CHAPTER 7

TOXICITY EXPERIMENTS

LABORATORY INVESTIGATIONS INTO THE TOXICITY OF OIL AND DETERGENTS

The spraying operations at sea and the detergent treatment of the beaches were mounted with one main objective in view, namely to preserve—or at least minimize the damage to—the amenity value of the coastal resorts. The amenity so seriously and blatantly threatened was the cleanliness of the beaches to which summer visitors flock for recreation. The removal of oil from the holiday beaches was an urgent objective, even at the risk of destroying or damaging other amenities, such as the incomparable wild life of the Cornish coast and shores. For even if the natural animal population around the treated beaches were largely destroyed, the effect on the tourist industry might be negligible compared with the prospect of an ever-oily beach. Few will have had serious doubts; to most only one course was right—to fight the oil with detergents regardless of the cost, in every sense.

But did these actions put at risk other and possibly greater interests? Was there a possible danger to public health? Was there a danger to offshore and inshore fisheries? The answers to these and other vital and recurring questions had to wait upon the availability of accurate knowledge about the toxicity of the materials that were being applied, their persistence in the sea and the manner in which they spread.

We soon found that the scientific literature was deficient in relevant information and so a programme of toxicity tests was undertaken in the laboratory by several scientists whose previous experience enabled them to take up this work and develop it with the minimum delay. This work was carried out at the same time as the shore parties and the cruise personnel were making their observations on the beaches and at sea.

The urgency of the problem militated against the accurate refinement of the techniques employed, but in spite of this the experiments provided information of great value. In fact an answer to the all-important question of the persistence of the toxic qualities of the detergent emerged within the first few days.

From the toxicity experiments we were able to develop methods of bioassay which in turn made possible a more detailed investigation into the spread of the detergents.

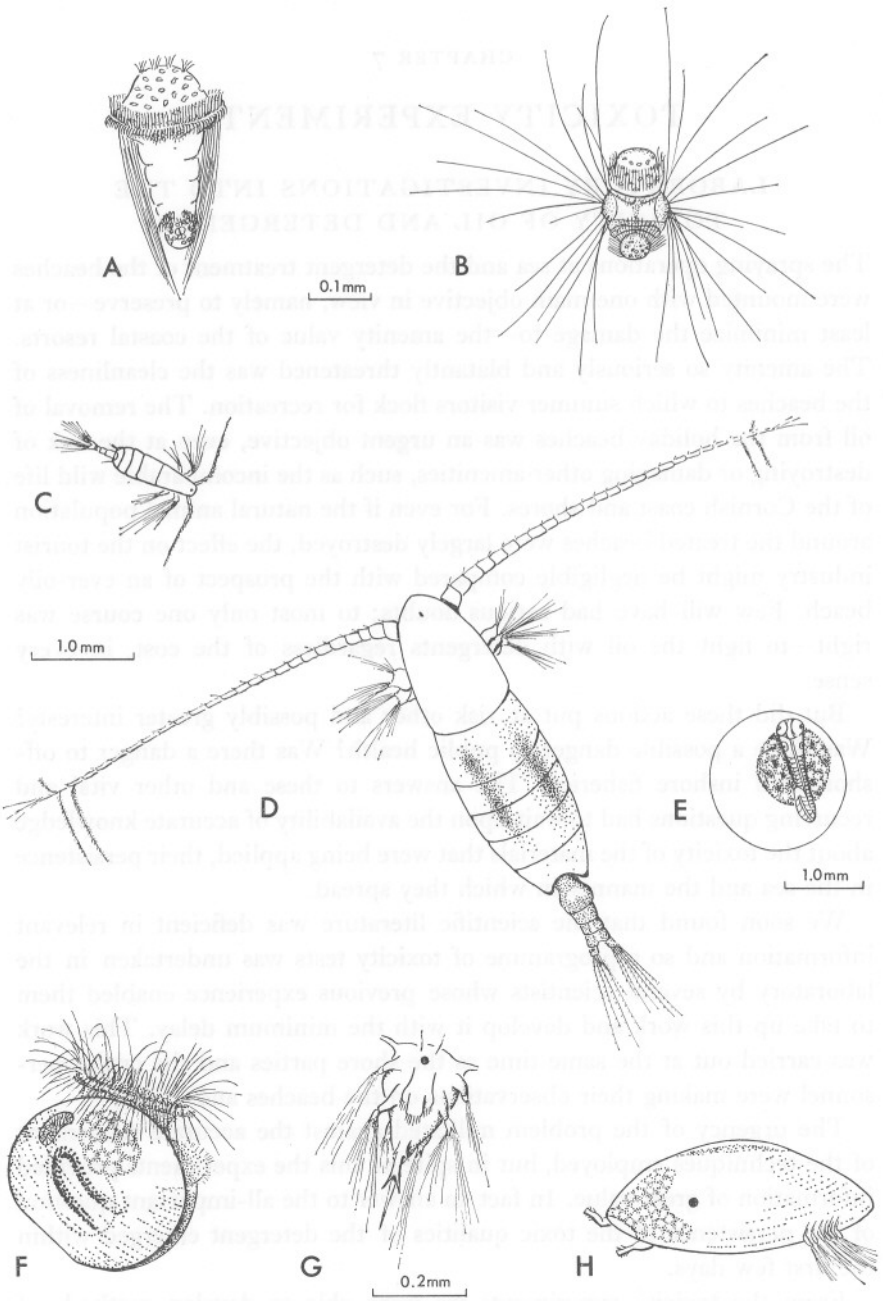


Fig. 22. Examples of zooplankton. A, *Sabellaria* larva, swimming. B, *Sabellaria* larva, irritated. C, The copepod *Acartia*. D, The copepod *Calanus*. E, Pilchard egg. F, Oyster larva. G, Nauplius larva of an acorn barnacle. H, Cypris stage of an acorn barnacle.

The results of the laboratory work are given in outline in the following pages; they represent much intensive effort. Where experiments are individually described they are examples of critical importance. It should perhaps be pointed out that when using living organisms as test material they are not necessarily continuously available; particularly is this true of larvae, which are of seasonal occurrence. Some zooplankton organisms tested are shown in Fig. 22 and phytoplankton in Fig. 5 (p. 28).

A survey of the contents of this chapter may help the reader to follow the somewhat complex subject-matter. The need for the analysis under section VI became obvious during early experiments in section I, and due care and allowance was made thereafter for the diversity of behaviour of the various ingredients of the detergents. At first several detergents were used and their toxicities compared. It became necessary to choose one detergent as a standard, and BP 1002 was chosen because it was one very widely used on the beaches, and with the willing co-operation of the BP Trading Co. Ltd its components could be separately investigated.

SUMMARY OF EXPERIMENTS

I. Toxicity studies on zooplankton
   Effects of detergents
      Elminius modestus larvae, barnacles
      Calanus finmarchicus and *Acartia clausi*, copepods
      Ostrea edulis and *Crassostrea gigas* larvae, oysters
      Lacuna vincta and *Nassarius reticulatus* larvae, shore gastropods
      Young fish
   Notes on toxicity of crude oil and of oil with detergent
   Conclusions from early experiments

II. Longer-term effects on zooplankton species during rearing
   Sabellaria larvae, polychaete worm, showing immediate transitory effects and longer-term effects (for transitory effects on settlement see page 86)
   Elminius larvae to metamorphosis
   Echinus esculentus larvae

III. Toxicity studies on phytoplankton

IV. Toxicity studies on intertidal and sublittoral organisms
   Intertidal algae
   Intertidal animals—toxic effects of BP 1002
      Various animals and in particular mussels, limpets, winkles and top-shells, barnacles (both recently metamorphosed and adult)
   Sublittoral animals—toxic effects of BP 1002
      Various sublittoral organisms including *Echinocardium* (heart-urchin) and its commensals, and *Crangon* (shrimp)

V. Bioassay methods developed to enable water samples from the field to be assessed rapidly

VI. Toxicity and stability of components of BP 1002, etc.—solvent, surfactant and stabilizer
Elminius larvae
Sabellaria larvae
Crangon (shrimps)

I. TOXICITY STUDIES ON ZOOPLANKTON

Effects of detergents

Barnacle larvae

Stage II nauplius larvae of the barnacle *Elminius modestus* were used, being obtained by hatching out fully developed embryos from the adult barnacle. Preliminary tests showed that the detergents BP 1002, Gamlen and Slipclean all rendered the larvae completely motionless in under 2 h, at a concentration of 10 ppm. At high concentration (100 ppm) the animals were rendered quiescent in a few minutes. By contrast, Teepol L, chosen as an example of an ordinary domestic detergent, was found to be far less harmful, concentrations between 27 and 270 ppm of active ingredients being needed to stop swimming activity. Immobility was here taken to be an indication of toxicity. Although a larva may regain mobility if removed from dilute detergent solution and replaced in fresh sea water, it was generally found that the loss of swimming activity was irreversible.

These hastily contrived experiments led us to more detailed studies. The method followed that used by Corner & Sparrow (1956), but is here given in outline.

In these later experiments a series of concentrations of each of several detergents was prepared in sea water: about 100 nauplii were placed in 5 ml of sea water of each concentration contained in a corked tube. The percentages rendered motionless were recorded for increasing times of immersion. These data, when plotted as percentage motionless against time, gave a sigmoid curve from which the time at which 50 per cent of the test sample had lost all activity could be estimated. This time (i.e. TD 50) was then plotted against concentration of detergent (as ppm) on a logarithmic scale. The results obtained are shown in Fig. 23. It is obvious from this that Teepol L is far less toxic than any of the other detergents tested; also that Teepol L may have a separate mode of toxic action, as the shape of the dose/time curve is markedly different from those characterizing the other four detergents, each of which has a sharp inflection above which the concentration must be greatly increased in order to produce any sensible change in TD 50. As these inflections occur at different points in each of the four curves and as the slopes also vary, it is only possible to compare

Table 13. *Relative toxicities of detergents to* Elminius *nauplii*

	Number of times as toxic as active ingredients of Teepol L			
TD 50 (min)	BP 1002	Slipclean	Gamlen	Dasic
10	60	30	17	13
30	28	12	25	7

toxicities by referring to a particular TD 50 value. Thus, taking a TD 50 of 10 minutes, the relative concentrations needed (as ppm) are 7 (BP 1002), 14 (Slipclean), 24 (Gamlen), 32 (Dasic) and 1550 (Teepol L). By contrast, if a TD 50 of 30 minutes is used the concentrations are 3·5 (BP 1002), 3·9 (Gamlen), 7·8 (Slipclean), 15 (Dasic) and 370 (Teepol L). Relative toxicities based on these two TD 50 values are therefore different (Table 13).

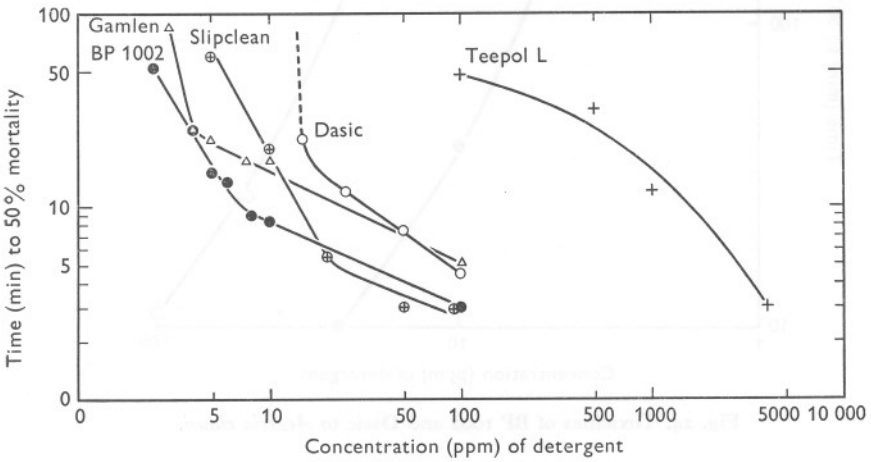


Fig. 23. Toxicities of various detergents to stage II *Elminius* nauplius larvae at 16–20 °C. Note: concentrations of Teepol L refer to the commercial preparation, which contains about 27 per cent active ingredients. The curve for pure Teepol L would be appropriately closer to those for the other detergents (as in Corner, Southward & Southward, 1968).

Copepods

These animals are important as food for pelagic fish and thus play a central role in the oceanic food-chain. Unlike *Elminius* their complete life-cycle is spent in the zooplankton: not just the young stages. The first experiments were 'rough and ready'. Samples of mixed zooplankton, mainly consisting of small copepods, were used in toxicity tests with BP 1002. Samples of the plankton were immersed in concentrations of 2·5 and 5·0 ppm of BP 1002 for 24 hours and then the fraction rendered inactive estimated by eye. The

indications were that both these concentrations had a deleterious effect on the animals.

(*a*) *Calanus finmarchicus* (Fig. 22D). More detailed experiments were carried out at Millport by Dr S. M. Marshall, F.R.S., who has kindly allowed us to use her results. The test animal used was the copepod

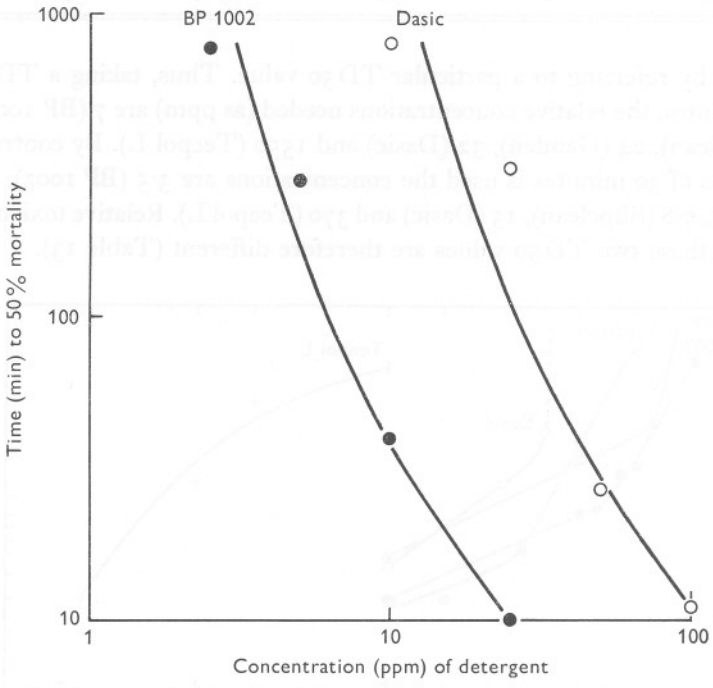


Fig. 24. Toxicities of BP 1002 and Dasic to *Acartia clausi*.

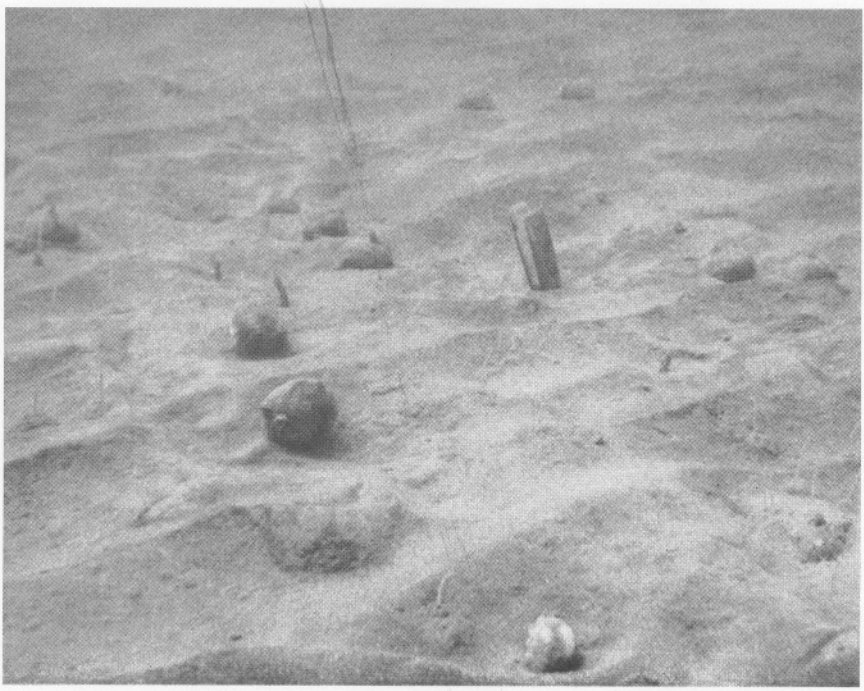
Calanus finmarchicus, and experiments with BP 1002, Gamlen, Dasic, Molyslip, and Houghton Solv. 112 showed that concentrations of 50 ppm were lethal in an hour; 5–10 ppm killed most specimens within two to three days, and 1 ppm, although not lethal, had some effect in that the

PLATE 24

Porthleven, depth 15 metres, 19 April. Photographs of the rippled sand bottom about half a mile offshore. **A**, *Echinocardium cordatum* on the sand surface. The sand humped up around the two urchins in the foreground suggests their recent emergence from the sand, these specimens being probably still alive. Those lying on the sand at the top had probably been there some time and were moribund or dead. **B**, Moribund *Echinocardium cordatum* and a razor-shell, *Ensis siliqua*, half projecting from the sand. In both photographs the slender arms of the brittle-star *Acrocnida brachiata*, which remained healthy, may be seen projecting above the sand surface.

PLATE 24

A



B

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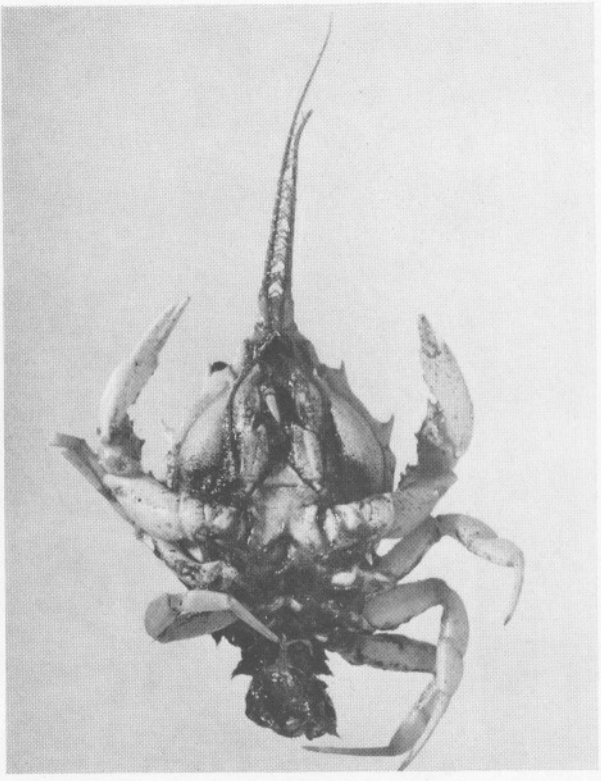
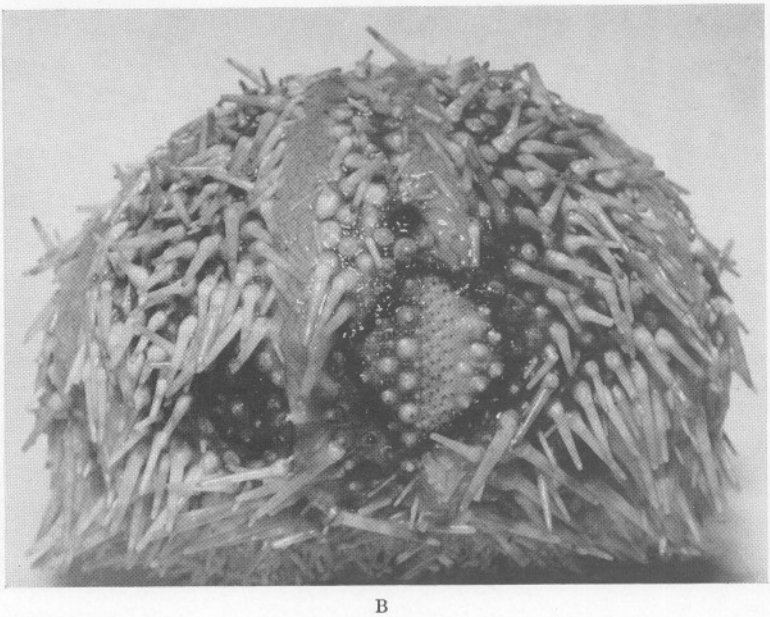
PLATE 25



A



B

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treated copepods were not as active as those used as controls. These data indicate that *C. finmarchicus* is more resistant than *Elminius* nauplii to the various detergents used.

(*b*) *Acartia clausi.* Another species used as a food by fish, and one that spends its entire life-cycle as a member of the zooplankton, is the small copepod *Acartia clausi* (Fig. 22 c). Experiments with this animal were carried out by Mr B. W. P. Sparrow (International Paints Laboratory, Newton Ferrers), who has kindly allowed us to quote his results, summarized in Fig. 24. It will be seen here that the dose/time curves for BP 1002 and Dasic are almost parallel, so that their toxicity ratio can be stated for a wide range of values for TD 50. As in earlier studies with other species, BP 1002 is more toxic than Dasic, the ratio being approximately 5:1. Moreover, the resistance of the very small copepod *Acartia clausi* to detergents is far less than that of the much larger species *Calanus finmarchicus*: thus a concentration of 50 ppm BP 1002 was lethal to *Calanus* in 1 hour, whereas half this concentration (25 ppm) killed *Acartia clausi* in only a few minutes.

There is a general indication that among similar animals the resistance to detergent poisoning is related to size in such a way that the bigger the animal the more resistant it is.

Oyster larvae (Fig. 22 F)

Because of the possible effects of detergents on oysters, particularly when the animals might be spawning, experiments were carried out at the Fisheries Laboratory (Conway) by Dr P. R. Walne, who has kindly allowed us to quote his results. The animals used were embryos of the oyster *Crassostrea gigas*, a relatively warm-water Australian species. The developing eggs were placed in solutions of various detergents for 24 hours at 23 °C, and the proportion of swimming larvae that had developed to the so-called D-stage was then estimated. The results, shown in Table 14, demonstrate that all the detergents tested were toxic to oyster larvae at

PLATE 25

A, *Corystes cassivelaunus*, the masked crab. This specimen was alive when collected from Gunwalloe, 600 metres from the shore in 10 metres depth on 15 April (dive no. 12; see Table 12). It has an accumulation of black material on the setae of the ventral side which is not usual in healthy individuals. This material had collected along the region of the crab's respiratory currents. **B**, A living edible sea-urchin (*Echinus esculentus*) collected from Sennen Cove on 24 April (dive no. 20; see Table 12). The animal is enfeebled and shows scars in which the surface epidermis and spines are gone and which are surrounded by an area of black tissue. The tube feet adjacent to these scars are responsive to tactile stimulation. Toxicity tests showed that the coelomic fluid of these urchins contained traces (2–5 ppm) of the kerosene fraction from the detergent. It is concluded that the scars are the direct or indirect result of pollution since these marks have not been previously recorded from normal populations.

Table 14. *Effects of detergents on development of oyster larvae*

Concn. (ppm)	Proportions of swimming larvae developed to D-stage						
	Control	Polyclens	Houghton Solv. 112	BP 1002	Slip-clean	Dasic	Gamlen
0	+++	.	.	.	.	.	.
0·5	.	o	o	+++	+++	+++	+++
1·0	.	o	o	+++	++	+	++
3·0	.	o	o	+	+	o	+

+++ = all, ++ = some, + = a few, o = none developed.

a concentration of 3 ppm and that some, particularly Polyclens and Houghton Solv. 112, appeared to have effects at 1·0 and 0·5 ppm.

In earlier experiments, made with *Ostrea edulis*, six types of detergent were tested for their effects on the growth of larvae of *O. edulis*. The results are shown in Fig. 25, from which it is apparent that concentrations of detergent in the range 2·5–7·5 ppm can halve the normal rate of development over two days.

Lacuna vincta (*the banded chink shell*) *and* Nassarius reticulatus (*the netted dog-whelk*)

These are larvae of shore-living gastropod molluscs, but like the nauplii of the barnacle *Elminius* they spend their life in the plankton until they settle on the shore and turn into adults. They were hatched from egg capsules and maintained in filtered sea water at 10 °C. When treated with detergent the larvae became opaque and were invaded by ciliates, which removed the soft tissues to leave an empty shell. The results are shown in Table 15.

Nassarius is obviously the more resistant species. Further experiments showed that at a concentration of 1·0 ppm of detergent the larvae of *Nassarius* recovered their customary activity after 36 hours and, ten days later, were still swimming and feeding normally.

Table 15. *Effect of BP 1002 on week-old larvae of* Lacuna *and* Nassarius

Species	Concn. (ppm)	% dead after:		
		2 days	4 days	10 days
Lacuna vincta (banded chink shell)	20	100	.	.
	2	100	.	.
Nassarius reticulatus (netted dog-whelk)	20	.	100	.
	10	.	.	70

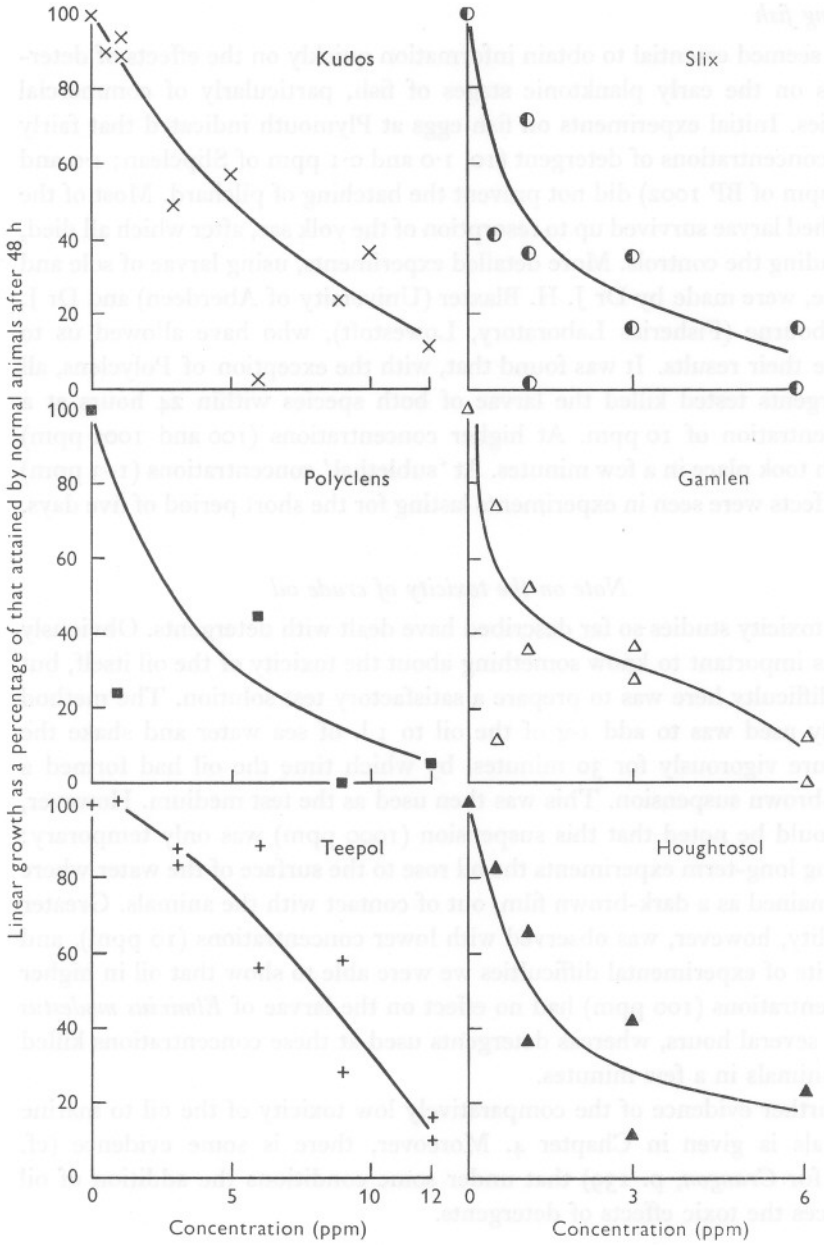


Fig. 25. Effect of various detergents on the growth of larvae of *Ostrea edulis*. Note the difference in the horizontal scale on the two sides of the figure, and the greater toxicity of the hard detergents on the right.

Young fish

It seemed essential to obtain information quickly on the effects of detergents on the early planktonic stages of fish, particularly of commercial species. Initial experiments on fish eggs at Plymouth indicated that fairly low concentrations of detergent (10, 1·0 and 0·1 ppm of Slipclean; 1·0 and 0·1 ppm of BP 1002) did not prevent the hatching of pilchard. Most of the hatched larvae survived up to resorption of the yolk sac, after which all died, including the controls. More detailed experiments, using larvae of sole and plaice, were made by Dr J. H. Blaxter (University of Aberdeen) and Dr J. Shelbourne (Fisheries Laboratory, Lowestoft), who have allowed us to quote their results. It was found that, with the exception of Polyclens, all detergents tested killed the larvae of both species within 24 hours at a concentration of 10 ppm. At higher concentrations (100 and 1000 ppm) death took place in a few minutes. At 'sublethal' concentrations (1–3 ppm) no effects were seen in experiments lasting for the short period of five days.

Note on the toxicity of crude oil

The toxicity studies so far described have dealt with detergents. Obviously it was important to know something about the toxicity of the oil itself, but the difficulty here was to prepare a satisfactory test solution. The method finally used was to add 1 g of the oil to 1 l. of sea water and shake the mixture vigorously for 30 minutes, by which time the oil had formed a pale-brown suspension. This was then used as the test medium. However, it should be noted that this suspension (1000 ppm) was only temporary: during long-term experiments the oil rose to the surface of the water where it remained as a dark-brown film, out of contact with the animals. Greater stability, however, was observed with lower concentrations (10 ppm), and in spite of experimental difficulties we were able to show that oil in higher concentrations (100 ppm) had no effect on the larvae of *Elminius modestus* after several hours, whereas detergents used at these concentrations killed the animals in a few minutes.

Further evidence of the comparatively low toxicity of the oil to marine animals is given in Chapter 4. Moreover, there is some evidence (cf. data for *Crangon*, p. 139) that under some conditions the addition of oil reduces the toxic effects of detergents.

Conclusions

The studies described in this section were of an exploratory nature, having been conducted over a period of ten weeks in response to a local disaster.

Nevertheless the results are unambiguous and the experiments left no doubt that the detergents used for emulsifying crude oil from the 'Torrey Canyon' were extremely toxic to marine planktonic animals. The experiments also showed that the very small members of the zooplankton are particularly susceptible to the toxic effects of detergents, and this could be serious in the sense that these animals—the microzooplankton—are now regarded as an extremely important part of the food-web in the sea (Johannes, 1961).

The overall biological effect of the detergents in the sea clearly depends on the persistence of the toxic principles. It was therefore desirable to know the relative toxicity of the several components of the detergents and their likely persistence. The experiments described in section V of this chapter are relevant to this question.

II. LONGER-TERM EFFECTS ON ZOOPLANKTON

In describing the experiments on the *Sabellaria* larvae (p. 145) the presence of a delayed effect was noted. To examine this further, and to see if it was evident in other species, some longer-term experiments were undertaken using sublethal concentrations of detergent. The species used for these experiments were *Sabellaria spinulosa*, *Elminius modestus* and *Echinus esculentus*. All are coastal animals having free-swimming planktonic larvae.

Sabellaria *larvae*

Larvae of *Sabellaria spinulosa* (Fig. 22A) were placed in Monax dishes, about a third to a half full, loosely covered with watch-glasses. Concentrations of 1 ppm and 0·5 ppm BP 1002 were tested with thirty larvae per dish. There was an immediate reaction to 1 ppm detergent, the animals flexing their bodies ventrally and erecting their provisional bristles to point in all directions (Fig. 22B). However, in the 0·5 ppm solution only about half the larvae reacted strongly: the remainder continued to swim but were irritable compared with the control animals. A little *Isochrysis* culture was added to all dishes after 2 hours, and overnight there was complete recovery of the larvae in 0·5 ppm, but not until two days had elapsed did those in 1 ppm appear to behave normally. At this time new larvae put into this dish showed no irritation, the irritant factor having disappeared. These two dishes and the control dish were kept supplied with *Isochrysis* for food, and all the larvae were apparently healthy and normal three weeks later. However, the experiment was continued, with the interesting result that after four weeks all the larvae in the solution which had originally contained 1 ppm of detergent were found to be lying motionless on the

bottom of the dish, in very poor condition. They were still surviving after six weeks, but were then in an even worse state and had hardly grown. Meanwhile, those which had originally been in a solution of 0·5 ppm detergent had become sluggish—but were still growing—after five weeks; however, during the sixth week they lay motionless, apart from an occasional twitch of the bristles. During all this time the animals used as a control sample were healthy, active and growing well.

The experiment therefore showed that the toxicity of BP 1002 still persisted, even after the organic solvent had evaporated, for after a prolonged period of apparent normality the larvae eventually succumbed.

Elminius *larvae*

Freshly liberated stage II larvae of *Elminius* (cf. Fig. 22G) were reared in small vessels, with diatoms as food. The cultures were subsampled at intervals to estimate the percentage of mortality and the stage of development reached. All cultures were kept in loosely covered dishes and it is believed that the initial toxicity of those containing detergent was lost within the first few days. Thus, the experiments were equivalent to a short exposure to poison followed by a long period of recovery.

In the first series of experiments, using BP 1002, Gamlen and Slipclean at concentrations of 5·0, 1·0 and 0·5 ppm, and also 'Torrey Canyon' oil at 100 ppm, *Phaeodactylum tricornutum* was used as the main food. This diatom is known to be an indifferent food for *Elminius* (Moyse, 1963) and in addition the cultures were rather crowded. The final mortality was therefore high in all cultures, although initially much higher in those containing detergent at 5·0 and 1·0 ppm and oil at 100 ppm. The results are given in Table 16 and Fig. 26. At low concentrations of detergent (0·5 ppm) there was a slight delay in development compared with the controls, but after a week little difference was discernible. In the presence of oil and with medium concentrations of detergent (1 ppm) development was delayed by about two or three days throughout the experiment, the least effect being shown by Gamlen and Slipclean, and the most by BP 1002: all these cultures reached the cyprid stage, although those in BP 1002 failed to metamorphose and settle even when provided with suitably prepared surfaces—that is, cleaned shells of *Mytilus edulis* which had borne live *Elminius modestus* (see Knight-Jones, 1956). At the higher concentrations of detergent (5 ppm) more than 50 per cent of the larvae died the first day. Of the survivors, those in Gamlen failed to develop at all and died while at stage II. Most of the Slipclean survivors reached stage III four days behind the controls and then died, although a few individuals developed as far as stage VI. The survivors of BP 1002 recovered after seven days at stage II

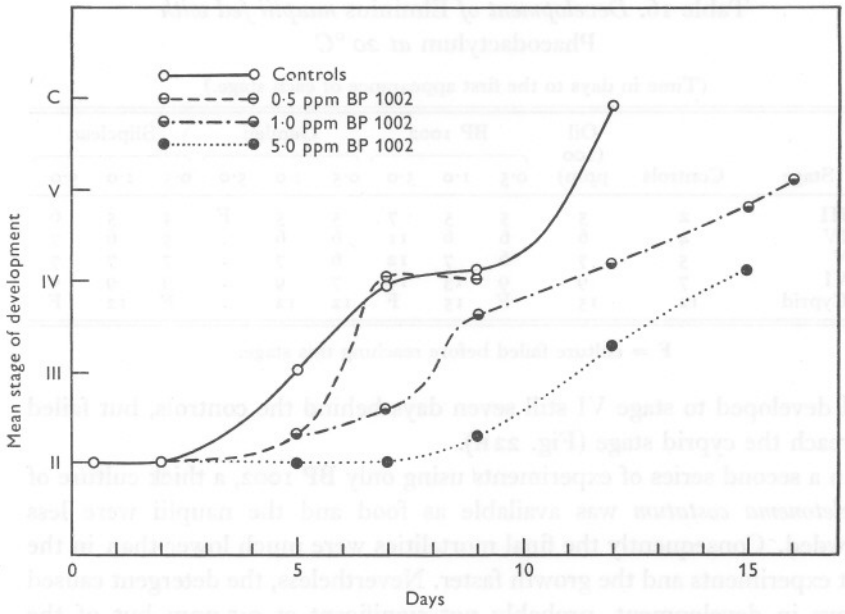


Fig. 26. Rate of development of larvae of *Elminius modestus* reared on *Phaeodactylum tricornutum* at 20 °C in the presence of different concentrations of BP 1002. Nauplius stages shown in Roman numerals; C, Cypris stage.

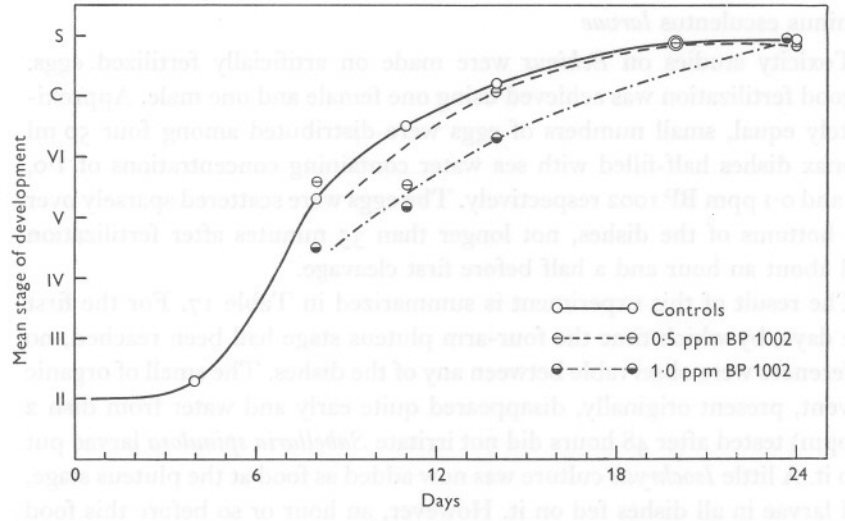


Fig. 27. Rate of development of larvae of *Elminius modestus* reared on *Skeletonema costatum* at 20 °C. Nauplius stages shown in Roman numerals. C, Cypris stage; S, settlement and metamorphosis.

Table 16. *Development of* Elminius *nauplii fed with* Phaeodactylum *at 20 °C*

(Time in days to the first appearance of each stage.)

Stage	Controls	Oil (100 ppm)	BP 1002			Gamlen			Slipclean		
			0·5	1·0	5·0	0·5	1·0	5·0	0·5	1·0	5·0
III	2	5	5	5	7	5	5	F	4	5	6
IV	4	6	6	6	11	6	6	.	5	6	7
V	5	7	6	7	12	6	7	.	7	7	7
VI	7	9	9	13	14	7	9	.	9	9	9
Cyprid	12	15	F	15	F	12	12	.	F	12	F

F = culture failed before reaching this stage.

and developed to stage VI still seven days behind the controls, but failed to reach the cyprid stage (Fig. 22 H).

In a second series of experiments using only BP 1002, a thick culture of *Skeletonema costatum* was available as food and the nauplii were less crowded. Consequently the final mortalities were much lower than in the first experiments and the growth faster. Nevertheless, the detergent caused delays in development, probably not significant at 0·5 ppm but of the order of three days at 1 ppm (see Fig. 27). At the latter concentration only a few cyprids were produced and very few of them metamorphosed and settled (0·6 per cent of the original larvae) compared with the controls (10 per cent of the original larvae).

Echinus esculentus *larvae*

Toxicity studies on *Echinus* were made on artificially fertilized eggs. A good fertilization was achieved using one female and one male. Approximately equal, small numbers of eggs were distributed among four 50 ml Monax dishes half-filled with sea water containing concentrations of 1·0, 0·5 and 0·1 ppm BP 1002 respectively. The eggs were scattered sparsely over the bottoms of the dishes, not longer than 35 minutes after fertilization and about an hour and a half before first cleavage.

The result of this experiment is summarized in Table 17. For the first five days, by which time the four-arm pluteus stage had been reached, no differences were observable between any of the dishes. The smell of organic solvent, present originally, disappeared quite early and water from dish 2 (1 ppm) tested after 48 hours did not irritate *Sabellaria spinulosa* larvae put into it. A little *Isochrysis* culture was now added as food at the pluteus stage, and larvae in all dishes fed on it. However, an hour or so before this food was added, and some hours after assessment of their condition recorded in Table 17, some of the plutei in dish 2 began to 'reduce' the lengths of their

A

B

C

D

(*Facing p.* 129)

Table 17. *Development of* Echinus esculentus *in concentrations of BP 1002*

Date	Dish 1 (sea-water control)	Dish 2 (1 ppm)	Dish 3 (0·5 ppm)	Dish 4 (0·1 ppm)
5. iv. 67	Eggs distributed to all dishes half an hour after fertilization			
6. iv. 67	Normal blastulae	Normal blastulae	Normal blastulae	Normal blastulae
7. iv. 67	Normal gastrulae	Normal gastrulae	Normal gastrulae	Normal gastrulae
10. iv. 67	Normal plutei	Normal plutei	Normal plutei	Normal plutei
11. iv. 67	Majority normal, a few with reduced arms	All with much reduced arms	Majority with partially reduced arms	Majority normal, a few with reduced arms
12. iv. 67	Majority normal and growing	All poor, with stumpy arms	Majority with short arms, some normal	Majority normal and growing

arms; that is to say the flesh of the arms began to shrink down the skeletal rods, which, left protruding, broke off. This is always a sign of ill health. By next day all plutei in this dish had all their arms reduced to mere stumps, although their stomachs were well filled with food. In dish 3 (0·5 ppm) arm-reduction began later, not all plutei were affected and there was some recovery subsequently. But there was very little recovery in dish 2. Almost all the plutei in dish 1 (control) and dish 4 (0·1 ppm) continued to be in excellent growing condition, only a very few in both dishes having reduced arms (as almost invariably happens under even the best rearing conditions).

Too much should not be read into this single experiment. It is of interest only in that it confirms the earlier demonstration of a long-term toxic effect on other species of some component of BP 1002

III. TOXICITY STUDIES ON PHYTOPLANKTON

In order to grow phytoplankton organisms for experimental work it is necessary to keep the culture media well aerated by vigorous bubbling.

PLATE 26

A, 130–150 metres from the breakwater at Porthleven in 5 metres of water, 5 April. Fluorescent orange *Delesseria sanguinea* which has been affected by detergent and was found growing at the top of a gully on the sea-bed. The *Delesseria* is normally the same colour as the alga seen growing behind it. **B**, Sennen Cove, 7 April. Underwater photograph of a dead *Echinus esculentus* (edible sea-urchin) resting upside-down in a gully between rocks at a depth of 10–11 metres. Normally in healthy individuals the tube feet are conspicuously extended. **C**, Porthleven Reef, 28 April. Underwater photograph of the sea-bed at a depth of 17 metres (dive no. 21). Dead *Echinocardium cordatum* and *Mactra corallina* with healthy *Asterias rubens* and *Marthasterias glacialis* feeding on them. **D**, Constantine Bay, 8 April. Shells of recently killed molluscs in the drift line. Mussels, limpets, winkles and dog-whelks are all represented.

This greatly reduced the level of the organic solvent 'Kex' in BP 1002 (see page 145), and for this reason the following section deals in effect with only the surfactant components of the detergent.

The six following phytoplankton species were used: 64, *Phaeocystis pouchetii*; 81, *Dunaliella primolecta*; 85, *Chlorella stigmatophora*; 92, *Coccolithus huxleyi*; 205, *Halosphaera minor*; 207, *Gymnodinium* sp. The numbers are those used in the Plymouth culture collection.

The growth curves for each of these species were measured at 12 °C in sea water, initially filtered through an Oxoid membrane and autoclaved, and then enriched as follows: nitrate 10^{-4} g ions/l.; phosphate 10^{-5} g ions/l.; vitamin B_{12} 5·5 μg/l.; manganese 2 μg/l.; iron (as citrate) 50 μg/l. The cultures were subjected to alternating periods of 12 hours of darkness and 12 hours of light at about 600 foot-candles, and were vigorously aerated. One hour before inoculation, BP 1002 with known proportion of surfactant was added to the sea water at different concentrations. The aeration during the subsequent hour has been shown to cause the removal of most of the volatile kerosene solvent in the emulsifier. A fifth culture vessel containing no BP 1002 was used as a control.

Cell counts were made on each culture vessel 1 hour after inoculation and then daily for 12 days, using a Coulter Counter Model F; the inocula were obtained from Plymouth stock cultures.

From plots of the logarithm of cell numbers per ml against time, the length of the initial slow-growing phase (lag phase) and the mean generation time for each culture have been obtained. These are given in Tables 18 and 19.

Inhibition of growth is indicated by an increase in the length of the lag phase and the mean generation time in relation to the control. The two genera least affected by the surfactants were *Dunaliella* and *Chlorella*, both of which are characteristic of brackish-water plankton. At the highest concentration examined (1·2 ppm of surfactant) the lag phase for *Chlorella* was increased by a factor of three relative to the control, a result indicating some retardation in metabolism, but it grew at the same rate as in the other concentrations. At 1·2 ppm surfactant the other genera examined were destroyed in the hour between inoculation and the first cell count. The *Gymnodinium* proved to be the most vulnerable to the surfactant, with the growth rate almost halved at surfactant concentrations of $1{\cdot}2 \times 10^{-2}$ and $1{\cdot}2 \times 10^{-1}$ ppm, although, surprisingly, the length of the lag phase was about the same as in the control. An apparently paradoxical effect was noted at the lowest concentrations with certain species, notably *Coccolithus*. Here the mean generation time was shortened by about one-third, indicating a growth-promoting effect of the detergent. There are, however,

Table 18. *The length of the lag phase (in days) at various concentrations of the surfactant component of BP 1002*

Phytoplankton	Surfactant concentration (ppm)				
	0	$1{\cdot}2 \times 10^{-3}$	$1{\cdot}2 \times 10^{-2}$	$1{\cdot}2 \times 10^{-1}$	1·2
64 *Phaeocystis pouchetii*	—	1·4	0·4	0·4	Cells killed
81 *Dunaliella primolecta*	2·2	1·2	2·0	2·0	2·5
85 *Chlorella stigmatophora*	0·8	0·9	0·9	0·9	2·5
92 *Coccolithus huxleyi*	0·25	0·35	0·35	0·35	Cells killed
205 *Halosphaera minor*	0·2	0·3	0·5	0·3	Cells killed
207 *Gymnodinium* sp.	1·2	2·6	1·7	0·9	Cells killed

Table 19. *The mean generation time (in days) at various concentrations of the surfactant component of BP 1002*

Phytoplankton	Surfactant concentration (ppm)				
	0	$1{\cdot}2 \times 10^{-3}$	$1{\cdot}2 \times 10^{-2}$	$1{\cdot}2 \times 10^{-1}$	1·2
64 *Phaeocystis pouchetii*	—	1·0	1·0	1·0	Cells killed
81 *Dunaliella primolecta*	1·2	1·1	1·1	1·1	1·0
85 *Chlorella stigmatophora*	1·2	1·1	1·1	1·1	1·2
92 *Coccolithus huxleyi*	1·6	1·1	1·1	1·1	Cells killed
205 *Halosphaera minor*	1·6	1·5	1·2	1·6	Cells killed
207 *Gymnodinium* sp.	3·8	3·2	7·3	7·3	Cells killed

indications that cells grown under such artificial stimulatory conditions are abnormally fragile (Kidder, Dewey & Heinrich, 1954).

Non-ionic surfactants adsorb on to cell membranes by interaction of the hydrophobic portion of the surfactant with the lipoidal constituents of the membranes. This results in an increase in the permeability of the cell wall, facilitating the passage of dissolved substances both into and out of the cell. At sufficiently high surfactant concentrations the cell constituents are able to leak out from the cell, causing its death (Hotchkiss, 1946). The brackish-water *Dunaliella* and *Chlorella*, which proved the most resistant to the surfactant, are well adapted for ionic regulation (that is, controlling the passage of ions across their cell walls); they might therefore be expected to tolerate changes in the permeability of the cell wall more easily than the strictly marine species.

IV. TOXICITY STUDIES ON INTERTIDAL AND SUBLITTORAL ORGANISMS

Intertidal algae

The familiar seaweeds of the shore were often exposed to very high concentrations of detergent during the beach-cleaning operations. To

investigate the effect on the shore vegetation, detailed tests were made by Dr A. D. Boney of Aberystwyth on four chosen intertidal species. These were the green filamentous alga *Cladophora rupestris*, the brown knotted wrack *Ascophyllum nodosum*, the red algae *Polysiphonia lanosa* (a filamentous form often epiphytic on *Ascophyllum*) and the thalloid *Porphyra umbilicalis* (sometimes known as laver).

The technique employed was to immerse the weed in detergent solutions of a wide range of strengths for varying periods, usually 3 or 6 hours, in some ways simulating conditions which might have been encountered on the shore, except that no freshwater mixtures were used. After immersion the weeds were rinsed with clean sea water and any gross damage could be seen at once. They were kept in clean sea water for 24 hours before being examined microscopically for signs of cell damage, such as shrinking of the protoplasm, loss of pigment, etc. The results are here given only in outline. On the whole, seaweeds are very much more tolerant of detergent than are intertidal animals. Indeed *Porphyra umbilicalis* and *Polysiphonia lanosa* showed no damage detectable by the microscope even after 6 hours immersion in the undiluted detergent. These short-term experiments suggested an unexpectedly strong resistance to detergent treatment not in accord with the bleaching of *Porphyra* and discoloration of other red algae often seen on the shore, although this could generally have been due to the action of fresh water with which the detergent was diluted. Tests carried out on the sublittoral red alga *Delesseria* (see page 137) perhaps suggest a further reason for the apparent discrepancy, in that this alga may take several days to show the effects of damage in sea water. The cell walls of *Porphyra* and *Polysiphonia* must presumably be very impermeable to the constituents of the detergents when uninfluenced by fresh water.

With *Ascophyllum nodosum* (one of the brown shore weeds) the investigation was confined to the reproductive cells which were active at the time of the investigation and were chosen as being likely to be the most sensitive indicators of toxicity. Six hours immersion in a 25 per cent solution of detergent caused irreversible cell damage to the reproductive cells themselves and also to the cells of the receptacle in which the reproductive cells lie before release. In detergent at 12 per cent concentration cell damage was very slight or absent, depending on the type of detergent used. In fact six proprietary brands of detergents were used for all the tests, but the difference in the degree of damage caused by the different brands was not significant.

After they were released from the parent plant the reproductive cells, the spermatozoids and the oospheres, were extremely sensitive, a brief exposure to 0·01 per cent solutions (that is, 100 ppm) being sufficient to kill them.

The green seaweed *Cladophora rupestris* was the most sensitive of the species tested. Here the reproductive cells were not accessible to study and the examination was concentrated on the apical cells of the filaments, which, being the growing points, were judged to be the most sensitive. Here, severe damage was noted after 6 hours immersion in 6 per cent solutions of all detergents (except BP 1002, which was apparently harmless at this concentration). There was less severe, but irreversible damage down to about 1 per cent concentration.

Intertidal animals—toxic effects of BP 1002

During shore-spraying operations the intertidal animals and plants were exposed to very high concentrations of detergent for periods of up to several hours and the expected mortalities described in Chapter 4 were soon evident to the shore observers. Nevertheless, the intertidal species are, as a group, constitutionally very tough, and many of them, the bivalves for example, are able to seal themselves off from a hostile environment for long periods, later to emerge unharmed. It therefore seemed profitable to examine some of these animals for their resistance to detergent poisoning.

The first experiments were only crude, and involved placing animals in sealed containers filled with sea water to which various amounts of detergent were added. The concentrations ranged from 0·2 to 100 ppm. The animals were left in the sealed containers for 24 hours before being removed to fresh sea water and their recovery observed. The containers were large enough to avoid any danger of oxygen deprivation. The results of these preliminary tests are summarized in Table 20.

A few species were selected for more careful study.

Mussels. Specimens of the common mussel, *Mytilus edulis*, 40–50 mm long collected from low-water neap-tide level were used in two sets of experiments. All survived 24 hours exposure to 5 ppm detergent (BP 1002), but 10 ppm and over was lethal within 24 hours. A good guide to the condition of *Mytilus* is its ability to reattach itself to the substratum after disturbance by the extrusion of new byssal threads. In 1 ppm detergent all the mussels had attached in 24 hours and in 5 ppm 60 per cent of the animals had attached, but there was no sign of new byssal threads in 10 ppm and over. Exposed to crude oil in 1000 ppm suspension the mussels all survived the 24-hour period but there was no attachment.

Experiments with *Mytilus galloprovincialis* in the Black Sea (Aljakrinskaya, 1966) reveal that high levels of oil in sea water (up to 2 per cent) can be tolerated by mussels, which remove it from suspension by means of their cleansing mechanisms. It should not be overlooked, however, that

Table 20. *Toxicity of BP 1002 to some intertidal species at 12 °C*

Species	Popular name	Conc. (ppm) needed to kill majority in 24 h	Notes
Coelenterata			
Actinia equina	Beadlet anemone	25	Some young animals survived 25 ppm
Anemonia sulcata	Snakelocks anemone	50	Some looked dead but later recovered
Annelida			
Nereis diversicolor	Rag-worm	25	A few survived this concentration
Crustacea			
Eurydice pulchra	Isopod	10	Some killed at 5 ppm
Carcinus maenas	Shore-crab	25	—
Cancer pagurus	Edible crab	10	—
Palaemon serratus	Prawn	5	—
Crangon vulgaris	Shrimp	2	—
Mollusca			
Nucella lapillus	Dog-whelk	100 +	Became detached at 10 ppm
Monodonta lineata	Top-shell	100	—
Littorina littorea	Winkle	100	Some could recover from 100 ppm
Calliostoma zizyphinum	Painted top-shell	10	Became detached at 2 ppm
Aplysia punctata	Sea hare	50	Became detached at 10 ppm
Patella vulgata	Limpet	5	Dying limpets frequently but not always release their attachment to the substrate

even low levels of detergent may inhibit the natural cleansing mechanism and thus reduce the mussel's tolerance to oil.

Limpets. The common limpet *Patella vulgata* was found in abundance dead and dying on the detergent-treated shores.

Experiments were made with small animals (about 25 mm length) carefully collected from an unpolluted beach, while they were actively moving. This avoids damage to the foot, as occurs if the animals are prised from their seats. The limpets were allowed to attach to glass plates kept in clean sea water overnight, and the water was then replaced by the test solutions shown in Table 21, which summarizes the results of the experiments.

The sensitivity to low concentrations of detergent is in accord with the high mortality of limpets noted in the field observations.

Top-shells and winkles. An experiment on the top-shells *Monodonta lineata* and *Gibbula umbilicalis*, and the winkle *Littorina littorea*, showed that the normal climbing response, observed when the animals are kept in

Table 21. *Toxicity of BP 1002 to* Patella

		BP 1002		
	Control	100 ppm	10 ppm	1 ppm
Behaviour of pallial tentacles	All expanded	All withdrawn	All withdrawn	Withdrawn at first but 75% recovered after 3 h
% attachment of foot after 18 h	100% living	All dead	All dead or dying	80% recovered

Table 22. *Effect of oil and BP 1002 on cirral (limb) beat of very young* Elminius modestus

		BP 1002		Kuwait crude oil	
	Control	100 ppm	10 ppm	Film (1000 ppm)	Suspension (100 ppm)
Initial Activity	60–80% fast beat	50% stopped cirral beat	50% normal beat	50% normal beat	50% normal beat
1 h	60–80% fast beat	50% stopped cirral beat	Pumping beat only	50% normal beat	50% normal beat
24 h	.	Dead	Few active	.	.
48 h	40% fast beat 40% normal beat	.	Dead	28% active	10% active

beakers of sea water, was inhibited by 10 ppm of BP 1002. The animals were partly withdrawn into the shell, and only the *Monodonta* survived three days immersion (by which time the toxicity was reduced by evaporation) and were able to climb out of the water. A detergent concentration of 1 ppm impaired activity in all three species but did not prove lethal.

The results of laboratory tests with the above species are again reflected in the observations on treated shores (Chapter 4) where there has been heavy mortality of winkles and top-shells. In places where the treatment has been light enough to give the animals a chance of survival *Monodonta* and *Nucella* have been the most tenacious.

Barnacles. Experiments made on *Elminius modestus* larvae (p. 118) were followed by others on the susceptibility of later stages to detergent poisoning. The effect on settlement and metamorphosis was first examined because this is a very critical stage in the life of a barnacle. Cleaned shells of *Mytilus* that had borne *Elminius* adults were placed in dilutions of BP 1002, and ten cyprids added to each. At 5 ppm and over the cyprids ceased swimming (see Fig. 30, page 141) and died in two days. At 3 ppm swimming stopped

after 24 h and the cyprids died in four days without settling. At 1 ppm swimming was unaffected and many of the cyprids settled and metamorphosed. The newly settled adult form ('spat') is another critical stage on which experiments were also made. Shells bearing recently settled spat were exposed (1) to different concentrations of BP 1002, (2) to a film of Kuwait crude oil on the surface of sea water, and (3) to sea water that had been mechanically shaken for 5 minutes with the oil. The results are shown in Table 22, cirral activity (that is, limb movements) being assessed in the way described by Crisp & Southward (1961).

These data show that the young, recently metamorphosed barnacle is more resistant than the larval stages to poisoning by detergents. Nevertheless, BP 1002 at a concentration of 10 ppm was ultimately lethal. Moreover Kuwait oil had an obvious depressing effect on cirral activity, and hence on feeding, and would thus inhibit growth.

Fully grown specimens of *Elminius* attached to mussel shells collected from low water of neap tides were also tested. They were treated with various concentrations of BP 1002, and with water that had been mechanically shaken for 30 minutes with 'Torrey Canyon' oil collected from a beach at St Ives during the first few days contamination. The vessels were not covered and so it is probable that the detergent lost toxicity in 24 hours. At a concentration of 100 ppm the barnacles became inactive and some died; the rest showed some cirral activity after 24 hours.

Detergent at concentrations of 5 and 10 ppm and oil at concentrations of 100 ppm slowed the rate of cirral beating by 25–35 per cent.

Sublittoral organisms—toxic effects of BP 1002

The observations reported in Chapter 6 show how detergents sprayed on the shore can lead to an expanding front of toxic water spreading to appreciable distances along the coast, over the surface and down to the sea-bed. Divers exploring offshore reaches of the sea-bed found a variety of dead and moribund animals whose habits and habitat precluded any suggestion that they had been killed on the shore and subsequently washed seawards. To complete this picture toxicity experiments were made with a selection of sublittoral species. The results of these are reported below and are sufficient to verify that the divers with their restricted range of vision and coverage saw a typical, but only a small, sample of the offshore consequences of the beach-cleaning operations.

Tolerance of various sublittoral species. The method used was the same as that already described on page 133. Table 23 gives some results for a number of species that live below the low-water mark. As might be expected, they are more sensitive than the intertidal animals. However, for a crustacean,

Table 23. *Toxicity of BP 1002 to some sublittoral species at 12 °C*

Species	Common name	Concn. (ppm) needed to kill majority in 24 h	Notes
Coelenterata			
Calliactis parasitica	Sea anemone	25	Stayed closed at 5 ppm
Crustacea			
Corystes cassivelaunus	Masked-crab	10	.
Portunus holsatus	Swimming-crab	5	.
Diogenes pugilator	Hermit-crab	25	.
Mollusca			
Nassarius reticulatus	Netted whelk	2·5	Some survived 2·5 ppm
Chlamys opercularis	Queen scallop	1	Affected at 0·5 ppm (tended to gape)
Laevicardium crassum	Smooth cockle	1	Affected at 0·5 ppm (tended to gape)
Spisula subtruncata	Trough-shell	2	Affected at 1 ppm (tended to gape)
Ensis siliqua	Razor-shell	0·5	.
Echinodermata			
Asterias rubens	Common starfish	25	Climbing stopped at 10 ppm
Ophiocomina nigra	Brittle-star	5	Affected at 2 ppm
Algae			
Delesseria sanguinea	Red seaweed	10	Took several days to change colour

Diogenes (a hermit crab) is remarkably resistant. Another fairly resistant species was the common starfish, *Asterias rubens*, which during the diving programme (p. 113) was seen feeding on other animals killed by the detergent.

Although it is one of the most sensitive seaweeds and becomes 'fluorescent' orange when killed (Plate 26A), the red weed *Delesseria* when treated with 0·001 per cent detergent took several days to change colour.

Bivalve molluscs were the most sensitive of the animals which were examined. Of these the razor-shell *Ensis siliqua* is the most susceptible to poisoning and was killed by 0·5 ppm of detergent. Results of the diving programme (p. 113) show that *Ensis*, together with the clam, *Mactra stultorum*, was killed at a distance of at least 1 kilometre off Porthleven. It therefore seems likely that detergent–oil patches having concentrations of about 0·5 ppm at the level of the sea-bed had moved through these areas.

Shrimps. Details of the use of the shrimp *Crangon vulgaris* in toxicity experiments are given under 'Bioassay'. The same method was used in order to test the relative toxicity of several detergents and the results are

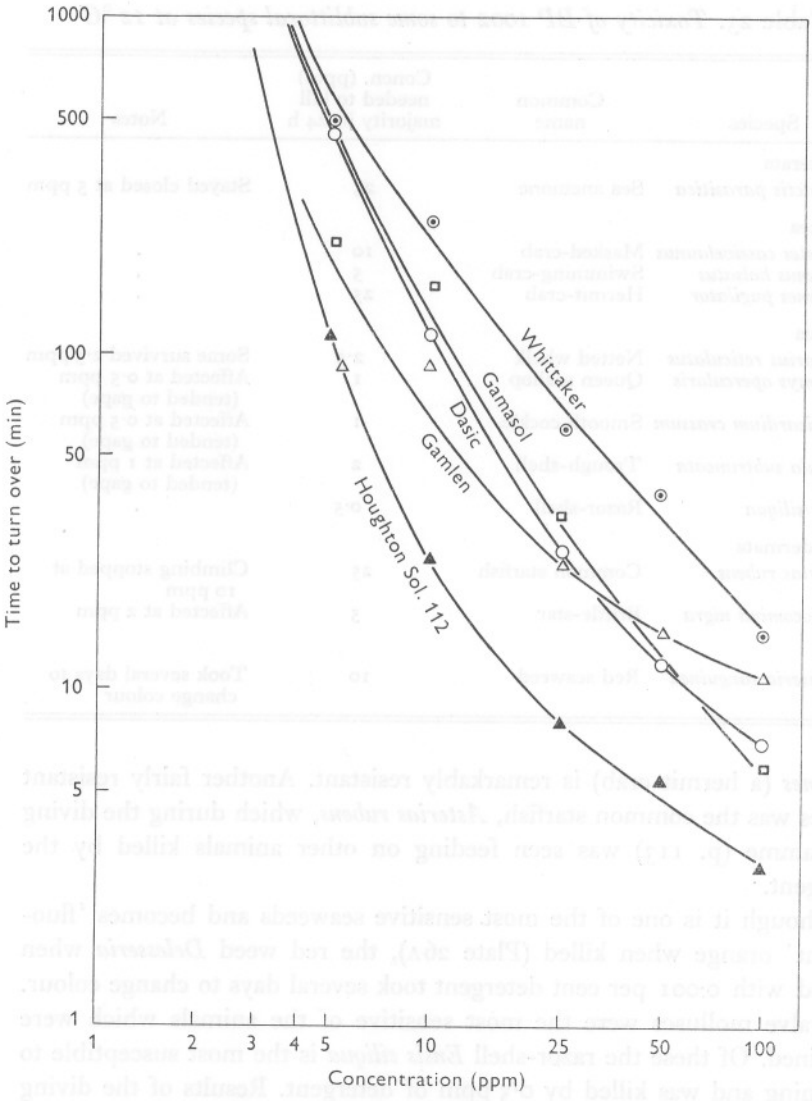


Fig. 28. Relationship between concentrations of different detergents and the mean times taken by *Crangon vulgaris* to turn over. (For 'Gamasol' read 'Gramosol'.)

shown in Fig. 28. As with similar experiments carried out on *Elminius*, the curves were not parallel: thus, whereas Gamlen was less toxic than Dasic and Gramosol at 100 ppm, it was more toxic at 5, 10 and 25 ppm. Comparison of these data with those for BP 1002 in Fig. 29 shows that at concentrations below 10 ppm BP 1002 was the most toxic detergent; this

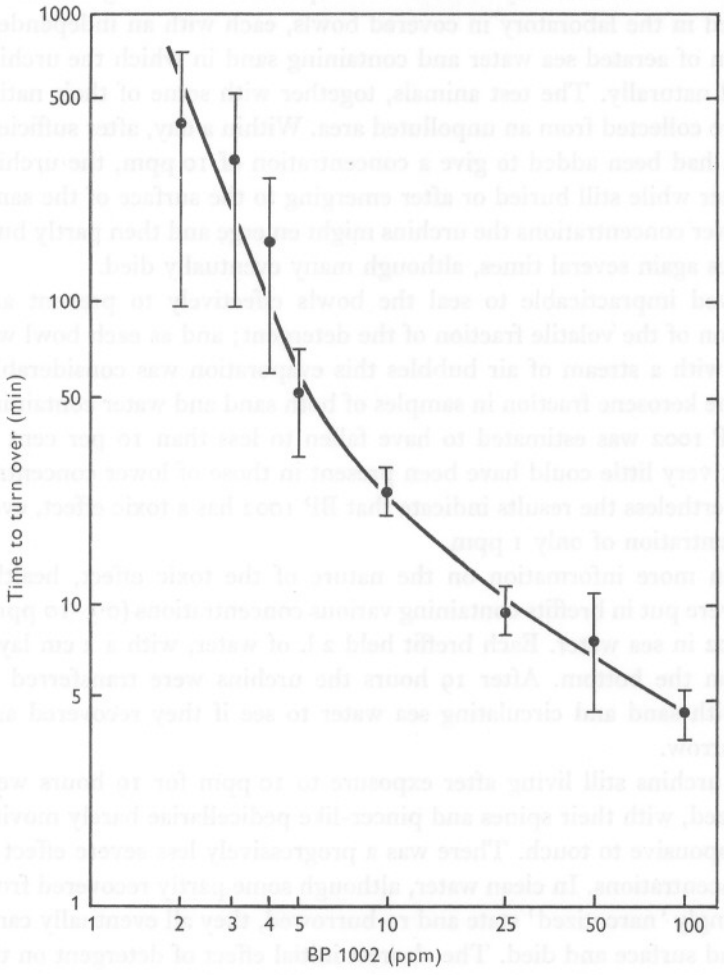


Fig. 29. Relationship between concentration of BP 1002 and the mean time for *Crangon vulgaris* to turn over. Vertical lines show the range of the standard deviation at each concentration.

again agrees in general with the *Elminius* results. In addition, it was found that a mixture of BP 1002 and Kuwait crude oil (equal volumes giving a concentration of 10 ppm detergent) was less toxic than the detergent used alone, the toxicity being reduced by some 30–40 per cent.

Sand-urchins and their 'commensals'. In view of the extensive mortalities of the heart-urchin, *Echinocardium cordatum*, off beaches cleaned with toxic chemicals, the effect of the detergent BP 1002 on it was tested in the laboratory.

The sand-urchin normally burrows to a depth of about 10–15 cm. It was maintained in the laboratory in covered bowls, each with an independent circulation of aerated sea water and containing sand in which the urchins burrowed naturally. The test animals, together with some of their native sand, were collected from an unpolluted area. Within a day, after sufficient detergent had been added to give a concentration of 10 ppm, the urchins died, either while still buried or after emerging to the surface of the sand. But at lower concentrations the urchins might emerge and then partly bury themselves again several times, although many eventually died.

It proved impracticable to seal the bowls effectively to prevent any evaporation of the volatile fraction of the detergent; and as each bowl was supplied with a stream of air bubbles this evaporation was considerable. In fact, the kerosene fraction in samples of both sand and water containing 5 ppm BP 1002 was estimated to have fallen to less than 10 per cent in five days; very little could have been present in those of lower concentration. Nevertheless the results indicate that BP 1002 has a toxic effect, even at a concentration of only 1 ppm.

To gain more information on the nature of the toxic effect, healthy urchins were put in breffits containing various concentrations (0·5–10 ppm) of BP 1002 in sea water. Each breffit held 2 l. of water, with a 2 cm layer of sand on the bottom. After 19 hours the urchins were transferred to a bowl with sand and circulating sea water to see if they recovered and would burrow.

Those urchins still living after exposure to 10 ppm for 19 hours were immobilized, with their spines and pincer-like pedicellariae barely moving and unresponsive to touch. There was a progressively less severe effect at lower concentrations. In clean water, although some partly recovered from the seemingly 'narcotized' state and re-burrowed, they all eventually came to the sand surface and died. The clearest initial effect of detergent on the activities of burrowed urchins was an arrested forward movement. A toxic effect was found above about 0·5 ppm. A small bivalve, *Montacuta ferruginosa*, and an amphipod crustacean, *Urothoë grimaldi*, both under 1 cm long, are common 'commensals' with the sand-urchin. Animals were tested in small, sealed bottles (150 ml capacity) holding various concentrations (0·1–50 ppm) of BP 1002, at least two specimens of each species being placed in each bottle for 12 hours. The *Urothoë* died when the detergent was above 5 ppm and appeared unaffected by lower concentrations. *Montacuta*, on the other hand, showed a graded effect: the bivalves died quickly in 50 ppm, but recovered in clean sea water from a 'narcotized' state with their valves gaping after exposure to lower concentrations. Concentrations below 1 ppm did not seem to have any effect.

V. BIOASSAY

The chemical methods of analysis described in Chapter 2 (p. 19) are potentially very accurate for estimating detergent concentration under well-controlled conditions, but they are attended by several defects which make them unsuitable for the testing of water samples, collected at sea or from the shore. The worst of these defects is that they estimate only

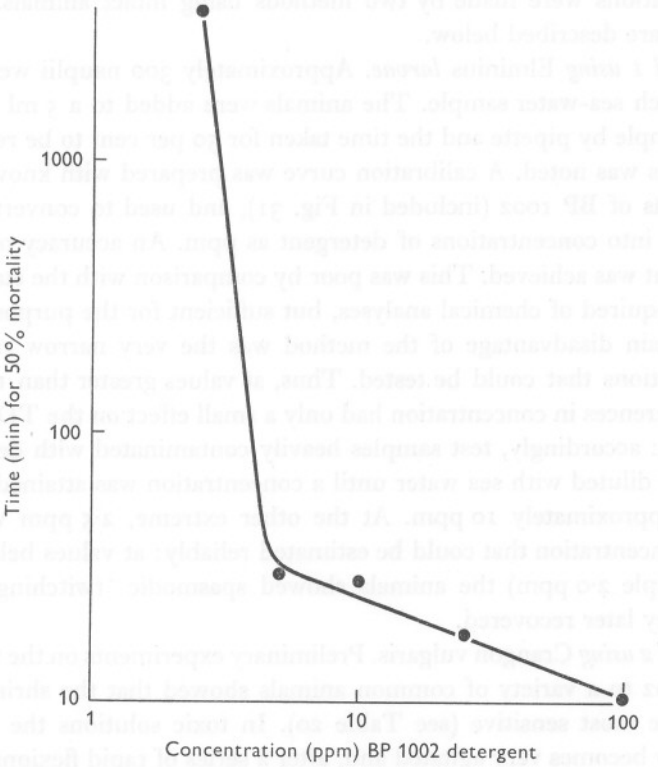


Fig. 30. Effect of different concentrations of BP 1002 on swimming activity of cyprids of *Elminius modestus* at 20 °C. See page 135.

one component of the detergent mixture; in two of the three methods this is the surfactant component which differs in density, persistence and toxicity from the main solvent component. Thus the concentration measured does not necessarily reflect the toxicity of the sea-water sample.

From the experience gained in the many toxicity tests earlier described we were able to devise simple methods of bioassay which, although they may fall short of the standards required in the analytical laboratory, are

easy to perform, are reliable, and directly measure the toxicity of the water sample. It was these methods which made possible the important series of observations on detergent drift reported in Chapter 6.

Several methods were tried. One which showed promise involved observing the effect of detergent solutions on the ciliary beat of isolated strips of the gill membranes of the mussel *Mytilus edulis*. However, the little time available in the early stages of the 'Torrey Canyon' operations prevented us from developing this method and, in the end, bioassay determinations were made by two methods using intact animals. These methods are described below.

Method 1 using Elminius *larvae*. Approximately 300 nauplii were used to test each sea-water sample. The animals were added to a 5 ml portion of the sample by pipette and the time taken for 50 per cent to be rendered motionless was noted. A calibration curve was prepared with known concentrations of BP 1002 (included in Fig. 31), and used to convert values of TD 50 into concentrations of detergent as ppm. An accuracy of about 10 per cent was achieved. This was poor by comparison with the standards usually required of chemical analyses, but sufficient for the purpose.

The main disadvantage of the method was the very narrow range of concentrations that could be tested. Thus, at values greater than 10 ppm, large differences in concentration had only a small effect on the TD 50 (see page 118): accordingly, test samples heavily contaminated with detergent had to be diluted with sea water until a concentration was attained representing approximately 10 ppm. At the other extreme, 2·5 ppm was the lowest concentration that could be estimated reliably: at values below this (for example 2·0 ppm) the animals showed spasmodic 'twitching' from which they later recovered.

Method 2 using Crangon vulgaris. Preliminary experiments on the toxicity of BP 1002 to a variety of common animals showed that the shrimp was one of the most sensitive (see Table 20). In toxic solutions the shrimp eventually becomes very agitated and, after a series of rapid flexions of the abdomen, turns over. Turning over is a fairly sharp end-point and the time taken for this to occur can be related to the concentration of detergent in the water. Occasionally, shrimps that have turned over in the presence of low concentrations of detergent subsequently recover if returned to fresh sea water. However, if at 25 ppm this recovery has not taken place after 5 minutes the toxic effect may be assumed to be irreversible.

Water samples for testing were collected in 200 ml clip-top glass bottles. The top 50 ml was removed for other tests and a shrimp weighing 1–2 g then placed in each bottle, which was subsequently sealed. Times for animals to turn over at 12 °C were recorded and converted into concen-

trations of BP 1002 as ppm using the calibration curve shown in Fig. 29. The method is not very accurate but seems to be reliable within its limits of accuracy. Thus, within 24 hours, 2 ppm of BP 1002 was always toxic but 1 ppm was not. Beyond this time, control shrimps died through lack of oxygen. The detergent does not seem to act on the respiratory system.

This second method has the same drawback as that using *Elminius* larvae, namely that the range of good sensitivity is restricted. In addition, as only one animal is used to test the sample the results are more variable; moreover, the volume of sea water needed to accommodate the test animal is much greater than the sample used in tests with *Elminius* (5 ml). However, a more serious criticism applying to both methods, when relating them to field observations, is that results are expressed as ppm BP 1002. In the field, toxic effects were often caused by some other detergent, or even by fresh water used in hosing down the beaches.

To sum up—in spite of their recognized limitations, two methods of bioassay were usefully applied in testing water polluted by detergents.

VI. TOXICITY AND STABILITY OF THE COMPONENTS OF DETERGENTS

Details have already been given in Chapter 2 of the chemical composition of various detergents, and it will have been noted that the largest constituent is the *organic solvent* (e.g. kerosene extract or 'Kex'). In addition there is a *surfactant* (or emulsifying agent); and a *stabilizer*.

None of the three components dissolves easily in sea water and stock suspensions were therefore prepared by mechanically shaking 1·0 ml with 1 l. of sea water for 30 minutes. These suspensions were then quickly diluted to provide test media representing an appropriate range of toxic concentrations. The organic solvent, 'Kex', was obviously unstable and evaporated continuously from the sea-water suspensions. Accordingly, all tests of the toxicity of the 'Kex' fraction were carried out in sealed vessels (the same precaution having been taken when tests were made with the whole detergent).

Studies with Elminius *larvae*

Toxicity. Stage II animals were used, as in the previous toxicity experiments. Data for the solvent 'Kex' are compared with those for BP 1002 in Fig. 31, from which it will be seen that 'Kex' used alone has a toxicity very close to that of BP 1002. By comparison, the stabilizer was notably less toxic than 'Kex' and the surfactant notably less toxic than the stabilizer (see Table 24). Figure 31 also includes data for Shellsol R, the organic solvent used to prepare the Dasic detergent. Compared with BP 1002,

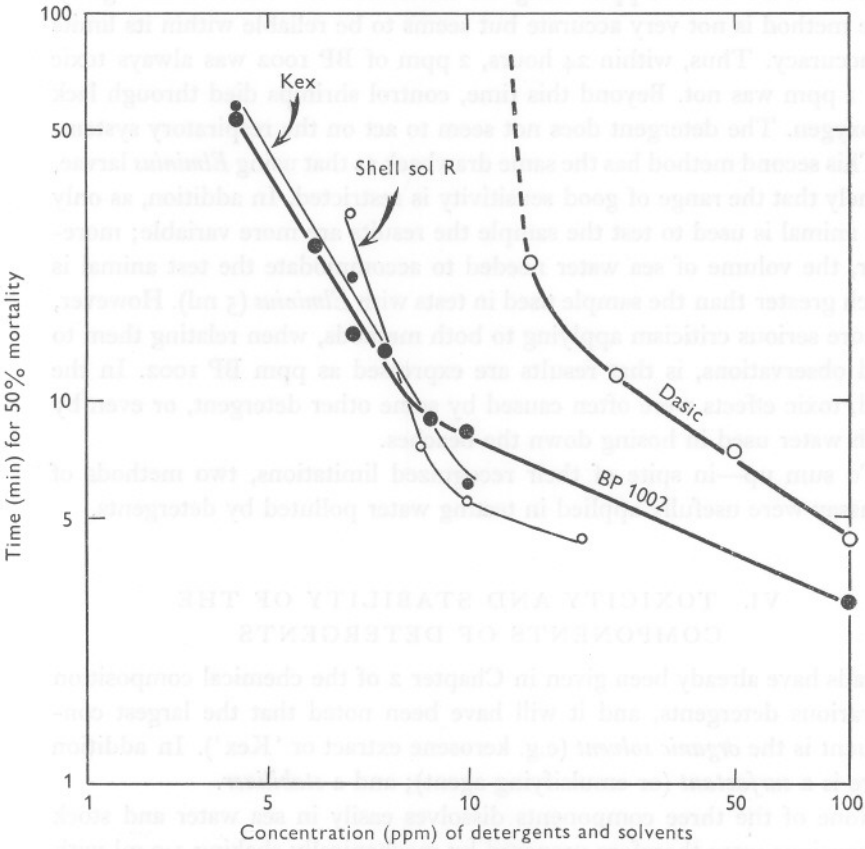


Fig. 31. Toxicities of detergents and their components to stage II *Elminius* nauplius larvae at 16–20 °C.

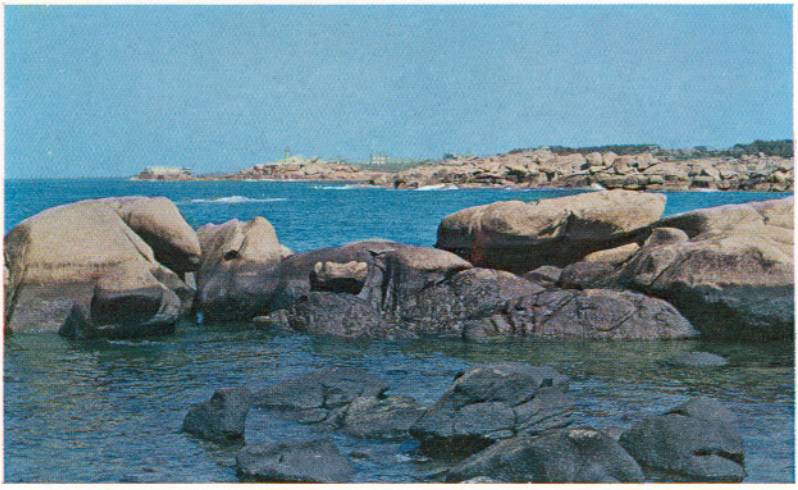
Dasic is much less toxic, but somewhat surprisingly, its organic fraction Shellsol R has almost the same toxicity as 'Kex'. The proportion of Shellsol R used in Dasic is slightly less than the proportion of 'Kex' in BP 1002. However, this difference is too small to account for Dasic being much less toxic than BP 1002. Possibly the other ingredients in Dasic may reduce the toxicity of Shellsol R.

Thus, in the case of BP 1002 and Dasic, and probably Gamlen and

PLATE 27

A, Oil residue on rocks west of Fort Le Crocq, Guernsey, 10 July. Shingle beach in distance mainly clear but with some residues above high-water springs. Rocky reefs were also oil covered at high-water neaps level. **B**, Oiled rocks on Ile Renot (Côtes du Nord), 21 June. The oil darkened to this colour after two neap tides. Mean Ruz lighthouse in background. **C**, Oiled salt marsh and sand near Trégastel (Côtes du Nord), 21 June. Note that the rushes, although presumably oiled nearly two months earlier, appear healthy.

A



B

C

(*Facing p.* 144)

Table 24. *Toxicity data for the components of BP 1002*

Concentration (ppm)	TD 50 (min)			
	Surfactant	Stabilizer	'Kex'	Total Mixture
500	50	—	—	—
50	120	18	2	4
25	200	38	4	5½
5	Non-toxic	212	21	17

Slipclean, the high toxicity of the 'detergent' is due mainly to the organic solvent on which it is based: in BP 1002, for example, the surfactant used is ten times less toxic (25 ppm) than the solvent (2·5 ppm). This finding appears to contrast with results obtained in fresh water, where some pure non-ionic surfactants, including the nonylphenyl-ethylene oxide condensate used in BP 1002, are toxic at concentrations below 10 ppm (see Marchetti, 1965). Possibly the cell membrane mechanisms are more sensitive in the fish that were used in Marchetti's freshwater experiments.

Stability. The stability of BP 1002, and of its three constituents, were also tested. Sea-water solutions were aerated for various periods of time in open vessels, and it was found that all the test solutions lost toxicity. The most marked effect was with 'Kex', a solution of 10 ppm (which originally killed 50 per cent of the test animals in 8 minutes) possessing no detectable toxicity after 2 hours aeration. The experiment was repeated using wide-mouthed dishes open to the atmosphere, but no aeration. The half-life of 'Kex' used at concentrations of 1–100 ppm was less than 24 hours in these conditions. The detergents Gamlen and Dasic also lost toxicity when similarly treated.

Solutions of the three components of BP 1002 were prepared with a heavy bacterial contamination by using sea water which had contained decaying barnacles. The stabilizer was found to have lost toxicity overnight, but the other components seemed unaffected.

Studies with Sabellaria *larvae*

Tests were made with *Sabellaria spinulosa* larvae that varied in the stage of their development from that figured by Wilson (1929, plate v, fig. 6) to the later stage drawn in fig. 7 on the same plate. The larvae had been reared in the laboratory from an artificial fertilization. The concentrations of BP 1002, and later of its constituents, were prepared in the same unfiltered sea water as used for the controls. All experiments were carried out under north-window illumination at a controlled temperature of about 15 °C.

To prevent loss of the toxic solvent during the course of the experiment, the sea water control and a solution of 1 ppm BP 1002 were each put into 100 ml glass-stoppered conical flasks, completely filled to leave no air-spaces under the stoppers. There were ten larvae in each flask, but no food was added. The larvae in the control were healthy and active three weeks later, although their flask was still well stoppered. But those in the detergent solution never recovered, remaining motionless on the bottom, bodies flexed ventrally and bristles erect. When the flask was unstoppered after 48 hours they were found to be dead. Moreover, water from the flask, shortly after unstoppering, was still extremely toxic to new larvae, quickly rendering them motionless with erect bristles. Air was now bubbled through the flask for several hours, the smell of the organic solvent disappeared and the water no longer had any toxic effect on fresh larvae immersed in it.

Tests were next made with sea-water solutions of the surfactant (10 and 1 ppm) and the stabilizer (10 ppm) prepared in open dishes. At 10 ppm both substances killed the larvae within a few hours, after first slowing their speed of swimming. There was no sudden raising of the bristles characteristic of treatment with the detergent. The larvae died with straight bodies, and with the bristle bundles only partly raised. In fact, in the solution of stabilizer, the bristles were barely lifted away from the sides of the body, the posture in death being almost as in life. In the 1 ppm concentration of surfactant, larvae showed no immediate reaction, but gradually their rate of swimming slowed and they became increasingly irritable. After 12 days, in spite of *Isochrysis* added for food, they were in poor condition and a few days later most were dead, the rest dying. All this time larvae used as a control were healthy, active and growing, and remained so five weeks after the experiment began.

A more extensive series of tests of the ingredients of BP 1002 was next made. These are listed in Table 25 and the results briefly summarized. 'Kex' at 2 ppm and 1 ppm had at first very little effect on the larvae: it merely made them slightly irritable. Overnight the slight smell of the solvent disappeared and from then on the larvae behaved normally for nearly four weeks; however, after this they lost activity until they lay motionless with only an occasional twitching of their bristles. The surfactant at 5 ppm and 2·5 ppm killed the animals, and the same concentrations of the stabilizer gradually slowed the swimming and killed within 20 hours. When treated with these components the larvae died as before with bodies straight and bristles scarcely raised. In another dish, containing 1 ppm of BP 1002, the immediate reaction was the usual ventral flexure of the body with well-raised bristles, the animals then remaining

Table 25. *Toxicity tests with BP 1002 and its components.*

(Ten larvae of *Sabellaria spinulosa* 29 days old were put into each dish.)

Date	Time	Dish 1: Control	Dish 2: 2 ppm 'Kex'	Dish 3: 1 ppm 'Kex'	Dish 4: 5 ppm surfactant	Dish 5: 2·5 ppm surfactant	Dish 6: 5 ppm stabilizer	Dish 7: 2·5 ppm stabilizer	Dish 8: 1 ppm BP 1002
14. iv. 67	3.00 p.m.	Put in	.	.	Put in	Put in	Put in	Put in	.
14. iv. 67	3.05 p.m.	Normal	.	.	Motionless	Normal	Normal	Normal	.
14. iv. 67	3.40 p.m.	Normal	.	.	Motionless	Slow	Slow	Slightly slow	.
14. iv. 67	3.45 p.m.	.	Put in	Put in	.	.	.	.	.
14. iv. 67	4.07 p.m.	.	.	.	.	.	.	.	Put in
14. iv. 67	4.25 p.m.	Normal	Irritable	Irritable	.	.	.	.	Motionless
15. iv. 67	10.08 a.m.	Normal	Almost normal	Normal	Dead	Poor	Dead	Dead	Slight recovery
17. iv. 67	2.30 p.m.	Normal	Normal	Normal	.	Dead	.	.	Poor
25. iv. 67	10.30 a.m.	Normal	Normal	Normal	.	.	.	.	Very poor
28. iv. 67	5.00 p.m.	Normal	Normal	Normal	——Ten new larvae put into dishes 4–7——				Three dead
29. iv. 67	10.40 a.m.	Normal	Normal	Normal	Poor	Slow	Poor	Almost normal	Bad
1. v. 67	10.10 a.m.	Normal	Normal	Normal	Dead	Very slow	Dead	Slightly slow	Bad
2. v. 67	12.20 p.m.	Normal	Normal	Normal	.	Almost motionless	.	Slow	Bad
4. v. 67	10.50 a.m.	Normal	Normal	Normal	.	Five dead	.	Slow	Another dead
11. v. 67	12.15 p.m.	Normal	Less active	Less active	.	Eight dead	.	Slow	All dead
17. v. 67	11.45 a.m.	Normal	Motionless	Motionless	.	All dead	.	Almost normal	.

motionless for some considerable time. There followed a period of apparent partial recovery but progressive deterioration soon set in.

The animals were not fed for two weeks after the beginning of the experiment. Some *Isochrysis* culture was then added to all dishes. On the same day, healthy larvae were put into the dishes of surfactant and stabilizer (Table 25, dishes 4–7) where the original larvae lay dead and decayed. In both these components at 5 ppm the larvae soon died with straight bodies and bristles held almost normally. In dishes containing these components at a concentration of 2·5 ppm there was a more gradual slowing of the swimming speed and the larvae survived longer than did those originally put into these same dishes. In fact, although they eventually died in this surfactant concentration, the larvae in the 2·5 ppm stabilizer showed distinct signs of recovery by the end of the experiment. The stabilizer was evidently no longer present in toxic concentration.

These experiments, like those conducted with *Elminius*, demonstrate that the solvent fraction of BP 1002 is quickly lost from sea water exposed to air. But there is evidence of chronic poisoning resulting from fairly brief exposure. These open-dish experiments differ from those with *Elminius* larvae in that the effect of the whole detergent is considerably more severe than that of the 'Kex' alone.

Studies with Crangon

Methods described in an earlier section (see page 142) for estimating the toxicities of detergents to shrimps were used in further experiments concerned with testing the relative toxicities of the components of BP 1002 and the stabilities of these components in sea water. Experiments with shrimps showed that the organic solvent 'Kex' is the most toxic fraction; and that aeration of sea-water solutions of BP 1002 causes loss of toxicity.

CONCLUSIONS AND SIGNIFICANCE OF THE TOXICITY EXPERIMENTS

The account of the results of the range of toxicity experiments is already in a much summarized form. The results as a whole may be drawn together in a few comments.

They exhibit the expected variation of tolerance as between one species and another, and it would be impossible to define a generally 'safe level' of detergent concentration in sea water. All that can be said is that acute effects in some animals are detectable at less than 1 ppm of detergent and that as the concentration increases so the effects mount progressively and extend over a wider variety of species. At 10 ppm exposure for 1 hour is lethal to most planktonic and sublittoral animals and, whereas the inter-

tidal animals are more tolerant, they were exposed to much higher levels of detergent concentration in the type of beach-cleaning operation employed in the situation under study.

The experiments were conducted under conditions of great urgency, for the detergent spraying was begun in the absence of any detailed and reliable information on its likely biological effects.

There is, it is true, abundant information in the scientific literature on the toxicity of detergents. However, this is largely concerned with ionic detergents and with a freshwater environment. There are good physiological reasons for supposing that the action of non-ionic detergents in sea water could be very different.

The assumed toxicity was quickly verified in the first experiments and the effect of the operation hinged upon two interwoven questions. How far will the poison spread? And how long will it last?

The many experiments performed to establish toxic levels together with the observations reported in Chapter 6 throw light on the first question, and the experiments described under 'Toxicities and stabilities of the components of detergents' were undertaken to help answer the second.

The various reports in the literature describing toxicity experiments with detergents are almost wholly concerned with the surfactant fraction of the oil-spill detergents or their equivalent. It was therefore natural to suppose that the toxicity of the oil-spill detergents was largely in the surfactant fraction. The toxicity of this fraction to some species, at least, has been demonstrated, and the possibility of accumulation in food-chains, though perhaps slight in open waters, should not be forgotten. The surfactants used in the manufacture of oil-spill detergents are 'hard'; that is, only slowly degraded, so that it seemed that the toxicity was likely to persist in the coastal waters of the Channel, and the prognosis was indeed gloomy.

Hence it is of crucial significance that our experiments show that the toxicity of the oil-spill detergents in sea water is almost entirely in the organic solvent fraction and, moreover, that this fraction rapidly disappears by evaporation, at least when in low concentration. This result is of central importance for the whole of the spraying operation, for had it not been for this previously unknown and unsuspected fact the biological consequences in the English Channel would have been vastly worse than they were.

Nevertheless, it should be noted that besides this important demonstration of the severe but transient toxicity of the detergents used there is also a longer-term effect on the organisms tested.