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Toxicity of Nuvan® and Dichlorvos Towards Marine Phytoplankton[1]

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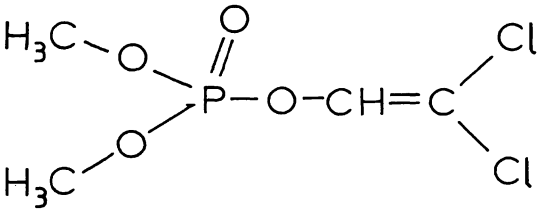
Abstract

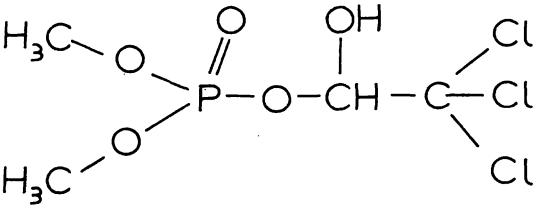
The pesticide Nuvan® and its active component dichlorvos were examined for toxicity to photosynthetic CO_2 fixation by natural assemblages of phytoplankton and by five unialgal cultures. Nuvan® was toxic to all organisms at concentrations which contained 1.0 ppm dichlorvos or higher. Aqueous stock solutions of Nuvan® stored at room temperature for more than 3 weeks retained their activity. Dichlorvos itself did not decrease photosynthetic activity. The toxicity of Nuvan® to these algae is either due to a degradation product of dichlorvos, to the carrier, to emulsifiers used in preparing the commercial formulation, or to a combination of such factors.

Introduction

The organo-phosphorus compound dichlorvos (Fig. 1A) is the active ingredient in a number of commercial products used as insecticides. Dichlorvos is an anti-cholinesterase compound and formulations containing it have been used against mosquitoes and fleas, as a worming agent for pigs and horses, and as an agricultural insecticide. It can also methylate bacterial DNA weakly (Rosenkranz, 1973, Rosenkranz and Rosenkranz 1972, Wennerberg and Lofroth 1974, Wright *et al.* 1979) and can act as a mutagen in some microorganisms (Carera *et al.* 1976, Dean 1972, Dyer and Hanna 1973, Mohn 1973, Shirasu *et al.* 1976, Wild 1973) and in plants (Singh *et al.* 1980). The toxicity, mutagenic activity and metabolism of dichlorvos have been reviewed (Anonymous, 1974, Wright *et al.* 1979). It is classed as a 'Probable Human Carcinogen' by the U.S. Environmental Protection Agency (1987). The Health and Safety Executive in



A. Dichlorvos



B. Trichlorfon

Fig. 1. Chemical structure of A, Dichlorvos (2,2-dichlorovinyl dimethyl phosphate; phosphoric 2,2-dichlorovinyl ester); B, Trichlorfon (0,0-dimethyl-2,2,2-trichloro-1-hydroxy ethyl phosphonate).

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the United Kingdom (cited in Ross and Horsman 1988) advises that workers using products containing dichlorvos should be monitored regularly for cholinesterase activity.

In the salmon farming industry dichlorvos is used to control infestations of sea lice [*Lepeophtheirus salmonis* (Kroyer) and *Caligis elongatus* von Nordmann] which can cause both loss of growth and mortality in fish held under farm conditions. It is toxic at concentrations below the recommended working concentration of 1 ppm dichlorvos to a number of non-target animals including species of crustacea and fish (reviewed by Ross and Horsman 1988).

The use of dichlorvos by the aquaculture industry is hotly debated. Salmon growers indicate that it is the only means for treating lice infections and those opposed to its use cite its toxicity to commercial and non-commercial species as well as to workers since it can be taken up by inhalation, ingestion or penetration through the skin. The United Kingdom has recently (1989) licensed the product 'Aquaguard', which contains dichlorvos, for a one-year trial period in Scottish salmon farms.

Little is known of the action of products containing dichlorvos on phytoplankton which are key components of marine food webs. In a community of freshwater periphyton attached to glass slides, a commercial product containing 50% dichlorvos decreased the number per unit area of *Chlorella pyrenoidosa* Chick, the dominant algal species. However, it was only the commercial formulation which was toxic; dichlorvos alone had little effect on the species composition of periphyton (Stanislawska-Swiatkowska and Ranke-Rybicka 1976). With *Chlamydomonas moewusii* Gerloff dichlorvos did not produce significant inhibition of algal growth as measured by estimating cell mass produced on plates, nor did it inhibit zygospore germination (Cain and Cain 1984). Krzeckzowska-Woloszyn (1979) compared the phytoplankton communities in freshwater ponds treated with neguvon with untreated control ponds. Though differences were noted between ponds, she concluded that the compound, which contains the dichlorvos precursor trichlorphon (Fig. 1B), had no direct and evident influence on phytocenoses in the treated ponds.

Because of the lack of information on interactions between dichlorvos and marine algae and because marine phytoplankton are a key component of aquatic ecosystems, we examined the effect of a commercial preparation containing dichlorvos, Nuvan®, used in the salmon farming industry and of dichlorvos itself on CO_2 fixation by unialgal cultures of marine algae and on natural populations.

Methods

Organisms

Unialgal cultures of *Chaetoceros calcitrans* (Paulsen) Takano strain CCAP 1010/1, *Isochrysis galbana* Parke strain CCAP 927/1, *Pavlova lutheri* (Droop) Green strain CCAP 931/1, *Pseudoisochrysis paradoxa* (Ott, nom. nud.) strain CCAP 949/1 and *Skeletonema costatum* (Greville) Cleve, strain unknown were obtained from the Shellfish Research Laboratory of University College Galway, Carna, Co. Galway, Ireland. These cultures were maintained in prefiltered seawater (Whatman GF/C). Each litre of seawater was supplemented with 1.0 mL of a solution which contained 77 g $NaNO_3$ and 66 ml of Bio Plant Food (N 5.2%, P 2.3%, K 5.0%) (Pan Brittanica Indust. Ltd., Waltham Cross, Herts., UK) per litre. Cultures were maintained at room temperature (21 ± 2 °C) with air bubbling through them continuously. Illumination was provided by a bank of 20 W fluorescent lights (Phillips, 'Coolwhite') providing an irradiance of approximately 40 W m^{-2} (400 – 700 nm) on the exposed side of the culture. Cultures were transferred at approximately 60 h intervals using a 20% inoculum in order to maintain actively growing populations, and all experiments were performed within four days of obtaining the stocks.

Natural assemblages of phytoplankton were collected from the sea at Black Rock, Salthill, Co. Galway (53°15.5'N; 9°03.5'W). Samples were coarse-filtered through a 100 µm nylon mesh and used within 2 hours of sampling.

Chemicals

Nuvan 500 EC® (Ciba-Geigy) was obtained commercially. The manufacturers advised that it contained 50% dichlorvos in di-*n*-butyl phthalate. Dichlorvos (99.5%) was obtained as a gift from Ciba-Geigy, Wexford, Ireland. Stock solutions of these chemicals were prepared in deionised water immediately before use. Di-*n*-butyl phthalate (98%) was obtained from BDH Chemicals Ltd., Poole, England.

Photosynthetic rate determinations

Photosynthetic rates were measured using the ^{14}C technique (Strickland and Parsons 1975). Samples of unialgal cultures or natural populations were transferred into 50 mL straight-sided plastic tissue culture bottles. Most experiments involved duplicate bottles at each Nuvan® concentration, but as many as four replicates were used in some experiments. Aliquots of stock Nuvan® or dichlorvos solution were added to give a final concentration in the range 0 – 10 ppm

dichlorvos, calculations involving Nuvan® based on a 50% dichlorvos content by volume. Finally, 0.2 mL (cultures) or 1.0 mL (natural populations) aliquots of a stock $NaH^{14}CO_3$ containing 10 μCi mL^{-1} were added to each bottle. The bottles were then incubated under water at room temperature at an irradiance of 150 W m^{-2} for 4 h. The contents of the bottles were then filtered through Millipore HA grade membrane filters, fumed in HCl and their radioactivity counted under liquid scintillation. Values obtained were corrected for background and for activity in dark bottle controls. Activities for samples were then compared to activities in control samples which did not receive dichlorvos. For each experiment, chlorophyll determinations were also made using the spectrophotometric method after extraction with 90% acetone (Strickland and Parsons 1975).

Results

Nuvan® inhibited photosynthetic CO_2 fixation by natural assemblages of phytoplankton (Table I). Inhibition was dose-related, having a notable effect at doses which yielded concentrations above 0.5 ppm dichlorvos. Variation in results between experiments was evident at the lower concentrations used, but the overall effect on natural assemblages was the same as that observed using unialgal cultures (Fig. 2). Phytoplankton concentrations in these experiments gave chlorophyll *a* concentrations in the range 0.4–1.5 μg L^{-1} for the natural populations and 10–50 μ L^{-1} for cultures. Variations in phytoplankton biomass for cultures within this range did not affect the observed toxicity of Nuvan® at the 2.0 ppm dichlorvos level, and was not responsible for the observed variation in results between experiments. In every experiment, however, at least 85% of the photosynthetic activity was inhibited at concentrations of Nuvan® which gave 10 ppm dichlorvos.

In order to determine if Nuvan® lost its toxicity when stored as an aqueous solution, a stock solution containing 100 μL Nuvan® per 100 mL of deionised water was stored in a volumetric flask at room temperature (21 ± 2 °C) under room illumination for 23 days. A fine white precipitate developed which was resuspended immediately before dispensing the solution to samples. In experiments with each of the five algae Nuvan® which had been stored was as toxic as freshly-prepared Nuvan® (data not shown).

To establish whether the effects of Nuvan® on photosynthetic CO_2 fixation were due to dichlorvos or some other component of the formulation, cultures were exposed to Nuvan® and to concentrations of dichlorvos which gave equivalent levels of dichlorvos. Aqueous solutions of dichlorvos did not develop a precipitate. Table II clearly shows that the toxicity of Nuvan® was not due to dichlorvos alone.

Discussion

Nuvan® can be toxic, to phytoplankton at a concentration of 1 ppm dichlorvos, the level recommended by the manufacturer for treating sea lice in salmon farms (Table I, Fig. 2). Moreover, toxic compounds in the Nuvan® formulation have the potential to be concentrated on suspended particulates and they might be bioaccumulated in organisms in the food web. Dichlorvos is a weak mutagen for bacteria. It is not a mutagen in animal systems because animals degrade it (reviewed by Anonymous, 1974 and Wright *et al.* 1979). It has not been examined as a mutagen for phytoplankton. Such studies should be undertaken in order to understand the effect of Nuvan® and related products in aquatic ecosystems.

Table I. Effect of Nuvan® on photosynthetic CO_2 fixation by natural assemblages of marine phytoplankton.

Experiment No.	Relative photosynthetic activity (%)[a] at dichlorvos concentrations[b] (ppm) of:				
	0.1	0.5	1.0	2.0	10.0
1	77	–	53	–	10
2	83	86	54	43	–
3	93	96	76	42	–
4	87	85	76	49	15

[a] Activity relative to control samples.
[b] Nuvan®, containing 50% dichlorvos, was added to yield the dichlorvos concentrations indicated.

The toxicity of Nuvan® was due to some compound(s) other than dichlorvos alone (Table II). It was not possible to determine if the carrier di-*n*-butyl phthalate was the active agent because it formed a biphasic suspension when added to deionised water. The manufacturers communicated to us that emulsifiers were used in formulating Nuvan®. Thus, Nuvan®'s toxicity is due to the carrier, to a degradation product of dichlorvos, to another chemical present in the formulation such as an emulsifier, or to a combination of such factors. Unfortunately we were not given details of the concentration or type of emulsifier used nor were we able to obtain samples of it or Nuvan® formulated with the omission of dichlorvos.

If the toxic effects of dichlorvos are considered to be due to its inhibition of cholinesterase (Anonymous 1974), it is not surprising that our experiments failed to demonstrate its toxicity to phytoplankton. In insects and animals, cholinesterase is a key enzyme in the cholinergic nervous system. No significant role

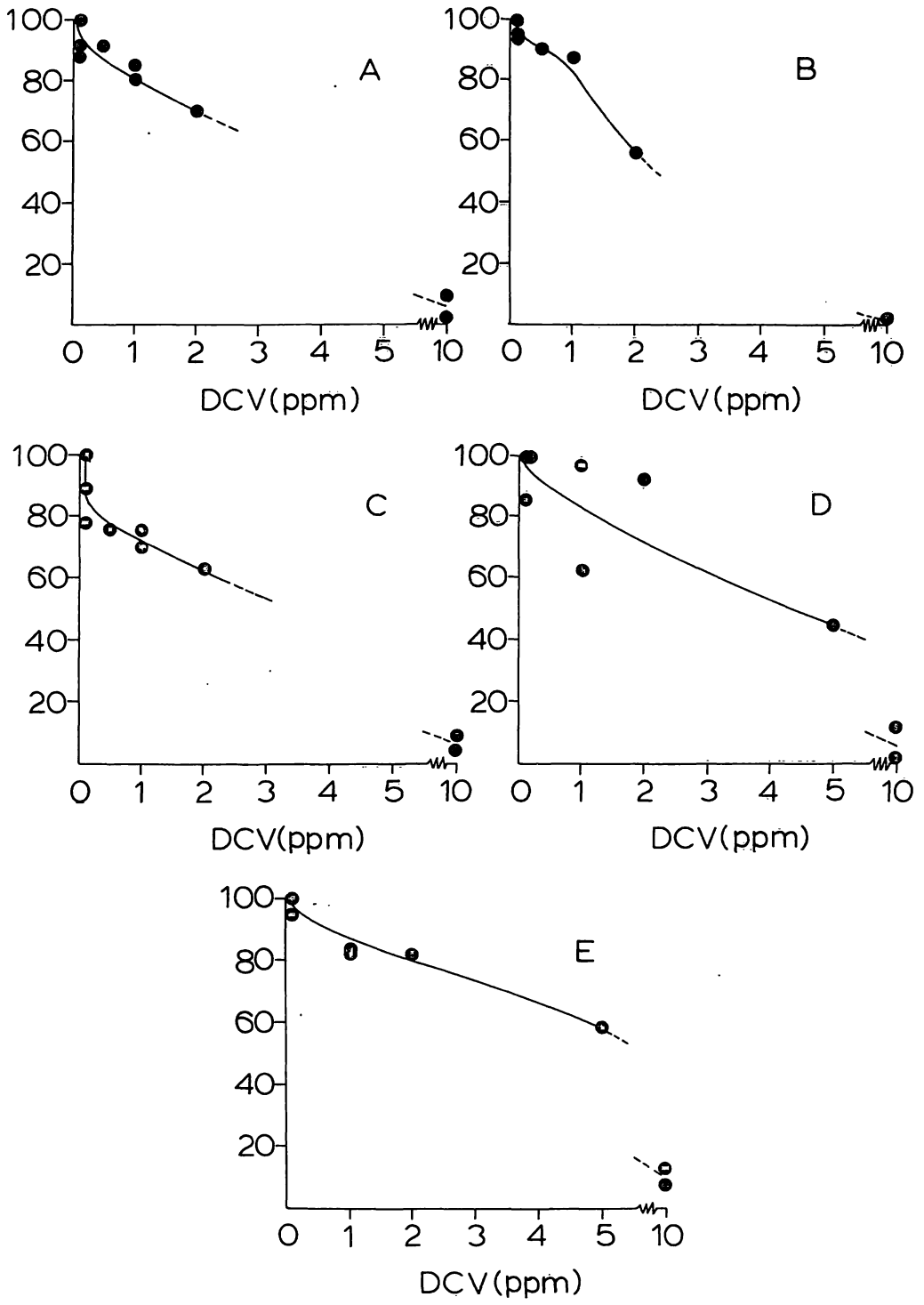

Fig. 2. Effect of Nuvan® on photosynthetic activity of unialgal cultures. A, *Chaeotoceros calcitrans*; B, *Skeletonema costatum*; C, *Pavlova lutheri*; D, *Isochrysis galbana*; E, *Pseudoisochrysis paradoxa*. See text for strain numbers.

for it has been reported for algae. Dichlorvos may have other effects. It has been shown to be toxic to the bacterium *Escherichia coli* B (Wild 1973), and to inhibit cell division in the yeast *Saccharomyces cerevisiae* N 123 (Eger *et al.* 1987). As was described in the introduction to this paper, however, studies have thus far failed to show any toxicity of dichlorvos alone towards a unialgal culture or freshwater periphyton (Cain and Cain 1984, Stanislawska-Swiatowska and Ranke-Rybicka 1976). Significantly, in the latter study a commercial formulation containing dichlorvos as the active ingredient did produce changes in periphyton species composition.

The fate and effects of pesticides containing dichlorvos should be established before they are accepted or rejected for use in aquatic ecosystems. Investigations, at least where algae are concerned, should consider not only the active ingredient, but the whole formulation.

Table II. Effects of Nuvan® and dichlorvos on photosynthetic CO_2 fixation.

	Relative Photosynthetic Activity (%)[a]					
	Nuvan®[b] 2.0 (ppm)	DCV[c] 2.0 (ppm)	Nuvan®[b] 5.0 (ppm)	DCV[c] 5.0 (ppm)	Nuvan®[b] 10.0 (ppm)	DCV[c] 10.0 (ppm)
P. paradoxa	82 (±3)	106 (±11)	59 (±3)	91 (±3)	13 (±1)	94 (±5)
I. galbana	93 (±12)	108 (±9)	45 (±7)	104 (±12)	12 (±2)	102 (±11)
P. lutheri	61 (±4)	85 (±5)	–	–	–	–
Natural Assemblage	72 (21)	110 (22)	–	–	–	–

[a] Activity relative to control samples. Errors indicate maximum spread on differences between duplicate samples and controls, or standard error on the difference betwen the means (natural assemblage only: n = 4).
[b] Nuvan®, containing 50% dichlorvos, was added to yield the dichlorvos concentrations indicated.
[c] DCV = Dichlorvos.

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