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EFFECTS OF TWELVE PESTICIDES ON LARVAE OF OYSTERS (*CRASSOSTREA GIGAS*) AND ON TWO SPECIES OF UNICELLULAR MARINE ALGAE (*ISOCHRYSIS GALBANA* AND *CHAETOCEROS CALCITRANS*)

by

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ABSTRACT

The effects of seven herbicides, four insecticides and one molluscicide were tested at concentrations of up to 10 mg/l on larvae of oysters, *Crassostrea gigas* (9 days exposure), and on laboratory cultures of the algae *Isochrysis galbana* and *Chaetoceros calcitrans* (21 days exposure). All of the pesticides tested had significant toxic effects on at least one of the test organisms. The strongest effects were those of lindane and isoproturon on survival and growth of *C. gigas* larvae, and of isoproturon and carbetamide on growth of *Isochrysis* and *Chaetoceros* cultures.

The log-probit model for the relationship between dosage and effect, and Haber's rule for the relationship between duration of exposure and effect, hardly ever applied to our data. In some cases we found marked toxicity threshholds, and in others there were pronounced hormesis effects. We characterize five types of responses to prolonged toxicant exposure, including delayed toxic effects (sometimes following initial hormesis), and initial growth inhibition with subsequent recovery (sometimes ending in hormesis).

The great variability of response (depending on duration of exposure, toxicant concentration and test species), is a reminder that the effects of pollutants on the marine environment cannot be assessed by simple methods (e.g. short-term bioassays with one or two test species). This contrasts with the requirement for easy routine methods of pollution assessment for monitoring and management purposes.

INTRODUCTION

Agricultural pesticides are introduced into the coastal marine environment through natural runoff, and they may constitute a threat to the ecological stability of the marine environment, as well as to aquaculture operations. Pesticide toxicology has, however, focused on terrestrial and freshwater studies (e.g. Hayes & Laws 1991). The effects of pesticides on marine unicellular algae were first studied by Ukeles (1962) in five algal species, and on bivalve larvae by Davis (1961) in hard clams (*Mercenaria mercenaria*) and American oysters (*Crassostrea virginica*). The effects of pesticides in the marine environment have been reviewed by Walsh (1972).

The present study attempts to assess the potential threat to oyster culture posed by some pesticides commonly used in agricultural operations adjacent to the Charente Maritime oyster growing region of southwestern France (Chevalier & Masson 1988). Seven herbicides, four insecticides and one molluscicide were tested at the concentrations which might be expected in the marine environment in the case of an accidental spill. Pesticide effects on growth and survival of Pacific oyster (*Crassostrea gigas*) larvae were investigated; because oyster recruitment may also be hampered indirectly by pollutant effects on the phytoplankton on which the larvae feed (e.g. His et al. 1986), pesticide effects on the growth of laboratory cultures of two algal species were studied as well.

MATERIAL AND METHODS

Adult Pacific oysters (*Crassostrea gigas* Thunberg) were induced to spawn in the laboratory by thermal stimulation. Within 30 min. of spawning, about 60,000 eggs were fertilized in 2 l glass beakers containing the appropriate solutions of pesticide in 0.2 μm filtered seawater of 28 ppt salinity, and incubated at 24 $\pm$ 1 °C. The water was changed after 24 h and the larvae were grown under the same conditions by standard methods (Utting & Spencer 1991) at a density of 8000/l for 8 more days. They were fed daily with 50 cells/μl of (noncontaminated) *Isochrysis galbana* and of *Chaetoceros calcitrans*. The water was changed every two days; at each water change larval mortality was assessed on samples of 200 larvae, and larval growth was determined by measuring the shell height of 50 larvae.

Non-axenic cultures of *I. galbana* (Parke) and *C. calcitrans f. pumilum* (Takano), originating from stocks of M.A.F.F. Conwy (Wales), were grown by standard methods (Laing 1991) under continuous illumination at 20 $\pm$ 1 °C and 28 ppm salinity in 2 l Erlenmeyer flasks filled with 1 l of culture medium; the medium used was as described by Walne (1966). The flasks were agitated thrice daily during the incubation period, which lasted 21 days in most cases, by which time the control algae had generally attained the stationary phase. Algal densities were determined with a Coulter counter every two or three days.

The products tested were: isoproturon, chlorotoluron, metoxuron, 2-4 D (quinoxone), bromoxynil, carbetamide, mecoprop, lindane, fenitrothion, parathion methyl, carbofuran, and metaldehyde; most of these compounds are described in ACTA (1991) and in Hayes & Laws (1991). The pesticides were used at the following concentrations: 0 (controls), 0.025, 0.05, 0.1, 0.25, 0.5, 1, 2.5, 5, and 10 mg/l of seawater, except lindane (test concentrations of 0.01 to 1.0 mg/l) and fenitrothion (0.005 to 2.5 mg/l). Hydrophobic compounds were dissolved in acetone; in these cases, an amount of acetone corresponding to the highest experimental concentration was added to the incubation water of the controls.

RESULTS

Overall results at the end of the exposure periods are summarized in Table 1. Pesticide effects were almost never linear or log-linear with time or concentration; in some cases we found marked toxicity threshholds, and in others we found pronounced hormesis effects at the lower toxicant concentrations.

Table 1:

Effects of twelve pesticides on the larvae of *Crassostrea gigas*, and on the algae *Isochrysis galbana* and *Chaetoceros calcitrans* at the end of incubation. Assessment of the effects is based on the performance of the controls. Toxicity levels (interpolated data) are in mg of active product per l of seawater. - = no such effect at highest concentration tested.

		Larvae of Crassostrea gigas (after 9 days exposure)		Isochrysis (after 21 days exposure)		Chaetoceros (after 21 days exposure)	
Effect (compared to controls):		50% mortality	10% height reduction	reduction in cell numbers 50%	20%	50%	20%
Herbicides							
Isoproturon	$C_{12}H_{18}N_2O$	0.37	0.25	0.017	0.007	0.078	0.064
Chlorotoluron	$C_{10}H_{13}ClN_2O$	-	0.60	0.083	0.060	0.42	0.29
Metoxuron	$C_{10}H_{13}ClNO_3$	-	9.0	0.088	0.025	0.39	0.29
2-4 D	$C_8H_6Cl_2O_3$	-	-	-	-	9.2	0.024
Bromoxynil	$C_{13}H_7Br_2N_3O_6$	7.0	0.80	-	7.5	-	-
Carbetamide	$C_{12}H_{16}N_2O_3$	9.3	4.2	0.037	0.017	0.096	0.076
Mecoprop	$C_{10}H_{11}ClO_2$	4.2	0.13	-	-	-	-
Insecticides							
Lindane	$C_6H_6Cl_6$	0.17	0.13	0.48	0.13	0.75	0.54
Penitrothion	$C_6H_{12}NO_5P_5$	-	0.19	-	-	-	-
Parathion methyl	$C_8H_{10}NO_5P_4$	7.2	0.087	4.6	3.3	8.4	6.5
Carbofuran	$C_{12}H_{15}NO_3$	6.9	0.46	7.6	6.0	-	2.4
Molluscicide							
Metaldehyde	$C_{12}H_8O_4$	7.4	0.69	-	-	-	-

Time dependent responses followed a variety of patterns:

Type 1 (37.1% of the cases): No significant effect on growth.
Type 2 (1.2% of the cases): Hormesis effect lasting to the end of the incubation period (e.g. Fig. 1, 0.05 mg/l).
Type 3 (12.3% of the cases): Delayed growth inhibition (e.g. Fig 1, 0.10 mg/l), including cases of initial hormesis with subsequent decline to control levels.
Type 4 (17.0% of the cases): Initial growth inhibition with subsequent partial or complete recovery, sometimes ending in hormesis (e.g. Fig. 2, 0.025 and 0.05 mg/l).
Type 5 (32.4% of the cases): More or less continuous growth inhibition (e.g. Figs. 1 and 2, 1 and 10 mg/l).

The types of responses of the test organisms to the various toxicant concentrations are summarized in Table 2, and an overview of the response patterns is given in Fig. 3.

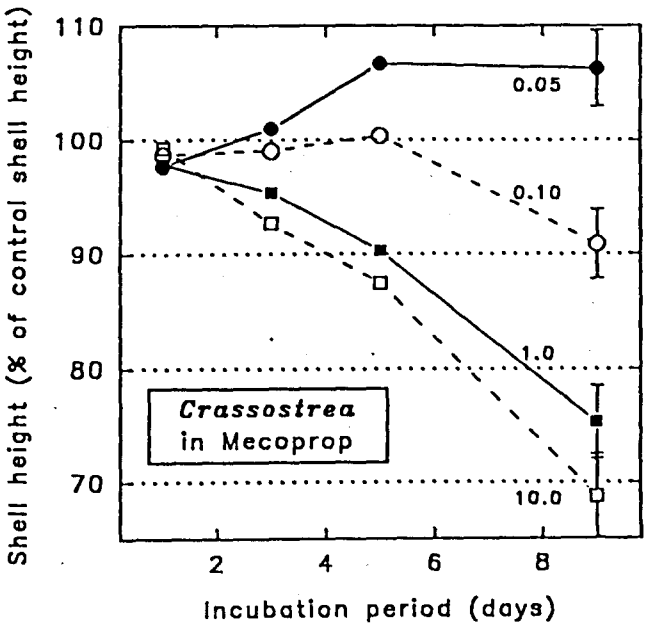

Fig. 1:

Effects of the herbicide mecoprop (concentrations in mg/l of seawater) on growth of *Crassostrea gigas* larvae. Vertical bars indicate 95% confidence intervals. The data for mecoprop concentrations of 0.025, 0.25, 0.5, 2.5 and 5 mg/l were intermediate, and have been omitted.

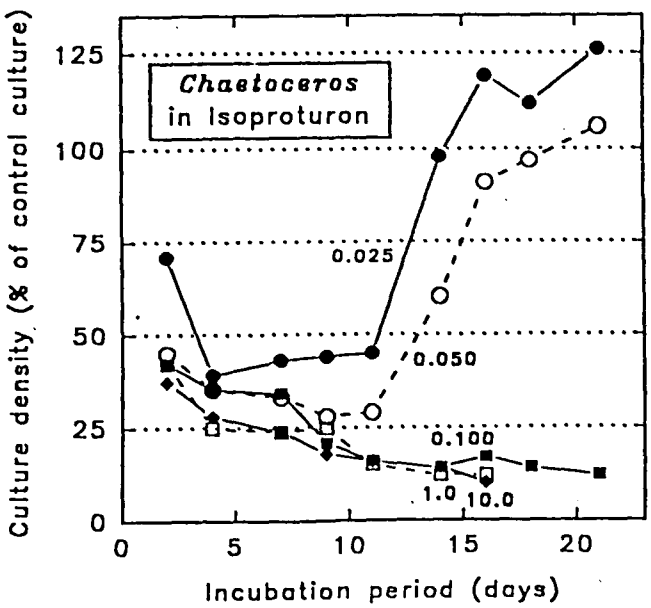

Fig. 2:

Effects of the herbicide isoproturon (concentrations in mg/l of seawater) on growth of laboratory cultures of *Chaetoceros calcitrans*. The data for isoproturon concentrations of 0.25, 0.5, 2.5 and 5 mg/l were similar to those for 0.1, 1 and 10 mg/l, and have been omitted.

Table 2:

Growth response patterns of the larvae of *Crassostrea gigas*, and of the algae *Isochrysis galbana* and *Chaetoceros calcitrans* during prolonged exposure to different concentrations of twelve pesticides. See text and Fig. 3 for characterization of the response types.

Contaminant concentration (mg/l):		0.010	0.025	0.05	0.1	0.25	0.5	1	2.5	5	10
Isoproturon	C. gigas		1	1	5	4	4	4	4	4	4
	Isochrysis		5	5	5	5	5	5	5	5	5
	Chaetoceros		4	4	5	5	5	5	5	5	5
Chlorotoluron	C. gigas		1	1	3	3	3	3	3	3	3
	Isochrysis		4	4	5	5	5	5	5	5	5
	Chaetoceros		3	1	4	4	4	5	5	5	5
Metoxuron	C. gigas		1	1	1	1	1	1	1	1	3
	Isochrysis		5	4	4	4	5	5	5	5	5
	Chaetoceros		3	4	4	4	5	5	5	5	5
2-4 D	C. gigas		4	4	4	4	4	4	4	4	4
	Isochrysis		1	1	1	1	1	1	1	1	3
	Chaetoceros		5	5	5	5	5	5	5	5	5
Bromoxynil	C. gigas		1	5	5	5	5	5	5	5	5
	Isochrysis		3	3	3	3	3	3	3	3	3
	Chaetoceros		1	1	1	1	1	1	1	1	3
Carbetamide	C. gigas		1	1	1	1	1	1	1	3	5
	Isochrysis		5	5	5	5	5	5	5	5	5
	Chaetoceros		2	2	3	3	3	3	5	5	5
Mecoprop	C. gigas		2	2	3	3	5	5	5	5	5
	Isochrysis		4	4	4	4	4	4	4	4	4
	Chaetoceros		4	4	4	4	4	4	4	4	4
Lindane	C. gigas	1	1	1	3	5	5	5			
	Isochrysis	5	5	5	5	5	5	5			
	Chaetoceros	1	1	4	1	4	4	5			
Fenitrothion	C. gigas	1	1	1	1	3	3	5	5		
	Isochrysis	1	1	1	1	1	1	1	1		
	Chaetoceros	1	1	1	1	1	1	1	1		
Parathion methyl	C. gigas		1	1	3	3	3	5	5	5	5
	Isochrysis		1	1	1	1	1	1	1	5	5
	Chaetoceros		1	1	1	1	1	1	1	1	5
Carbofuran	C. gigas		1	1	1	1	5	5	5	5	5
	Isochrysis		1	1	1	1	1	1	1	1	1
	Chaetoceros		1	1	1	1	1	1	5	5	5
Metaldehyde	C. gigas		1	1	1	3	3	3	3	3	5
	Isochrysis		1	1	1	1	4	4	4	4	4
	Chaetoceros		1	1	1	1	1	1	1	1	1

DISCUSSION

According to our results, an accidental spill of a few tons of most of the pesticides tested would cause significant toxic effects in a coastal area several square kilometers in size. In the case of the Charente Maritime oyster culture area, an important effect on the recruitment of the stock would have to be expected. At the highest pollutant concentrations high mortalities would have to be expected locally already after a short period of exposure. The effects would vary greatly from one plankton species to another, and low pollutant concentrations would drastically alter the composition of the natural population, as has already been pointed out by Ukeles (1962).

Our results are not fully comparable to those of Ukeles (1962; effects of pesticides on five species of algae for a duration of 10 days), Davis & Hidu (1969; effects on bivalve larvae for 10 to 12 days), and others, notably because of differences in the species studied and in the methods. Most such studies are conducted over shorter periods of time (often 96 h), and the shape of the response curve is rarely mentioned (only the effect at the end of incubation), although UNEP (1989) recommends that it be done in the case of phytoplankton tests. Response curves such as Types 2 and 3 have been described before (e.g. Bowes et al. 1971), and hormesis has been reviewed by Stebbing (1982).

In our study half the cases with a significant toxic effect do not conform to the log-probit model nor to Haber's rule. One obvious implication is that the assessment of a substance's toxicity is largely determined by the duration of the experiment. The responses to toxic exposure do not conform to theory for a variety of reasons, such as a decrease in pesticide concentration due to degradation and sorption effects (Muir et al. 1985); vector effects due to the uptake of toxicants by phytoplankton and their subsequent consumption by zooplankters; cumulative effects of continuous toxicant uptake in some test organisms, and development of resistance in others.

Another aspect, which we did not investigate in the present study, is the reaction of the test organisms to incubation in uncontaminated seawater after removal of the toxicant itself. Ukeles (1962) notes that when pesticides inhibited growth of marine algae, the test organisms were viable at the end of the experiments in some cases, and unviable in others. Seaman et al. (1991) found that incubation of bivalve larvae in certain turbidity regimes led to slower growth, but that during subsequent incubation in non-turbid water the larvae grew much faster and later overtook the controls in size.

These phenomena are likely to be even more complex *in situ*. The log-probit model, in which the toxicant effect is plotted on a probability scale against the logarithm of toxicant concentration (supposedly yielding a straight line), is the method most widely used to assess toxic effects (e.g. UNEP 1989). Its validity is still unproven, however (Hayes 1991), and our data provide a striking example for its limitations. There is still a considerable need for studies which will help to develop valid and reliable criteria for toxicity assessment (e.g. Vranken et al. 1986).

Simple and rapid methods, such as UNEP (1989), which proposes 96 h tests, and His & Seaman (1993), who propose an 18 h test with oyster embryos, are much in demand, not only because they produce quick results, but also because they provide simple data sets which are understandable to uninitiated decision makers. These simple tests, however, even if they are convenient, will generally lead to an underestimation of pollution effects.

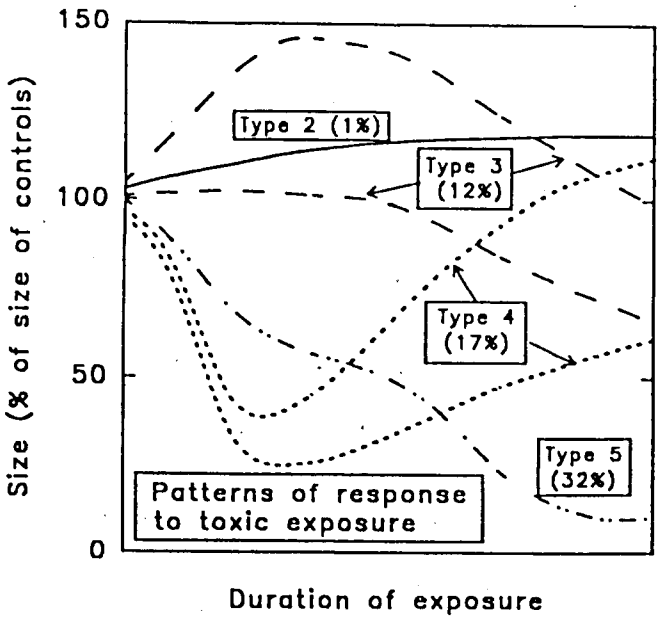

Fig. 3:

Generalized growth response patterns of marine bivalve larvae and of marine unicellular algae during incubation in contaminated seawater; percentages of each type of response in our data are in parenthesis (Type 1 = no significant effect: 37%).

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