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1. Introduction

In the years following 1967 when the wreck of the TORREY CANYON first focussed public attention on oil pollution there have been a number of large spills of crude oil and refined oil products around the British Isles (Table 1). Oils vary considerably and, as shown in Table 1, the oils spilt since 1967 have included substances as diverse as 'four star' petrol and heavy fuel oil. In addition, each oil is a complex mixture of numerous hydrocarbon and other components; in particular, crude oils from different sources possess a wide range of physical properties and chemical compositions. The toxicity of crude oils and oil products to a wide range of marine organisms has been assessed by a number of workers but very few comparable data are available for oils commonly transported and hence potentially liable to be spilt in the coastal areas of Northern Europe.

Chemical dispersants have been used to treat many of the spills listed in Table 1, but since 1974 dispersants have been subject to statutory control by licensing under the Dumping at Sea Act (Great Britain – Parliament, 1974). The assessment of toxicity for regulatory purposes is carried out at the MAFF Fisheries Laboratory at Burnham-on-Crouch using two toxicity tests which simulate the use of dispersants at sea and on beaches (Blackman *et al.*, 1977). Although a standard oil (fresh Kuwait crude) is used in these tests, little was known about the toxicity of other types of oil after treatment with dispersants.

1.1 Scope of the research programme

This report presents the results of a series of investigations carried out between February 1979 and May 1981 to assess the relative toxicities of a range of oils, alone and when treated with three representative dispersants. The tests were intended primarily to identify those oils which were likely to cause special environmental problems if spilt at sea, and secondarily to indicate whether more than one oil should be used in the standard MAFF tests for oil dispersants. Three types of toxicity test were carried out to determine:

(a) the acute toxicity of each oil to brown shrimps (*Crangon crangon* L) within a 96 h exposure period in order to obtain comparable toxicity curves and LC50 data;

Table 1 Major (> 1 000 t) oil spills off North-west Europe between 1967 and 1980

Date	Ship or oilfield	Area	Type of oil	Approximate quantity (10^3 tonnes)	Source
March 1967	TORREY CANYON	Cornwall	Kuwait crude	120	Smith, 1968
October 1970	PACIFIC GLORY	Isle of Wight	Nigerian crude	6-7	Department of the Environment, 1976
August 1972	DONA MARIKA	Milford Haven	'Four star' petrol	3	Blackman *et al.*, 1973
October 1974	UNIVERSE LEADER	Bantry Bay	Kuwait crude	2-3	O'Sullivan, 1975
November 1975	OLYMPIC ALLIANCE	Dover Strait	Iranian light crude	2-3	Department of the Environment, 1976
April 1977	Ekofisk oilfield	North Sea	Ekofisk crude	10-30	Bourne, 1977
March 1978	AMOCO CADIZ	Brittany	Iranian light crude Arabian light crude	120) 220 100)	O'Sullivan, 1978
May 1978	ELENI V	East Anglia	Heavy fuel	5	Blackman and Law, 1980
October 1978	CHRISTOS BITAS	Pembroke	Iranian heavy crude	2-3	Bourne, 1979
December 1978	ESSO BERNICA	Sullum Voe	Bunker C fuel	1-2	Richardson, 1979
January 1979	BETELGEUSE	Bantry Bay	Saudi Arabian crudes	2	Cross *et al.*, 1979

Table 2 – Details of imported oils used in the experimental programme

Source	Type	Viscosity at 15°C: Centipoise	Viscosity at 15°C: Shear rate (s^{-1})	Percentage of total carriage (1978) *
Abu Dhabi	Murban crude	6.2	945	5.2
Iran	Light crude	11.7	945)	
			)	19.7
Iran	Heavy crude	19.2	791)	
Iraq	Basrah crude	17.0	945	6.9
Kuwait	Crude	15.8	945	5.9
Libya	Brega crude	9.6	945	5.7
Nigeria	Crude	13.2	945	7.6
Saudi Arabia	Light crude	9.6	945	26.1

* Excluding carriage between the UK and Norway or France and the carriage of refined or part-refined products between North-west European ports. All other sources of crude oil, refined or part-refined products comprise less than 3% each (Warren Spring Laboratory, personal communication).

Table 3 – Details of North Sea oils used in the experimental programme

Field	Viscosity at 15°C		Production (10^6 t a^{-1})*	
	Centipoise	Shear rate (s^{-1})	1977	Estimated peak
Argyll	30	315	0.8	1.1
Auk	15.8	791	2.3	2.3
Beryl	7.2	945	3.0	5.0
Brent	7.3	945	1.3	23.0
Claymore (stabilised)	21.0	945	0.3	4.5
Ekofisk	12.2	627	20.0	30.0
Forties	10.9	945	20.1	24.0
Montrose	7.3	945	0.8	1.4
Murchison	6.8	945	0	7.2
Piper	10.4	945	8.6	12.6
Thistle	6.8	945	0	8.7

* Department of Energy, 1980

(b) the effect of three selected dispersants (a conventional and two concentrates) on the acute toxicity of each oil to brown shrimps under the conditions of the MAFF 'sea' test – a dispersant passes this test if the oil dispersion it produces is no more toxic than physically-dispersed oil;

(c) the relative toxicity of each oil to common limpets (*Patella vulgata*) under the conditions of the MAFF 'beach' test – a dispersant passes this test if it is no more toxic than the reference oil used.

These tests were of a short-term nature and carried out under standardised laboratory conditions to provide comparable data on a number of oils as a basis for evaluating the results of routine screening tests. The study was not intended to form a basis for predicting ecological effects caused by spilt or dispersed oils; such an exercise would require the provision of additional data from different tests (Wilson *et al.*, 1974).

The 19 crude oils selected for the tests were those most likely to be spilt in UK waters, including those crude oils imported in greatest quantity into the UK and those produced in the major North Sea oilfields. The eight imported oils listed in Table 2 represent over 75% of the total oil carried in UK waters in 1978, excluding oil carried between the UK and Norway or France and the carriage of refined or part refined products between any north-west European ports. Table 3 lists the eleven North Sea crude oils tested, and details of production from each field. The crude oils were tested in the 'fresh' state to provide a comparison with the fresh Kuwait oil used in the MAFF standard test. In an earlier series of experiments (Norton and Franklin, 1980) fresh oil which had been 'weathered' by exposing it to air for 24 h was greatly reduced in toxicity; tests of such oil would therefore require concentrations of oil greater than the 1 000μl l^{-1} and/or longer exposure periods than the 100 min of the standard test. Weathered oils are also less amenable to dispersion by chemicals and, in practice, only fresh oils are capable of being readily dispersed into the water column.

Six oil products, chosen to represent the major groups of oil products transported through UK waters, were also tested; these are listed in Table 4. Tests to determine the effects of dispersants on five of these products were included because, even though oil products are not normally considered to be amenable to dispersant treatment, there have been instances of such spills being sprayed (Blackman and Law, 1980). These tests are also useful in providing information on the relative effects of different dispersants when applied to oils with a wide range of physical and chemical properties. 'Four star' petrol was not included in the 'sea' and 'beach' tests because the results of the 96 h tests showed that 100% mortality occurred well within 100 min: furthermore, it is most unlikely that chemical dispersants would be used to treat a material of such high volatility and low viscosity as petrol.

Table 4 – Details of oil products used in the experimental programme

Product	Viscosity at 15°C	
	Centipoise	Shear rate (s^{-1})
'Four star'* petrol	0.83	Not dependent
Diesel	4.0	945
Gas oil	5.3	945
Lubricating oil	88	203
Medium fuel oil	1 090	78
Residual fuel oil	28 550	49

* Premium quality (Road Octane Number 97); maximum permitted lead content 0.4 g l^{-1} (CONCAWE, 1979)

The three dispersants used in the 'sea' tests were chosen to represent each of the major categories of formulation currently available, and included a conventional hydrocarbon solvent-based dispersant (BP 1100X), a concentrate (Synperonic OSD 20) and a 'self mix' concentrate (Corexit 9527*). An earlier series of experiments (Norton and Franklin, 1980) had shown that these dispersants span the range of toxic effects when mixed with fresh Kuwait oil and tested by the standard MAFF 'sea' test.

2. Methods

Experimental details of the tests used in this study are summarised below. A more detailed description of the 'sea' and 'beach' test apparatus and methods has been given by Blackman *et al.*, 1977.

2.1 Materials

All of the toxicity experiments described in this report were carried out with filtered (<10 μm) natural sea water of 28-35°/oo salinity at a temperature of 14 – 16°C.

Fresh Kuwait crude oil, taken from a 200 l drum obtained from the Warren Spring Laboratory, Stevenage, in August 1978 and stored in a sealed container, was used as the reference standard in all experiments. Other test oils were obtained in 5 or 10 l quantites from the Warren Spring Laboratory in October 1978, with the exception of the Iranian oils which were supplied by BP Trading Ltd, Sunbury in May 1979 and the 'four star' petrol which was obtained locally. Details of the oils are given in Tables 2, 3 and 4. The viscosity measurements were made by the Warren Spring Laboratory using a Ferranti portable rotational viscometer at optimised shear rates. Sub-samples of each oil were stored in 250 ml airtight metal cans to prevent losses due to evaporation; a fresh can was opened for each experiment. All oils were brought to the test temperature of 15°C before use, with the exception of residual

* The sample of Corexit 9527 used for these experiments was different from the formulation currently in production.

then randomly added to each tank and the lids put in place. After a further 2 h the aerators were removed and the motors switched on. The motor speeds were checked with the aid of a stroboscope and adjusted, if necessary, to between 1 350 and 1 450 r/min. The lids were then pushed to one side to allow the test material to be added. The concentrations used for the crude oils were 20, 50, 100, 200, 500, 1 000, 2 000 and 4 000 $\mu l\ l^{-1}$; a suitable range of concentrations was chosen for each oil product. Each concentration was tested in duplicate and controls of clean sea water were included in each test. The sensitivity of shrimps to Kuwait oil has been shown to vary seasonally (Norton and Franklin, 1980) and, as it was thought that this would also occur during the period of the test programme, two concentrations of Kuwait oil (200 and 1 000 $\mu l\ l^{-1}$) were included as a reference in each test. If the response times of the experimental animals exposed to these two reference concentrations were outside a narrow predetermined range the test was repeated; those data which were acceptable were then adjusted using the median Kuwait response as a standard, to take account of variations in shrimp sensitivity between tests. The oil concentrations were nominal, no measurements being made of the actual concentrations during the experiment. The oil was added to the tanks from a disposable syringe which was directed into the vortex created by the propeller to ensure immediate mixing a reproducible dispersion.

The solution of all oils except residual fuel oil were renewed after 48 h to counteract losses of the lighter fractions due to absorption by the test organism, degradation or volatilisation. Stirring of the tanks maintained the dissolved oxygen concentration at close to air saturation value (8.4 mg l^{-1}).

The tanks were inspected at frequent intervals, including 24, 48, 72 and 96 h after adding the oil, and dead animals, defined as those not responding to gentle prodding, were recorded and removed. Because of the possibility of cannibalism of freshly-moulted shrimps, the number of animals remaining alive at the end of the experiment was also noted so that the mortality attributable to the test treatment could be calculated. At the time of each observation, and for each tank, the cumulative percentage mortality was calculated using the formula $\frac{2m - 1}{2p} \times 100$, where m was the cumulative number dead and p was the total number of animals in the tank. Thus, in experiments which started with 20 shrimps in the tank, the death of the first shrimp was recorded as a 2.5% mortality, the second as 7.5% and the 20th as 97.5%. This is because the median response relates not to the response of the tenth animal out of a total of twenty but to a response between the tenth and eleventh animal (Lloyd, 1979).

Figure 2 shows how the mortality data were used to obtain a 96 h LC50 value for each oil, using Kuwait oil as an example. For each test tank the cumulative percentage mortalities were plotted against exposure time as described by Franklin (1980) and a time/mortality curve drawn by eye (Figure 2a). The time at which 50% mortality occurred (LT50) in each tank was then read off this graph. Estimates of the variability of the response of the test populations were made by calculating the 95% confidence limits from the slope of the line (Litchfield, 1949). Additionally, it was often useful to obtain approximate estimates of the concentrations of oil lethal to 50% of the test organisms (LC50) at fixed observation times. These were obtained from concentration/mortality curves in which the cumulative percentage mortalities at, for example, 96 h were plotted against the corresponding concentrations (Figure 2b). A line fitted by eye between the points allowed approximate values for the LC50 at these time intervals to be estimated. A concentration/median response (toxicity) curve was then obtained by plotting the values of the LT50 against concentration and the estimated values of the LC50 against the corresponding time, using log. – log. graph paper. A curve was fitted by eye through these two sets of points (Figure 2c). The LC50 values for 96 h exposure were derived from the toxicity curve. Since the concentration of oil present in the tanks was not measured and the actual concentration of the various oil components may not have remained constant during the period of the test, the term LC(I)50 has been used, where C(I) is the initial concentration of test substance (Lloyd and Tooby, 1979).

2.4 'Sea' tests

Sixteen tanks of the type shown in Figure 1 were each filled with 18 l of sea water and aerated for at least 1 h. Twenty shrimps were then randomly added to each tank and the lids put in place. After a further 2 h the aerators were removed and the lids moved to one side to allow the test material to be added. In each test, materials were added to these sixteen tanks as follows:

- Kuwait oil (3 tanks);
- test oil alone (4 tanks);
- test oil plus BP 1100X (3 tanks);
- test oil plus Synperonic OSD 20 (3 tanks);
- test oil plus Corexit 9527 (3 tanks).

It should be noted that the number of replicates used in this experimental programme was less than the five used when a dispersant is submitted for testing as part of the licensing procedure under the Dumping at Sea Act. Eighteen ml of oil were applied to the surface of the water in the tanks and, where appropriate, 18 ml of dispersant (or a 10% solution of dispersant concentrate) were distributed as evenly as possible over the surface of the oil. The concentrations were thus 1 000 $\mu l\ l^{-1}$ of the oil, and either 1 000 $\mu l\ l^{-1}$ of BP 1100X or 100 $\mu l\ l^{-1}$ of Synperonic OSD 20 or Corexit 9527. These concentrations of oil and dispersant were nominal.

One minute after adding dispersant the lids were replaced to cover the test tanks and the motors were switched on to give stirring speeds of between 1 350 and 1 450 r/min. A visual assessment of the degree of dispersion obtained in each tank was made at the start and end of the 100 min exposure period. After 100 min the motors were switched off, the lids removed and the surface oil and oily water

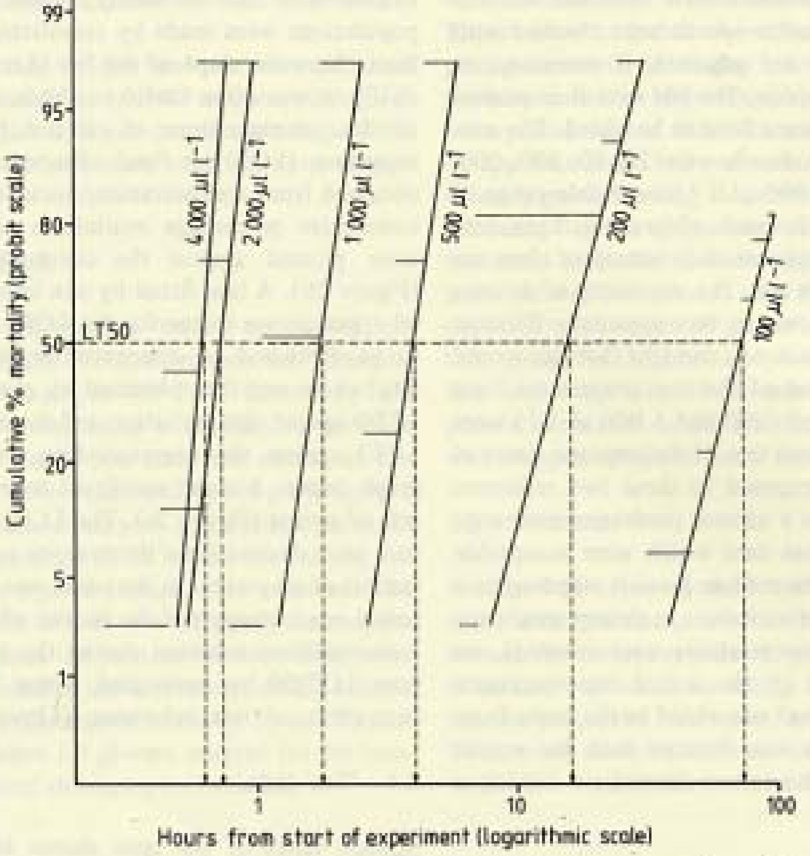

Figure 2 Example of how the mortality data for fresh Kuwait crude oil were used to obtain the 96 h LC50:

(a) time/mortality curves.

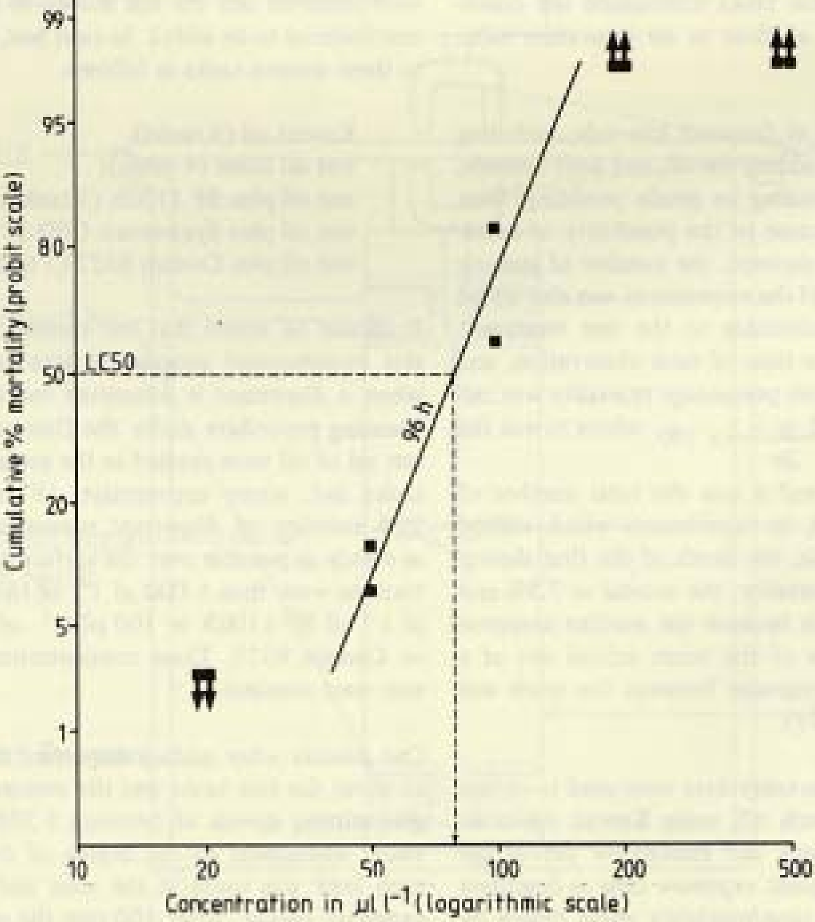

Figure 2 Example of how the mortality data for fresh Kuwait crude oil were used to obtain the 96 h LC50:

(b) concentration mortality curve.

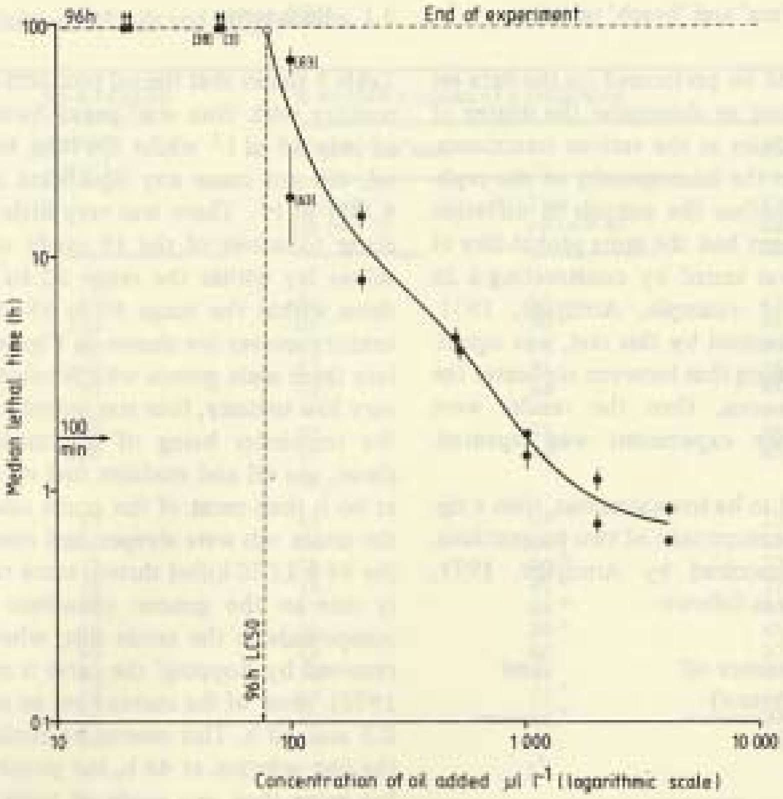

Figure 2. Example of how the mortality data for fresh Kuwait crude oil were used to obtain the 96 h LC50:

(c) concentration/median response (toxicity) curve.

⧫ = median lethal times and 95% confidence limits,
○ = median lethal concentrations,
[ ] = % mortality at end of experiment (if > 0 and < 100%).

siphoned out of the tanks. The test animals were then gently transferred to tanks of clean, gently-flowing, aerated sea water for 24 h so that those which were anaesthetised by the oil might recover. At the end of this period the number in each tank found to be dead (defined as lack of response to gentle prodding) was recorded. To account for any cannibalism of freshly-moulted shrimps the number of animals remaining alive at the end of 24 h was also noted to allow the mortalities caused by the test treatment to be calculated. The mortalities in each set of replicates were then compared statistically with those in the test oil control (using the method described below in Section 2.6) to determine (a) whether there was a significant difference between the toxicities of the test oil and Kuwait oil and (b) the extent to which the addition of each dispersant modified the toxicity of the test oil.

2.5 'Beach' tests

Two sets of five test plates, each with 20 limpets attached, were placed horizontally, attached animals uppermost, in spraying tanks. Each limpet on one set of plates was sprayed from a height of about 10 cm with 0.8 ml of Kuwait oil, from a hand-operated sprayer, to give an application rate of about 0.4 l m^{-2}, the average rate for beach application. The second set was sprayed in a similar manner with the test oil, except that the two fuel oils had to be poured onto the limpets, because of viscosity problems. The reference dispersants were not included in the 'beach' test series since in the standard test limpets are exposed to dispersant alone and not to a dispersant/oil mixture as in the 'sea' test.

The plates of sprayed limpets were left in moist air for 6 h before being washed for 15 s with running sea water. Each plate was then suspended vertically in a recovery tank and subjected to further rinsing with clean sea water by means of successive simulated 'tidal cycles', as described in section 2.2. Limpets which became detached from the plates were recorded as dead and were removed from the tanks after 24 h and 48 h. After 48 h any remaining limpets not firmly attached to their plates were gently detached and placed on the tank floor. Those failing to reattach to a surface within a further 24 h period were also counted as dead. The total mortalities of limpets after 72 h on the five plates treated with the test oil and the five plates treated with Kuwait oil were compared statistically using the technique described in section 2.6.

2.6 Statistical analysis of 'sea' and 'beach' test data

Before a statistical test could be performed on the data set from any 'sea' or 'beach' test to determine the degree of significance between mortalities in the various treatments, it was first necessary to test the homogeneity of the replicates, i.e., to determine whether the animals in different tanks with the same treatment had the same probability of dying. This homogeneity was tested by constructing a 2x contingency table (see, for example, Armitage, 1971, pp 207-211). If χ^2, as determined by this test, was significant at the 95% level, suggesting that between replicates the results were not homogeneous, then the results were regarded as invalid and the experiment was repeated.

If the replicates were found to be homogeneous, then a significance test based on the comparison of two proportions, unpaired case (e.g., as described by Armitage, 1971, pp 129-131) was carried out as follows:

	Reference oil (control)	Test
Total number dead =	r_1	r_2
Total number tested =	n_1	n_2
Proportion dead =	$p_1 = \frac{r_1}{n_1}$	$p_2 = \frac{r_2}{n_2}$

Pooled proportion dead, p = $$\frac{r_1 + r_2}{n_1 + n_2}$$

Difference between proportions dead, d = $$p_1 - p_2$$

95% confidence limits of d = $$d \pm 1.96\sqrt{\left[\frac{p_1(1-p_1)}{n_1} + \frac{p_2(1-p_2)}{n_2}\right]}$$

Standardised normal deviate, u = $$\frac{p_1 - p_2}{\sqrt{\left[p(1-p)\left(\frac{1}{n_1} + \frac{1}{n_2}\right)\right]}}.$$

The null hypothesis that $p_1 = p_2$ was rejected in favour of $p_1 \neq p_2$ if u was significant at the 95% level.

Both the homogeneity and significance tests were carried out using a programme written for a Hewlett Packard HP 97 calculator.

3. Results

The LC(I)50 values, or median lethal concentrations, of each oil to *Crangon* after 96 h exposure, as well as the 'sea' and 'beach' test data for the oils alone, are summarised in Table 5. The results of each of the three types of test are discussed separately below.

3.1 96 h tests

Table 5 shows that the oil products spanned a wide range of toxicity with 'four star' petrol having a 96 h LC(I)50 value of only 15 $\mu l\ l^{-1}$ whilst the least toxic product, lubricating oil, did not cause any significant mortality of *Crangon* at 4 000 $\mu l\ l^{-1}$. There was very little difference between the acute toxicities of the 19 crude oils: their 96 h LC(I)50 values lay within the range 32 to 140 $\mu l\ l^{-1}$, with 12 of them within the range 50 to 95 $\mu l\ l^{-1}$. The corresponding toxicity curves are shown in Figure 3: these appear to fall into three main groups with lubricating and fuel oil being of very low toxicity, four star petrol being the most toxic, and the remainder being of intermediate toxicity. Although diesel, gas oil and medium fuel oil were slightly more toxic at 96 h than most of the crude oils, the toxicity curves for the crude oils were steeper, and concentrations greater than the 96 h LC50 killed shrimp more rapidly. This was probably due to the greater quantities of more toxic volatile compounds in the crude oils; when these components are removed by 'topping' the curve is much shallower (Connor, 1972). Most of the curves have an inflection between about 0.3 and 10 h. This cannot be explained by the renewal of the test solution at 48 h, but possible causes are that oil (a) has more than one mode of toxic action, e.g., a chemical effect on physiological reactions and a physical effect on cell membranes, (b) contains a mixture of fast-acting substances toxic at high concentrations and slower acting compounds toxic at low concentrations, and (c) changes with time, i.e., 'weathering': all these reactions are likely to occur with a substance as chemically complex as oil, and their importance depends on the exact composition of each oil.

3.2 'Sea' tests

Although the results of the 96 h tests indicated that there was very little difference in toxicity between the various crude oils, the 'sea' test showed that nine of them were significantly more toxic than was Kuwait oil when tested for 100 min against the same population of shrimps (Table 5). All of the oil products tested (excluding petrol) were significantly less toxic than Kuwait oil.

Results showing the effect of dispersants on the toxicity of each oil are given in Table 6. The degree to which the toxicity of the test oil was affected by chemical dispersion depended on the dispersant used. In general, Synperonic OSD 20 did not modify the degree of toxicity of each oil, whilst BP 1100X tended to reduce its toxicity and 'Corexit 9527' to increase it. Since these experiments were undertaken, the formulation of Corexit 9527 has been changed, and a more recent sample tested by the MAFF 'sea' test did not significantly increase the toxicity of Kuwait oil. This dispersant has now been licensed for sea and beach use. The effects of dispersants on the degree of toxicity of the various oils, are illustrated in Figure 4 where the mortalities

Table 5 – The acute toxicity of 25 oils and oil products

Oils and products (ranked in order of increasing viscosity within each group)	96 h LC(I)50 to *Crangon crangon* ($\mu l\, l^{-1}$)	% mortality (mean of *n* replicates)			
		MAFF 'sea' test		MAFF 'beach' test	
		Kuwait oil (*n* = 3)	Test oil (*n* = 4)	Kuwait oil (*n* = 5)	Test oil (*n* = 5)
IMPORTED:					
Abu Dhabi	48	88	90	45	59
Libyan	120	88	98^{+}	46	47
Saudi Arabian	60	48	58	45	48
Iranian light	140	83	81	18	34^{+}
Nigerian	50	33	64^{+}	46	40
Kuwait	80	—	—	—	—
Iraqui	90	90	83	46	60^{+}
Iranian heavy	120	85	79	45	67^{+}
NORTH SEA:					
Murchison	95	92	95	20	58^{+}
Thistle	130	38	64^{+}	45	69^{+}
Beryl	37	58	90^{+}	27	58^{+}
Brent	32	18	61^{+}	27	46^{+}
Montrose	70	67	86^{+}	46	62^{+}
Piper	80	70	89^{+}	45	78^{+}
Forties	85	68	80	27	46^{+}
Ekofisk	50	78	84	44	43
Auk	70	19	59^{+}	44	33
Claymore	80	40	79^{+}	27	47^{+}
Argyll	80	73	66	46	54
PRODUCTS:					
'Four star' petrol	15	not tested			
Diesel	25	37	22^{-}	38	28
Gas oil	13	70	8^{-}	38	17^{-}
Lubricating oil	>4 000	65	0^{-}	38	4^{-}
Medium fuel oil	41	63	9^{-}	27	21
Residual fuel oil	2 600	32	1^{-}	27	10^{-}

– = significantly lower mortality than Kuwait oil at 95% probability ($P \leqslant 0.05$)
+ = significantly greater mortality than Kuwait oil at 95% probability ($P < 0.05$)

Table 6 – Results of MAFF 'sea' tests using 24 oils and oil products and three dispersants

Oils (ranked in order of increasing viscosity within each group)	% mortality (mean of *n* replicates)			
	Oil alone (*n* = 4)	Oil plus BP1100X (*n* = 3)	Oil plus Synperonic OSD 20 (*n* = 3)	Oil plus Corexit 9527*(*n* = 3)
IMPORTED:				
Abu Dhabi	90	67^{-}	77^{-}	93
Libyan	98	63^{-}	88^{-}	93
Saudi Arabian	58	30^{-}	50	73
Iranian light	81	90	81	90
Nigerian	64	60	72	78
Kuwait	59	46	64	78^{+}
Iraqui	83	70	85	82
Iranian heavy	79	70	87	88
NORTH SEA:				
Murchison	95	92	89	97
Thistle	64	58	65	85^{+}
Beryl	90	63^{-}	87	88
Brent	61	48	57	83^{+}
Montrose	86	82	85	95
Piper	89	70^{-}	80	98^{+}
Forties	80	74	80	93^{+}
Ekofisk	84	73	86	92
Auk	59	53	65	62
Claymore	79	33^{-}	87	82
Argyll	66	62	78	87^{+}
PRODUCTS:				
Diesel	22	2^{-}	15	28
Gas oil	8	0^{-}	7	23^{+}
Lubricating oil	0	0	0	0
Medium fuel oil	9	0^{-}	15	12
Residual fuel oil	1	0	0	2

– = significantly lower mortality than oil alone at 95% probability ($P \leqslant 0.05$)
+ = significantly greater mortality than oil alone at 95% probability ($P < 0.05$)
* = earlier Formulation, different from present day

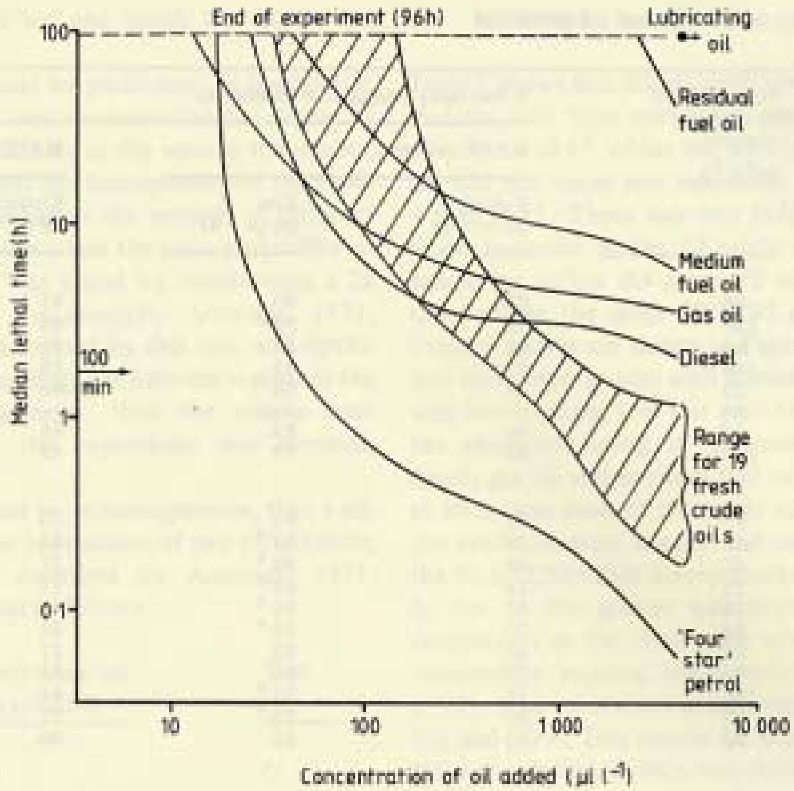

Figure 3. The toxicity of 25 oils to the brown shrimp (*Crangon crangon*) (curves derived as in figure 2).

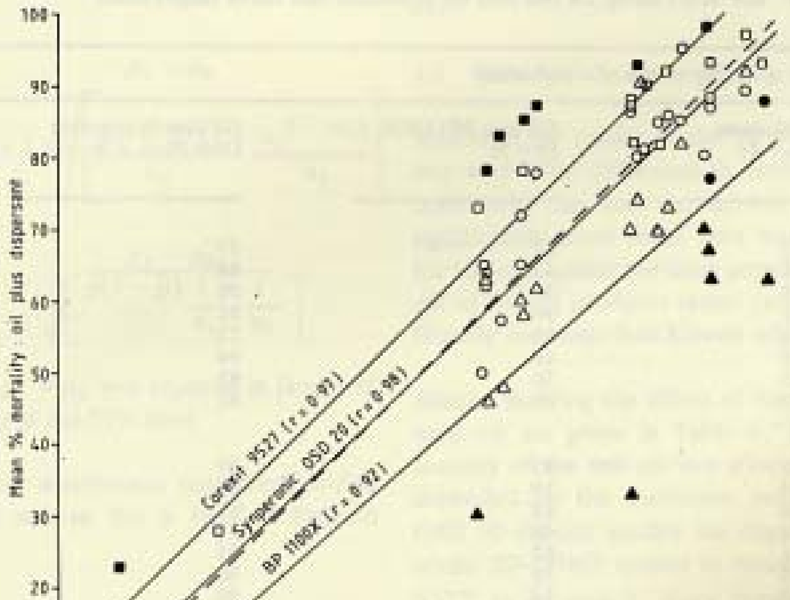

Figure 4. The effect of three dispersants on the toxicity of 24 oils to shrimps in the MAFF 'sea' test. Δ = BP 1100X, O = Synperonic OSD 20, □ = Corexit 9527 (earlier formulation): solid symbols denote oil plus dispersant mortality significantly different from mortality in oil alone at 95% probability ($P < 0.05$).

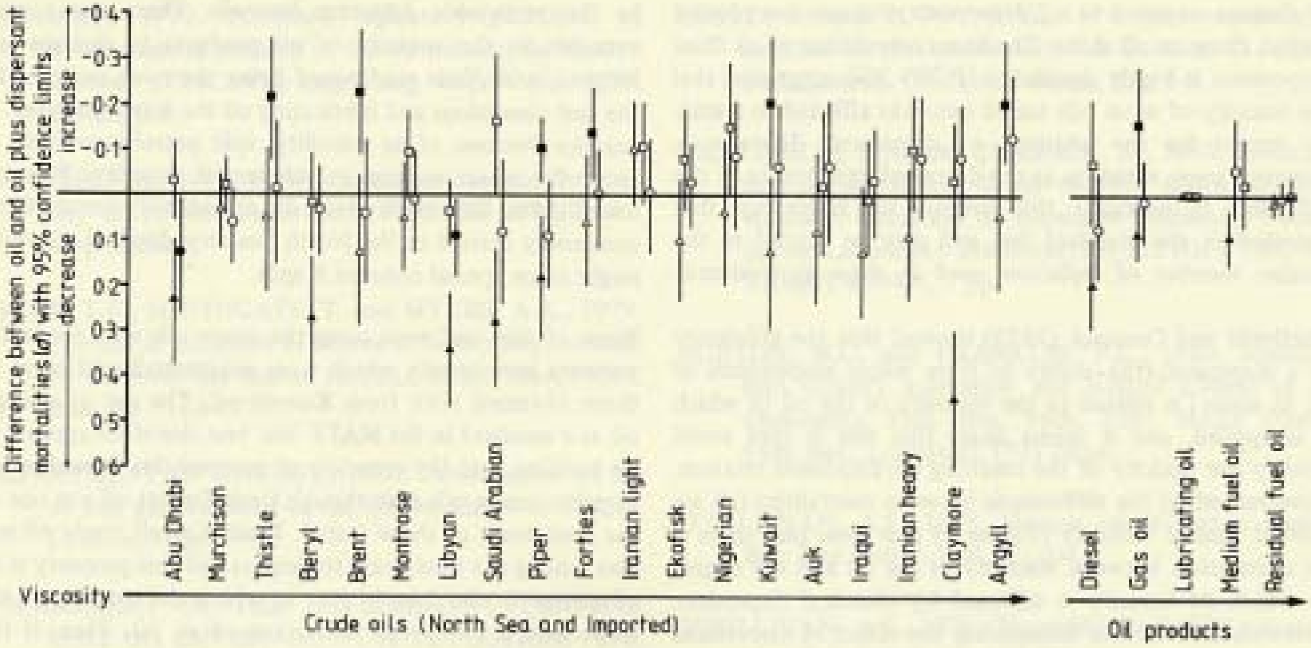

Figure 5. **The relationship between the toxicity of oil/dispersant mixtures and oil viscosity. Δ = BP 1100X, O = Synperonic OSD 20, □ = Corexit 9527 (earlier formulation): solid symbols denote oil plus dispersant mortality significantly different from mortality in oil alone at 95% probability ($P<0.05$).**

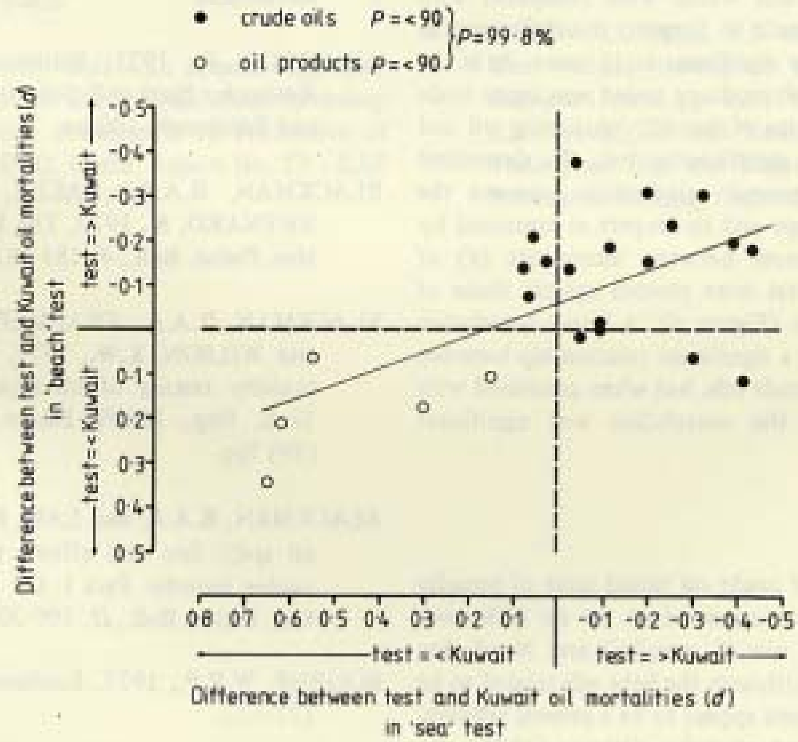

Figure 6. **The relationship between the results of 'sea' and 'beach' tests using 24 oils.**

of shrimps exposed to oil/dispersant mixtures are plotted against those in oil alone. The linear correlation of all three dispersants is highly significant ($P \geqslant 99.9\%$), suggesting that the toxicity of most oils tested here was affected to a similar extent by the addition of dispersant. There was, however, some variation in the degree of significance of the difference in mortality; this variation was larger than that obtained in the standard test and may be related to the smaller number of replicates used in these experiments.

Martinelli and Cormack (1979) showed that the efficiency of a dispersant (the ability to form stable suspensions of oil in water) is related to the viscosity of the oil to which it is applied, and it seems likely that this in turn could modify the toxicity of the resulting oil/dispersant mixture. However, when the differences between mortalities (d) are plotted against viscosity (Figure 5) it is clear that there is no correlation between viscosity of the oil and the degree to which its toxicity is affected by chemical dispersion. Research into factors influencing the effect of dispersants on oil toxicity is continuing.

3.3 'Beach' tests

The mortality of limpets treated with Kuwait oil ranged from 18 to 46% (Table 5) which may be attributed to seasonal variations in their sensitivity (Norton and Franklin, 1980). It seems likely that the sensitivity of limpets to other oils would vary similarly and repetition of several of the experiments at different times of year showed that there was very good agreement between tests, the differences between mortalities (d) remaining the same despite a differing response to Kuwait oil.

Most of the 18 crude oils which were compared with Kuwait oil were more toxic to limpets, the difference in toxicity being statistically significant in 11 cases. As in the 'sea' test, none of the oil products tested was more toxic than Kuwait, the toxicities of gas oil, lubricating oil and residual fuel oil being significantly less. To determine whether there was a general relationship between the toxicity of oils to shrimps and to limpets as measured by these tests, the differences between mortalities (d) of limpets in the 'beach' test were plotted against those of shrimps in the 'sea' test (Figure 6). A linear correlation analysis failed to detect a significant relationship between the two sets of data for crude oils, but when combined with data for oil products the correlation was significant ($P \geqslant 99\%$).

4. Conclusions

All of the 19 samples of crude oil tested were of broadly similar toxicity and there appeared to be no difference between fresh imported oils (8 samples) and North Sea crude oils (11 samples). Although the light oils tended to be the most toxic there did not appear to be a general relationship between toxicity and viscosity. Ottway (1971) also failed to detect any clear-cut correlation between the chemical composition of 20 crude oils and their toxicity to the periwinkle *Littorina littoralis*. There was a wide variation in the toxicity of oil products to shrimps and limpets, with 'four star' petrol being the most toxic under the test conditions and lubricating oil the least. However, in practice, because of its volatility, spilt petrol is unlikely to enter the water column in substantial quantity. Thus, in broad terms, the toxicity tests did not identify among those commonly carried in the North Sea an individual oil which might cause special concern if spilt.

None of the 'sea' tests using the crude oils with three dispersants gave results which were substantially different to those obtained with fresh Kuwait oil. The use of a single oil as a standard in the MAFF 'sea' test therefore appears to be justified and the criterion of acceptability would apply to other crude oils even though fresh Kuwait oil was one of the least toxic of those tested. Fresh Kuwait crude oil was also among the least toxic to limpets and this property is an advantage in the 'beach' test in which the toxicity of the dispersant is compared with that of an oil. Thus, if the toxicity of a dispersant were to be compared with that of one of the more toxic crude oils, it might just pass the test whereas it would fail if compared to a less toxic crude oil. Therefore, a dispersant which passes the criterion of the standard 'beach' test with fresh Kuwait oil as the reference would also satisfy the criterion if another oil was used, and there is no need to increase the number of reference oils used.

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