Garmat Ali region during 2008 (Figure 1). Thus, the barnacles were possible not hardened to oil contamination. In the laboratory, the naupliar mass from each individual of barnacle was removed and placed in a partially blackened Petri dish of filtered river water. The dish was illuminated with an overhead 60W table lamp. The positively phototrophic stage I nauplii were pipetted off, as needed, from the light corner of the dish. They were rinsed in the river water before being placed in a beaker of filtered river water. The concentration of the larvae was adjusted to 80±2 individuals/ml. The adults barnacle (still attached to cobbles) were cleaned by brushing and were then acclimated

in aerated, filtered river water at a temperature of 20±2°C for one day. Only individuals with active cirral activity were selected for experimentation.

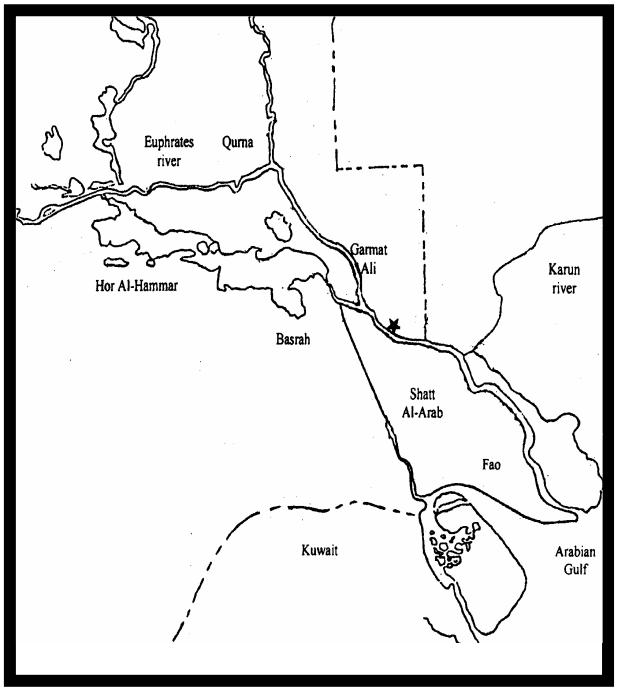


**Figure (1): Map of sampling location.**

## Preparation of test solution:

A dispersants (BP1100, BP1100, BP1100X and Corexit8666) and gas oil dispersion stock solution were prepared by adding 1ml. of chemical to a 1L volumetric flask contained a small amount of river water. The mixture was vigorously dispersed for 2h (for BP1100, BP1100, BP1100X and Corexit8666) and 30min (for gas oil) in a shaker. The mixture was then made up to 1L with additional filtered river water. Since both dispersants and gas oil were unstable, a fresh stock solution was prepared for each experiment. Various concentrations of these solutions were obtained by diluting an appropriate amount of stock solution with filtered river water immediately prior to each experiment. The salinity of the river water used in the experiments was 1.6‰±2 ‰.

To determine the concentration of chemicals used, the procedure of UNEP [9] was used. 100ml of nanograde carbon tetrachloride ($CCl_4$) was used in two successive 50ml extractions and the extracts were combined. The mixture was vigorously shaken to disperse the $CCl_4$ thoroughly throughout the water sample. The shaking is repeated several times before decanting the $CCl_4$. To these extracts a small amount of anhydrous sodium sulphate was added to remove excess water. The $CCl_4$ extracts were reduced in volume to less than 5ml by using a rotary evaporator. The reduced extract was carefully pipetted into a precleaned 10ml volumetric flask, making sure any residual particles of sodium sulphate were excluded and evaporated to dryness by a stream of pure nitrogen. The flask was then rinsed with fresh hexane and the rinsing used to make the sample volume up to exactly 5ml prior to UVF analysis (spectrofluorometer).

## Toxicity experiments:

The effect of BP1100 and gas oil on the nauplii of *B. amphitrite amphitrite* was studied by, introduced 1ml of river water containing nauplii into Petri dishes containing known concentrations of BP 1100 (i.e. 3 , 6 , 12 and 30ppm) and gas oil (i.e. 15, 30, 60 and 120ppm). The vaseline seal was applied to the Petri dishes to reduce the evaporation of BP1100 and gas oil. The numbers of motile and non motile individuals of nauplii were enumerated at intervals of 0, 4, 6, 12, 20, 40, 80min. Three replicates for each treatment were used. The mean number of non motile larvae subjected to the two chemicals was determined. Control barnacles in clean river water were established. The non motile individuals were isolated using a very fine pipette. They were rinsed with clean river water and placed in a dish of filtered river water for a recovery period of 2h. The number of non motile individuals was again counted and recorded, and the percentage recovery was calculated. Individuals remaining non motile were considered to be dead.

The effect of BP1100 on the adult barnacle was tested by immersing 40 individuals of adult barnacle (attached to cobbles) in the containers contained the test solutions of BP1100 in known concentrations (i.e. 70, 200 and 600ppm). The percentage inactive individuals (i.e. those failing to responded to being touched on their tergal and scutal plates) was determined after 3, 10, 20, 27 and 50h intervals. The test solutions were replaced every 24h by freshly prepared solutions. At the end of the tests, the inactive barnacles were then transferred to clean river water and allowed to recover for a further period of 48h Individuals falling to respond to a tactile stimulus at the end of this period were considered to be dead. The mortality was then calculated.

The effect of BP1100 on the cirral activity of adult barnacle was tested by immersing the barnacles (attached to cobbles) in the pre-aerated containers contained the test solution of BP1100 in the concentrations of 20ppm and 200ppm. The containers were covered with plastic bowl. The test solution was not aerated during the experiments. The time of 20 cirral beats of each of 10 barnacles was recorded at 0, 3 and 9h intervals. The cobbles were then removed from the test solution and the animals were rinsed with clean river water. The barnacles were then reimmersed in clean filtered river water for 18h The cirral activity recording continued for 9, 12 and 25h Control animals in clean river water were established.

The comparative toxicity of gas oil and the dispersants (BP1100, BP1100X and Corexit8666) to the adult barnacle was done by exposing 40 individuals of barnacle to 70ppm concentration of each chemical for 18h. The barnacles were then rinsed with clean river water and allowed to recover for 48h in filtered river water. The mortality was enumerated and recorded.

**Spectrofluorometer:**

The Shimadzu RF–540 spectrofluorometer equipped with a DR–3 data recorder was used to determine the concentration of chemicals used. The basis quantitative measurements were made by measuring emission intensity at 360nm with excitation set at 310nm and monochrometer slits of 10nm. The

reference oil used for calibration was Basrah regular crude oil obtained from South Oil Company.

**Calculation of $TC_{50}$ and $LC_{50}$:**
To compute the $TC_{50}$ and $LC_{50}$ values the method described by UNEP [9] was used.

**Results :**

Tables (1 and 2) showed the percentage of non motile individuals of nauplius larvae of *B.amphitrite amphitrite* exposed to various concentrations of BP1100 and gas oil at different time intervals and the $TC_{50}$ (i.e. that concentration which immobilizes 50% of the nauplii) values. The $TC_{50}$ values indicated that the BP 1100 dispersant was more toxic than gas oil. The effect of BP1100 and gas oil on the barnacle nauplii was approximately the same. The percentage of non motile individuals of nauplii of *B. amphitrite amphitrite* was directly proportional with the concentration and exposure time of the two chemicals. For both chemicals, the percentage of non motile nauplii sharply increased at 12min of exposure. The percentage of non motile nauplii decreased after 40min. of exposure in all concentrations of gas oil and only for lower concentration of BP1100 dispersant (i.e. 3 and 6ppm).
Tables (3 and 4) represented the percentage of mortality of naupliar larvae of *B. amphitrite amphitrite* exposed to various concentrations of BP1100 and gas oil at different time intervals followed by a 2h period of recovery in clean river water and the $LC_{50}$ (i.e. that concentration which is lethal to 50% of the larvae) values. The $LC_{50}$ values demonstrated again that the BP 1100 dispersant was more toxic than gas oil.
Table (5) showed the percentage of non active individuals of adults of *B. amphitrite amphitrite* exposed to various concentrations of BP1100 at different time intervals. The percentage of mortality of adults of *B. amphitrite amphitrite* exposed to various concentrations of BP1100 at different time intervals followed by a 48h period of recovery in clean river water was shown in (Table 6). The percentage of inactive individuals and the mortality of adult of *B.amphitrite amphitrite* was directly proportional with the concentration and exposure time of the BP 1100. The high mortalities were occurred after the 3h of exposure to BP1100 (i.e. 10, 20, 27 and 50h).
Table (7) illustrated the cirral activity of adults of *B. amphitrite amphitrite* exposed to various concentrations of BP1100 for 9h followed by a 18h period of recovery in clean river water. An increase in cirral activity was observed when the adults were returned to clean river water, however, the cirral activity was lower than that of the control following exposure to concentration of 200ppm of BP1100 followed by a period of recovery.
Table (8) illustrated the percentage of mortality of adults of *B. amphitrite amphitrite* exposed to 70ppm of three different dispersants and gas oil for a period of 18h. The BP1100 was the most toxic dispersant, while BP1100X was found to be the next most toxic dispersant. The Corexit8666 was relatively less toxic to the barnacle, while the gas oil was the least toxic.

**Table (1): The percentage of non motile individuals of naupliar larvae of *B.amphitrite amphitrite* exposed to various concentrations of BP1100 at different time intervals and the $TC_{50}$ values.**

| Concentration ppm | Time (min) | | | | | | |
|---|---|---|---|---|---|---|---|
| | 0 | 4 | 6 | 12 | 20 | 40 | 80 |
| | Percentage of non motile napulii | | | | | | |
| **3** | 0 | 6 | 7 | 12 | 13 | 12 | 11 |
| **6** | 2 | 8 | 13 | 50 | 75 | 71 | 54 |
| **12** | 10 | 33 | 45 | 90 | 92 | 94 | 97 |
| **30** | 21 | 90 | 95 | 98 | 98 | 98 | 98 |
| **$TC_{50}$** | 59.5 | 17.2 | 16.0 | 10.9 | 10.0 | 9.9 | 10.0 |

**Table (2): The percentage of non motile individuals of naupliar larvae of *B.amphitrite amphitrite* exposed to various concentrations of gas oil at different time intervals and the $TC_{50}$ values.**

| Concentration ppm | Time (min) | | | | | | |
|---|---|---|---|---|---|---|---|
| | 0 | 4 | 6 | 12 | 20 | 40 | 80 |
| | Percentage of non motile napulii | | | | | | |
| **15** | 0 | 0 | 2 | 5 | 6 | 22 | 20 |
| **30** | 7 | 8 | 11 | 24 | 30 | 41 | 35 |
| **60** | 13 | 23 | 28 | 49 | 74 | 70 | 48 |
| **120** | 20 | 61 | 83 | 98 | 97 | 96 | 86 |
| **$TC_{50}$** | 215.7 | 117.6 | 83.4 | 67.5 | 54.4 | 48.2 | 61.5 |

**Table (3): The percentage of mortality of naupliar larvae of *B.amphitrite amphitrite* exposed to various concentrations of BP1100 at different time intervals followed by a 2h period of recovery in clean river water and the $LC_{50}$ values.**

| Concentration ppm | Time (min) | | | | | | |
|---|---|---|---|---|---|---|---|
| | 0 | 4 | 6 | 12 | 20 | 40 | 80 |
| | Percentage of mortality of napulii | | | | | | |
| **3** | 0 | 0 | 0 | 1 | 1 | 1 | 1 |
| **6** | 0 | 1 | 1 | 1 | 1 | 4 | 5 |
| **12** | 1 | 3 | 4 | 7 | 6 | 10 | 20 |
| **30** | 1 | 12 | 19 | 20 | 24 | 27 | 32 |
| **$LC_{50}$** | --- | 114.0 | 79.6 | 68.7 | 62.0 | 54.9 | 39.2 |

**Table (4): The percentage of mortality of naupliar larvae of *B.amphitrite amphitrite* exposed to various concentrations of gas oil at different time intervals followed by a 2h period of recovery in clean river water and the $LC_{50}$ values.**

| Concentration ppm | Time (min) | | | | | | |
|---|---|---|---|---|---|---|---|
| | 0 | 4 | 6 | 12 | 20 | 40 | 80 |
| | Percentage of mortality of napulii | | | | | | |
| **15** | 0 | 0 | 0 | 0 | 0 | 0 | 1 |
| **30** | 0 | 0 | 0 | 0 | 1 | 2 | 2 |
| **60** | 0 | 0 | 2 | 5 | 4 | 5 | 6 |
| **120** | 1 | 3 | 5 | 11 | 12 | 14 | 11 |
| **$LC_{50}$** | --- | --- | 980.9 | 510.7 | 462.0 | 381.6 | 421.7 |

**Table (5): The percentage of non active individuals of adults of *B. amphitrite amphitrite* exposed to various concentrations of BP1100 at different time intervals.**

| Concentration ppm | Time (h) | | | | |
|---|---|---|---|---|---|
| | 3 | 10 | 20 | 27 | 50 |
| | Percentage of non active adults | | | | |
| **70** | 4 | 13 | 25 | 32 | 44 |
| **200** | 29 | 56 | 91 | 93 | 100 |

| | | | | | |
|---|---|---|---|---|---|
| **600** | 67 | 86 | 94 | 100 | 100 |

**Table (6): The percentage of mortality of adults of *B. amphitrite amphitrite* exposed to various concentrations of BP1100 at different time intervals followed by a 48h period of recovery in clean river water.**

| **Concentration ppm** | **Time (h)** | | | | |
|---|---|---|---|---|---|
| | **3** | **10** | **20** | **27** | **50** |
| | **Percentage of mortality of adults** | | | | |
| **70** | 4 | 9 | 13 | 15 | 22 |
| **200** | 14 | 57 | 85 | 100 | 100 |
| **600** | 45 | 96 | 100 | 100 | 100 |

**Table (7): The cirral activity of adults of *B. amphitrite amphitrite* exposed to various concentrations of BP1100 for 9h followed by a 18h period of recovery in clean river water.**

| **Concentration ppm** | **Time (h)** | | | | |
|---|---|---|---|---|---|
| | **0** | **3** | **9** | **12** | **25** |
| | **Cirral activity** | | | | |
| **20** | 0.49 | 0.61 | 0.72 | 0.75 | 0.78 |
| **Control** | 0.81 | 0.88 | 0.86 | 0.85 | 0.84 |
| **200** | 0.02 | 0.03 | 0.05 | 0.22 | 0.23 |
| **Control** | 0.60 | 0.58 | 0.64 | 0.63 | 0.56 |

**Table (8): The percentage of mortality of adults of *B. amphitrite amphitrite* exposed to 70ppm of three different dispersants and gas oil for a period of 18h.**

| **Chemicals** | **Percentage of mortality of adults** |
|---|---|
| **BP1100** | 21 |
| **BP100X** | 11 |
| **Corexit8666** | 8 |
| **Gas oil** | 2 |

## Discussion:

The toxicity of dispersants on the aquatic organisms was well known [10]. Greco *etal*. [11] found that the dispersants were very toxic to the barnacles and the larval stages were more sensitive to dispersants than the adults. It was suggested that the greater part of the toxicity in the dispersants were provided by their organic solvents [12]. Fuller *etal.* [10] tested the effect of surfactants, stabilizing fractions and organic solvents of dispersants on the larvae of fishes and found that a lower concentrations of organic solvents killed the larvae within hours or within a day or two, whereas a low concentrations of the surfactants and stabilizing fractions had little effect. In the present study, the BP 1100 and gas oil had a similar effect on nauplii and adults of barnacle *B. amphitrite amphitrite*. But the effect of gas oil on the barnacle was little than the BP1100. The percentage of non motile nauplii was sharply increased at 12 min of exposure to BP1100 and gas oil (Tables 1 and 2). This suggested an immediate and profound effect of these chemicals on the nauplii. Similarly, the percentage of inactive adults showed a sharp increase at 10h of exposure to BP 1100 (Tables 5). The percentage of non motile nauplii increased slowly after 12min of exposure to BP 1100 and gas oil until 40min and then showed decrease in all concentrations of gas oil and only in low concentrations (i.e. 3 and 6ppm) of BP1100. This trend may indicate the concentrations evaporation of the chemicals and possibly also to the acclimation of the nauplii to these concentrations. The reduce of concentrations of gas oil and BP1100 by evaporation may allow a few of the nauplii to recover and hence reduce the percentage mortality. There was no decrease of the

percentage of non motile nauplii in higher concentrations of BP1100 (i.e. 12 and 30ppm) (Table 1) indicated to stability of BP1100 in the presence of the stabilizer and the surfactant compounds compared with the gas oil. The increase of the percentage of non active individuals and the percentage of mortality was also found in the adults barnacle (Tables 5 and 6). There was no significant increase in the percentage of non motile nauplii when the concentration of BP1100 was from 12 to 30ppm (Table 1). This suggests that the concentration of 12 ppm of BP1100 is lethal to larvae. However, The never was not 100% non motile larvae in all the concentrations of BP1100 indicated that at least a small number of larvae were capable to tolerate high concentration of chemical. In the present investigation, the BP1100 was more toxic than the gas oil to the barnacles. The organic solvent of BP100 has a great amount of aromatic contents [13]. Farid [14] concluded, after reviewing recent literatures, that toxicity of oils and their derivatives was due to the chemical toxicity of soluble aromatics, rather than other compounds. Gas oil, having an aromatic content less than that of BP1100 would possess a proportionally lower toxicity to the barnacle larvae. The mortality curve of the BP1100 and gas oil suggested a mode of action by the chemicals on the barnacle and may give further support to the fact that gas oil and BP1100 share a common toxic element. The time–concentration response and the high recovery rate of nauplii exposed to BP1100 and gas oil suggested that the toxic elements in both chemicals were the damage and the degree of damage depends on the penetration rate of the toxic element (aromatic compounds) which may obstract certain metabolic pathways. The toxic element would similarly diffuse out in a negative concentration gradient and allow the nauplii to recover when placed in fresh water or in reducing the concentration of BP1100 or gas oil by evaporation. The percentage of mortality of nauplii exposed to the BP1100 was higher than the percentage of mortality of nauplii exposed to the gas oil (Tables 3 and 4). This may indicate the stability of BP1100 of the nauplii due to the presence of the stabilizer and the surfactant fraction in their tissues and that the diffusion rate of the BP1100 was much lower than that of the gas oil. Thus, the damage caused by BP1100 on nauplii was possibly more than that of the gas oil. Disregarding the chemicals used and the stage of life, a direct time–concentration response can be generally demonstrated in all the experiments of the present study. The nauplii and the adults of barnacle had the same affectability to the BP1100. However, the larvae were more susceptible to BP1100 than the adults (Tables 1, 3, 5 and 6). The cirral activity of the adults barnacle exposed to of BP1100 in 20 and 200ppm concentrations was reduced. But some trends were clearly evident that the cirral activity of barnacle increased at 3 to 9h of exposure to BP1100 (Table 7). This indicated an acclimation and/or evaporation of the test solution. The cirral activity showed an increase when the barnacle was returned to clean river water. However, the cirral activity of the barnacles exposed to 200ppm of BP 1100 was still lower than that of the control barnacles and the 20ppm of BP1100 exposed barnacles. This may suggest that the impairing effect of BP1100 on the cirral activity and hence the normal mechanisms of feeding and respiration of the barnacle or that the period of recovery is not a long enough for recovery. The toxicity experiments showed that the dispersants (BP1100, BP1100X and Corexit8666) and gas oil vary widely in their toxicity to the barnacle tested. The BP1100 was clearly the most toxic dispersant. BP1100X was the next most toxic dispersant. The dispersant Corexit8666 were relatively less toxic to the species tested, while the gas oil was almost non–toxic (Table 8). Fuller *etal*. [10] studied the toxicity of crude oil, BP1100 and BP1002 upon the barnacles and found that BP1002 was the most toxic to those animals.

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