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Effect of Acetone on the Toxicity of Four Chemicals to *Selenastrum capricornutum*

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Most procedures for the assessment of algal growth inhibition allow the use of a water miscible organic solvent as a carrier for chemicals sparingly soluble in water (ISO, 1984; OECD, 1983). In general the procedures limit the carrier solvent concentration in the final culture to approximately 100 mg l^{-1} (~100 µl l^{-1}). At this concentration the organic solvent would not be expected to have any significant effect on the solubility of the test chemical; so it is best described as a carrier solvent, rather than co-solvent which is sometimes used as an alternative.

Herzel and Murty (1984) showed that the water solubility of dieldrin and nitrofen was unaffected by acetone up to 500 mg l^{-1}, but that captan was 41 per cent more soluble. They express the view that, on the whole, the effect on solubility is insignificant. In any toxicity test, however, the important factor is what effect the presence of the solvent has on the toxicity of the test compounds.

A small number of carrier solvents have been used in algal growth inhibition tests. Hughes and Vilkas (1983) recommend N,N-Dimethyl formamide (DMF) as a solvent because of its resistance to metabolism by bacteria (which grow in close association with algae in non-axenic cultures) and Lundy et al (1984) used methanol as a carrier solvent when testing the effect of polychlorinated biphenyls, DDT and dieldrin on mixed cultures of marine diatoms. However, the most commonly used solvent in algal toxicity testing is acetone. Stratton and Corke (1981) studied the effects of atrazine and permethrin on

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Scenedesmus quadricauda and Chlorella pyrenoidosa in the presence of a range of acetone concentrations, up to 1 per cent (10 g l^{-1}). Short term inhibition of photosynthesis ($^{14}CO_2$ uptake) by acetone was measured. Although acetone alone was not inhibitory to the test algae there was a range of responses in the pesticide-treated cultures. Different acetone levels did not seem to affect permethrin toxicity but affected atrazine toxicity in an unpredictable manner. Kleppel and Mclaughlin (1980) tested for acetone toxicity against the marine diatom Skeletonema costatum in a study of the effects of PCB's on different cell densities. They found 0.1 per cent (~1000 mg l^{-1}) acetone had no effect on culture growth but did not study its effects on PCB toxicity.

Stratton (1984) used a method developed by Stratton et al (1982) for fungi to determine that acetone with a final concentration of 0.1 per cent in culture could be used as a carrier solvent in algal toxicity tests. He states that this level does not give inhibition values significantly different (ρ = 0.95) from the inhibition calculated for acetone-free cultures.

Although there have been studies of the effects of carrier solvents themselves on the growth of algae, there has been little work on the effects of carrier solvent on the toxicity to algae of chemicals being tested.

The study reported here investigates the effects of acetone at 100 mg l^{-1} (the recommended final concentration in the OECD guidelines) on the toxic values obtained for chemicals of different aqueous solubilities. Aniline and tebuthiuron (readily soluble), the poorly soluble butyl ester of 2,4 dichlorophenoxy acetic acid (2,4-D BE), and almost insoluble 1,1,1-trichloro-2,2-di(chloro- phenyl) ethane (DDT). All were tested against Selenastrum capricornutum using a standard test method. The freely soluble compounds were chosen to investigate acetone effects other than solubilization of the chemicals by the carrier solvent. For example Parasher et al (1978) and Weinberger et al (1983) suggest that solvents influence the uptake of chemicals by algae, possibly by interacting with cell membranes.

MATERIALS AND METHODS

The method used was that described as the 'PRL' method previously (Adams and Dobbs, 1984), modified to cope with the addition of test chemicals with and without acetone.

Stock solutions of aniline and tebuthiuron were made up in sterile particle-free deionised water whilst stock solutions of 2,4-D BE and DDT were made up in acetone 24 hours before their addition to the culture flasks. The concentrations of the

stock solutions were such that, when 10 µl aliquots were added to 100 ml cultures of exponentially growing S. capricornutum, the test chemicals were present at the required treatment level. A second set of DDT solutions was prepared that gave the required test concentrations when 20 µl aliquots were added to 100 ml cultures. In these, therefore, the final acetone concentration was ~200 mg l^{-1}; instead of 100 mg l^{-1}, the OECD recommended amount. Acetone free DDT and 2,4,-D BE acetone-free flasks were prepared by adding 10 µl aliquots of the stock solutions to autoclaved, empty 250 ml narrow necked conical flasks. The flasks were stored in an incubator at 22°C and shaken at 175 rpm for 24 hours while the acetone evaporated.

All experiments were carried out in triplicate, the test chemicals being added to 24 hour old cultures containing approximately 10^4 cells ml^{-1} of exponentially growing S. capricornutum. Aniline and tebuthiuron were added in particle free deionised water, immediately followed by 10 µl acetone for the solvent containing replicates. DDT and 2,4-D BE were added in acetone and their acetone free replicates were prepared by transferring the cultures, using aseptic technique, to the sterile flasks containing the compounds prepared 24 hours earlier. This was termed day 0. The cultures were incubated as above for 6 days and the cell count for each flask was recorded every 24 hours, using an Elzone 80XY electronic particle counter (Particle Data Ltd).

The cultures containing 5 mg l^{-1} DDT were analysed at the end of the experiment to establish that there was, in fact, DDT present. Firstly the DDT was extracted from the cultures with 15 ml and 5 ml of hexane. The hexane samples were combined, made up to 25 ml and stored at 4°C until required for analysis. The cultures were then lysed with a Soniprobe and hexane was again used to extract any remaining DDT, though the lysed cultures and hexane did not separate out clearly. All the samples were analysed by gas chromatography, using a Pye 104 (Pye Unicam Ltd) with electron capture detection.

RESULTS AND DISCUSSION

Using the daily replicate cell counts for tebuthiuron, aniline and 2,4,-D BE, the areas under the growth curve over different culture periods were calculated. The areas for each treatment level were compared to the controls using Dunnett's method (Zar, 1974), as described elsewhere (Adams et al, in press), to give the lowest concentrations tested that caused a significant effect ($\rho = 0.95$) (LCSE). LCSE values with and without acetone for tebuthiuron and 2,4-D BE (Table 1) are almost identical, and the close similarity of growth curves for tebuthiuron (Figures 1 and 2) support the view that acetone has had no effect. This suggests the use of acetone as a carrier solvent with a final concentration in culture of 100 µl l^{-1} is acceptable.

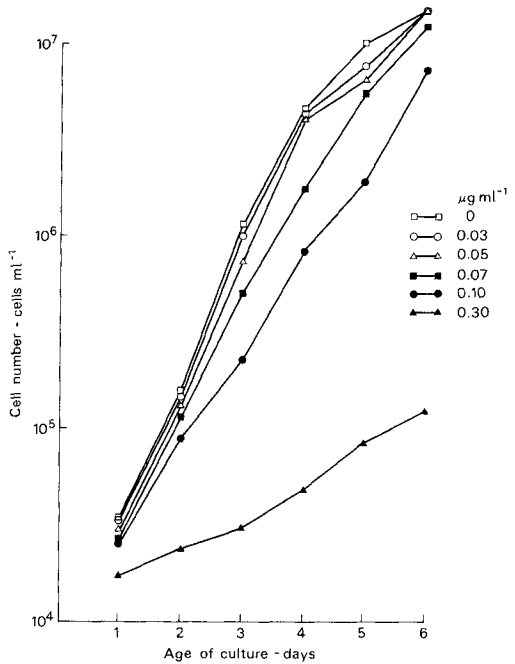

Figure 1 The effect of tebuthiuron without acetone on growth of *S. capricornutum*. Mean cell number of 3 replicate flasks is recorded at each treatment level.

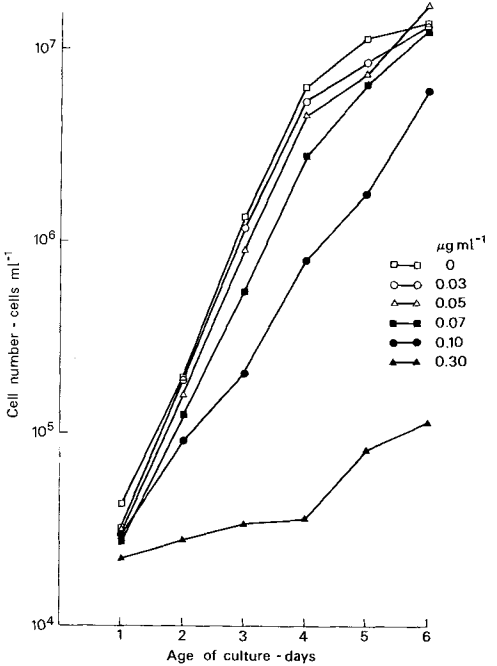

Figure 2 The effect of tebuthiuron with 100 µg ml^{-1} acetone on the growth of *S. capricornutum*. Mean cell number of 3 replicate flasks is recorded at each treatment level.

Table 1 Lowest concentration of test chemicals(μg ml^{-1}) causing a significant reduction (ρ = 0.95) in growth of Selenastrum capricornutum. Values calculated using area under the growth curve (cell concentration against time)

Days used in calculation	2,4-D BE		Tebuthiuron		Aniline	
	Acetone	None	Acetone	None	Acetone	None
1 to 3	3	3	0.07	0.05	1	5
1 to 4	3	3	0.05	0.05	1	3
1 to 5	3	3	0.05	0.05	5	3
1 to 6	5	3	0.05	0.05	5	3

The LCSE values are higher by day 5 for aniline with acetone and day 6 for 2,4-D BE with acetone because control culture growth is slowing as it reaches the stationary phase and the treated cultures are catching up. The tebuthiuron treated cultures in Figures 1 and 2 are also catching up with the controls, which are reaching the stationary phase; but it is not sufficient to affect the LCSE value recorded. The results obtained for aniline are comparable to those obtained by Calamari et al (1980) using S. capricornutum.

DDT at the concentrations tested in this experiment (5 μg ml^{-1} DDT with 200 μl l^{-1} acetone) did not affect the growth of S. capricornutum. Considering the wide variety of results obtained with other green algae the resistance of S. capricornutum is perhaps not surprising. Many researchers have found DDT has no effect on different species at concentrations up to 100 μg l^{-1} (Lal and Saxena, 1982) whilst others have found an effect on algal population density at concentrations as low as 1 μg l^{-1} (Goulding and Ellis, 1981). The GC analysis of 5 μg ml^{-1} DDT cultures indicated concentrations of approximately 4 μg ml^{-1} DDT (thus confirming that there was DDT present).

The results of this investigation suggest that the use of acetone as a carrier solvent with a final concentration of 100 μl l^{-1} is acceptable in algal growth inhibition tests. The results of the DDT experiment support the view that test species is a very important variable, and the results obtained depend heavily on that chosen. More work into the importance of test species is required.

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