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The toxicity of some herbicides used for *Spartina* control

by

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INTRODUCTION

The value of *Spartina* species in stabilizing marine mud flats and marshes has long been recognized and has led to the widespread introduction of these species. However, the features which make them ideal for this purpose (e.g. vigorous growth, rapid vegetative and seed dispersal) may at times produce adverse effects. Thus in many areas *Spartina* has spread from the mud flats where it was introduced on to nearby sandy bathing beaches, resulting in the loss of amenity areas. *Spartina* spp. have ousted other species, such as *Enteromorpha* and *Zostera* which are important in the diet of some wildfowl, and in some areas have led to the closure of navigation channels by allowing the accretion of silt. Clearly, in these areas, and others where stabilization of the mud flats is not wanted, control of *Spartina* sward is necessary.

There are several reported instances of chemical treatment for the control of the intertidal marine and estuarine weeds. For example, lime and bleaching powder are effective in controlling littoral fucoids, and chlorobenzenes in removing *Zostera*, while 2, 4, D and 2, 4, 5, T have been used on a limited scale in the USA to remove *Juncus* spp. For control of *Spartina* spp. dalapon (2, 2-dichloropropionic acid) and substituted urea compounds such as fenuron (NN dimethyl N'phenylurea), monuron (N'4-chlorophenyl-NN-dimethylurea) and diuron (N'-3, 4-dichlorophenyl -NN-dimethylurea) have been advocated most frequently.

For the most part, in control and eradication programmes, little attention has been given to the effects of herbicidal treatment on the associated fauna, mainly because the species involved have had no commercial or special scientific value. However, Pickett and Franklin (1973) described how *Spartina* has invaded the habitat of the cockle (*Cardium edule*) in the Burry Inlet, where it has been proposed that herbicides should be used. In this instance it is important to assess the effects of such treatment on the fauna, and to this end laboratory and field investigations were undertaken.

LABORATORY EXPERIMENTS

Initial experiments were carried out under the standard test conditions employed at Burnham-on-Crouch (Portmann and Wilson 1971) to assess the level of acute toxicity of the compounds in sea water. Thereafter, attempts were made to determine the effects of the chemicals under simulated field conditions.

a. Acute toxicity tests. The acute toxicities of Dowpon (= dalapon plus an adjuvant) and of fenuron to the brown shrimp, *Crangon crangon*, and to the cockle, *Cardium edule*, were determined. Adult cockles (2-3 year-old) and cockle spat were tested separately. Test solutions were made up by adding the appropriate weight of herbicide to 10 l of filtered sea water at 15°C. The tests were conducted in Perspex tanks held at a constant temperature of 15°C. The solutions were aerated gently and renewed every 24 hours. The tanks were inspected frequently and dead animals removed. At each concentration the mortality (%) was plotted against time on log-probability paper, and hence the time for 50% mortality (ET_{50}), together with its 95% confidence limits, was calculated. The ET_{50}s were plotted against their respective concentrations to give the toxicity curves shown in Fig 1.

Fenuron was more toxic to shrimps than Dowpon. However, the toxicity of fenuron to shrimps, which had a 48 hour LC_{50} of 600 parts/10^6, is still less than that of many other herbicides, for example diquat or paraquat. Dowpon was more toxic to cockles than fenuron. There was very little difference in response between adult and spat cockles, but in the longer term they were both more susceptible than the shrimps.

b. Simulated field exposures. Although standard toxicity tests are useful for assessing the relative toxicity of several compounds and the relative susceptibility of several species, they are of limited value for assessing the likely ecological effects. In order to provide more useful laboratory data on toxicity, the herbicides were applied to the animals in conditions more likely to pertain to the field.

Cockles and substrate were collected from a local cockle-bed; the muddy sand in which the cockles had been growing was placed in Perspex tanks (50 cm x 20 cm x 12 cm deep) to a depth of approximately 4 cm, and about 8 l of clean sea water (salinity c. 30°/oo temperature 15°C) was added to each. The contents were allowed to settle for 24 hours. Ten cockles were placed in each tank and allowed to burrow into the sand. The water was aerated and renewed every 24 hours. After acclimation to these conditions for 48 hours the tanks were completely drained of sea water and herbicide treatments applied. In addition to controls, each herbicide was administered at a 'normal' or recommended level of application, and at 3.3 times, 10 times, and 33 times normal application. For Dowpon the recommended rate for field application is 62 kg/ha as a solution of about 6 g/l. This application for the tank was calculated to be 0.62 g/100 ml sea water. For fenuron the recommended field application was 2700 kg/ha of 3-5% active material in a granular pellet. In the laboratory this rate of application was achieved by mixing 1.09 g of 100% active material with 26.9 g of sand which acted as the matrix or base material. An even application of this 4% active material over the substrate was thus possible.

After application of the herbicides each tank was left without sea water for 12 hours to simulate the maximum expected field exposure. Thereafter, the tanks were filled with clean aerated sea water which was changed every 24 hours. Observations were made daily to record mortalities and the number of cockles fully buried in the substrate. On day 5 (120 h) all the animals were dug out of the substrate, and dead animals were removed and live cockles held in sea water for a further 3 days.

The effect of the simulated field applications of the herbicides on the survival of the cockles is shown in Table 1, from which it is clear that rates of application above the recommended level have deleterious effects on survival. The effects on burrowing behaviour were even more marked, showing that sub-lethal effects were occurring under these conditions even at 'normal' rates of application (Fig 2).

FIELD EXPERIMENTS

These were considered necessary because there is very little information on the relationship between laboratory toxicity tests and effects in the field, where there are many more variables. Furthermore, in this instance one of the modes of application of the herbicide involved a commercial technique, the Biflon system, which could not be reproduced in the laboratory. This is a bi-fluid spraying system for the application of highly viscous formulations of pesticides as invert (water-in-oil) emulsions.

Two areas of beach were chosen, each bearing cockles, and one including *Spartina* clumps and the other without *Spartina*. In order to be able to detect effects of the herbicide directly on the fauna rather than as a result of effects due to the eradication of the *Spartina*, the experiments were carried out in November when herbicide treatment of the grass is less effective. Within each area, 10 m square plots were sprayed with dalapon or Dowpon (both as wettable powders), or dalapon with Biflon, or Dowpon with Biflon, or fenuron. A 10 m strip was left between each plot; there was one control plot in each area. Ten random samples of 1/10 m^2 of substrate to a depth of *c*. 10 cm were taken at each plot in order to estimate the density of *Spartina* and fauna before spraying. Only those animals retained in a 2 mm-mesh sieve were recorded.

Applications of the wettable powders (dalapon and Dowpon) were made as a spray (at a pressure of 2.8 kg/cm^2) with a Saville Knapsack at rates of 31.4 kg/ha in a volume of 280 l/ha. Application of the herbicide plus oil phase in the bi-fluid system was made at a pressure of 2.8 kg/cm^2 with a Biflon knapsack unit at 31.4 kg/ha in a volume of 158 l/ha of oil/water mixture. Fenuron was hand-applied in a granular form (5% active) at a rate of 2700 kg/ha.

The plots were resampled 2 weeks after spraying, in order to assess the impact of the herbicides on the fauna. It is not possible to set out all the results in this paper, but briefly the most commonly occurring species in both areas were Cardium edule, Macoma balthica, Hydrobia (= Peringia) ulvae, Corophium volutator and several species of smaller amphipods. Nereis diversicolor, Retusa sp., Carcinus maenas, Mytilus edulis and Littorina sp. occurred less frequently; Arenicola marina was found only in the area without Spartina. The populations showed very marked 'clumped' distributions within each plot and large differences in density between plots, so that analysis of variance techniques to determine treatment effects were not possible. Therefore, it was decided to determine the magnitude and direction of change in density of each species within each plot by calculating the significance of the differences between the mean densities from the 'before' and 'after' surveys. With the exception of Hydrobia and Macoma, where the values were generally larger and therefore distributed normally, the t-tests were performed on transformed data, using $\log_e (x + 1)$. The results of the t-tests are shown in Table 2.

In the area containing Spartina clumps no consistent signficant effects that could be attributed to herbicide treatment emerged. In the area without Spartina large differences in the density of several species were found. Since changes took place in both control and treated plots is seems likely that these changes were the result of the effects of hydrographic changes which affected the area during the 2 weeks between the surveys. However, relative to changes in the control plot, treatment effects were observed only with fenuron, where significant decreases in the fauna occurred in 4 of the 6 species considered.

CONCLUSIONS

With the exception of fenuron it seems unlikely that application at the recommended rates of the herbicides tested here will result in any direct adverse effect on the mud-dwelling animal communities. Clearly, under practical conditions of Spartina control faunistic changes will occur as the Spartina is removed and sediment structure alters. However, these changes are the indirect result of spraying rather than the result of the toxicity of herbicides.

More generally, there is considerable value in extending toxicological investigation from the laboratory to the field. Field experiments can amply demonstrate the limitation of laboratory tests; on the other hand, laboratory tests can explain results observed in the field. For an overall assessment of the potential hazard of materials applied to the marine environment both procedures should be adopted.

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The reference to the proprietary products in this report should not be construed as an official endorsement of these products, nor is any criticism implied of similar products which have not been mentioned.

REFERENCES

PICKETT, G. D. and FRANKLIN, A. 1973. The effect of recent topographical changes on stocks of cockles (Cardium edule L) in the Burry Inlet, South Wales. ICES C.M. 1973/K:23, 5 pp (mimeo).

PORTMANN, J. E. and WILSON, K. W. 1971. The toxicity of 140 substances to the brown shrimp and other marine animals. MAFF Shellfish Information Leaflet No. 22 (mimeo) Fisheries Laboratory, Burnham-on-Crouch, Essex.

TABLE 1

The effects of Dowpon and fenuron applied under simulated field conditions on the survival of cockles (expressed as the percentage of cockles dead after 8 days)

Herbicide	Rate of application				
	Control	Normal	3.3X	10X	33X
Dowpon	20	30	60	100	100
Fenuron	0	0	20	80	100

TABLE 2

Values of 't' for the significance of difference between mean densities from 'before' and 'after' surveys. The order of the treatment results reflects the order of the field plots.

*Differences of significance at or above 5% level

(+) and (-) Direction of change of mean density

Species	Treatment					
	Dowpon	Dalapon with Biflon	Control	Dalapon	Dowpon with Biflon	Fenuron
	Area with Spartina clumps					
Cardium edule	0.67	0.18	1.39	0.22	0.74	1.65
Macoma balthica	3.55 *(+)	1.06	0.52	1.41	0.12	1.08
Hydrobia ulvae	1.67	0.95	1.20	1.14	0.62	2.05
Corophium sp.	0.27	4.15 *(+)	4.53 *(+)	0.57	1.77	0.42
Other amphipods	1.15	2.53 *(-)	3.27 *(+)	0.03	0.90	1.84
Arenicola marina	-	-	-	-	-	-
Spartina (dry wt) g	0.15	4.25 *(+)	0.24	0.13	1.63	0.04
	Area with no Spartina clumps					
Cardium edule	1.68	0.83	2.34 *(-)	2.34 *(-)	1.86	3.22 *(-)
Macoma balthica	3.08 *(+)	2.42 *(+)	3.25 *(+)	1.73	1.32	1.50
Hydrobia ulvae	2.49 *(+)	1.77	1.15	0.94	0.93	0.05
Corophium sp.	4.92 *(+)	2.36 *(+)	4.14 *(+)	4.72 *(+)	5.16 *(+)	2.23 *(-)
Other amphipods	5.61 *(+)	2.85 *(+)	5.13 *(+)	3.12 *(+)	2.41 *(+)	2.41 *(-)
Arenicola marina	0.81	2.49 *(-)	0.35	1.00	1.82	8.14 *(-)
Spartina (dry wt) g	-	-	-	-	-	-

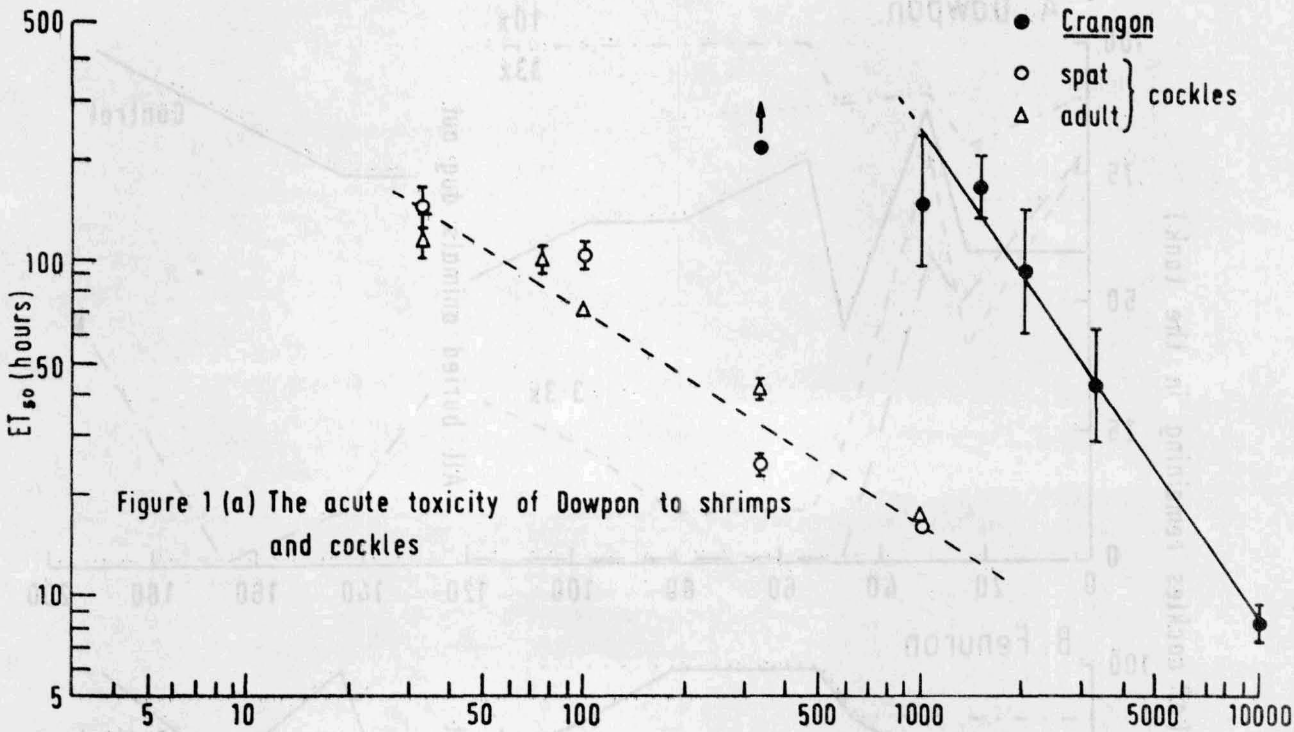
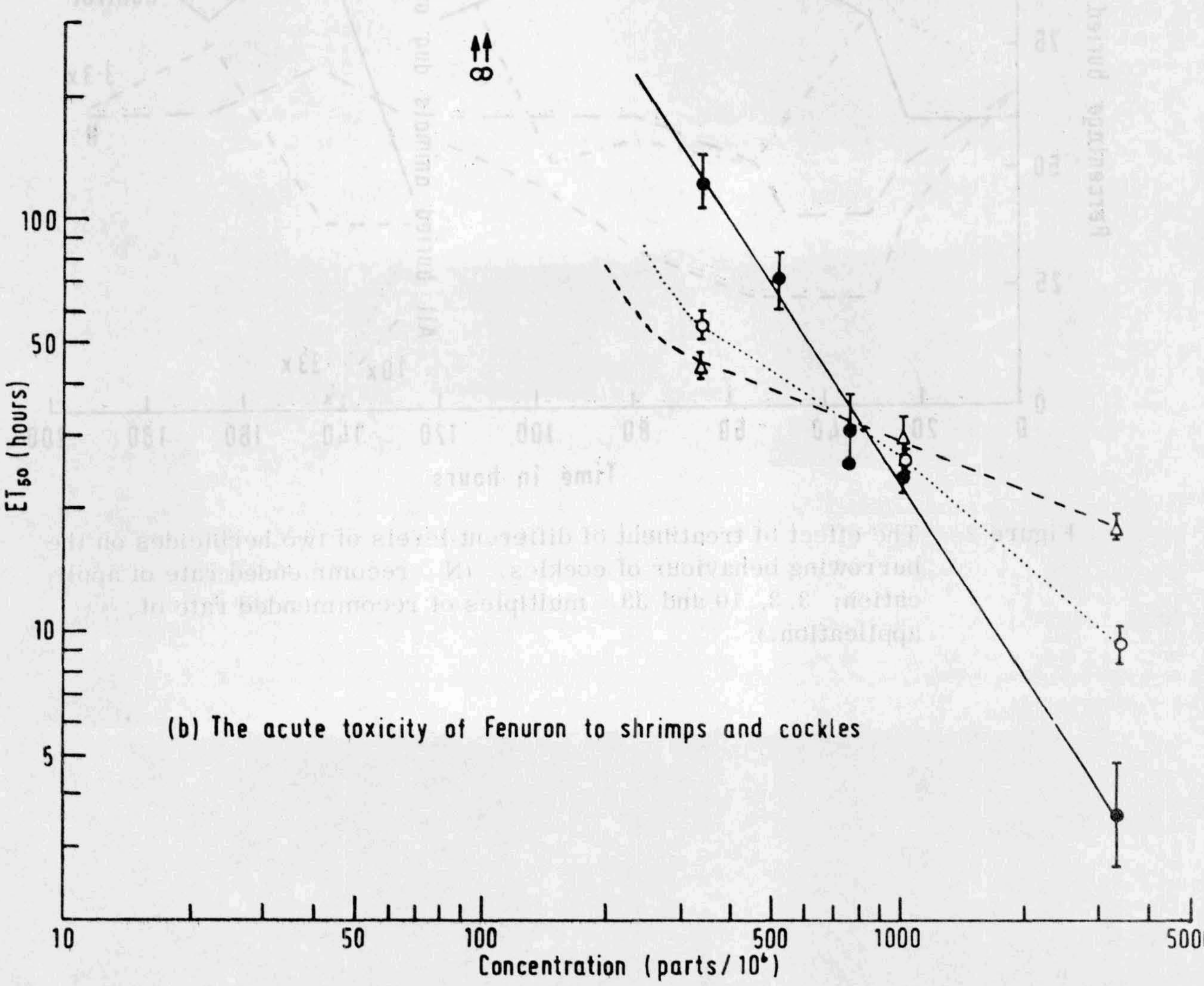


Figure 1 (a) The acute toxicity of Dowpon to shrimps and cockles



(b) The acute toxicity of Fenuron to shrimps and cockles

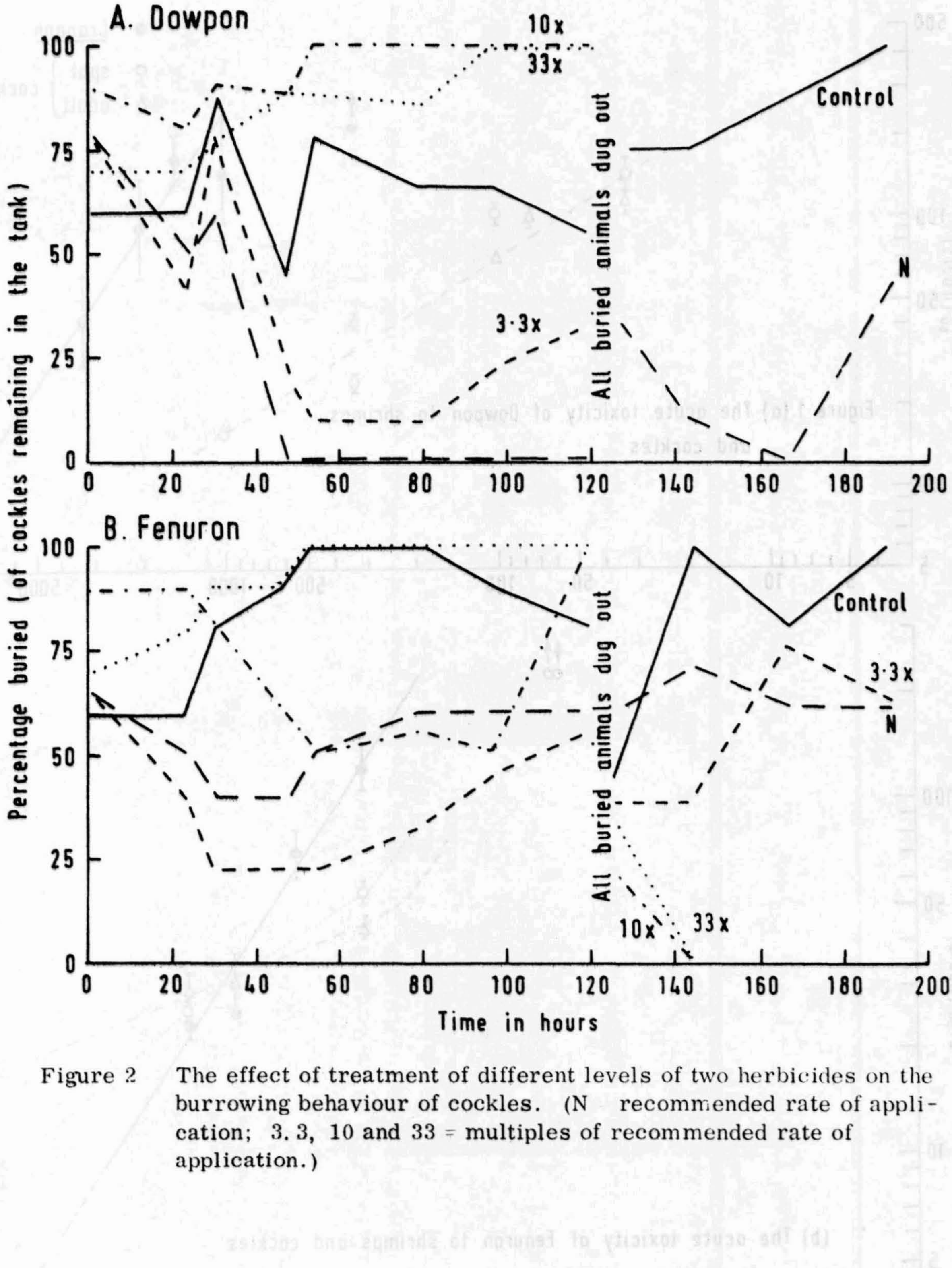


Figure 2 The effect of treatment of different levels of two herbicides on the burrowing behaviour of cockles. (N recommended rate of application; 3.3, 10 and 33 = multiples of recommended rate of application.)