Bull. Environm. Contam. Toxicol. 27, 13-16 (1981)

Interaction between Acetone and Two Pesticides towards Several Unicellular Green Algae

Glenn W. Stratton[1] and Charles T. Corke[2]

[1]Department of Biology, Nova Scotia Agricultural College, Truro, Nova Scotia, B2N 5E3, Canada; and [2]Department of Environmental Biology, University of Guelph, Guelph, Ontario, N1G 2W1 Canada

Use of organic solvents in pesticide bioassays is often necessary due to the low water solubility of many of these compounds. However, it has been shown that the solvent carrier used can have a significant effect on the response of a test organism to pesticides (MAJEWSKI et al. 1978, DALELA et al. 1979). Recently, a method was reported for analyzing the effects of solvent-pesticide combinations (BURRELL 1980). This involves treating an organism with various concentrations of solvent, and the same concentrations of solvent combined with one level of test pesticide. The net pesticide effect is determined by calculating the per cent inhibition in each solvent-pesticide treatment with reference to activity in the corresponding solvent control. Theoretically, the inhibition calculated at each level of solvent should be the same since all systems contain the same concentration of pesticide and all data are calculated using the appropriate solvent controls. This would be indicative of an additive interaction between the solvent and pesticide. Inhibition values significantly higher than expected represent synergism, and those significantly lower than expected represent antagonism (BURRELL 1980).

This technique has been used to evaluate the effects of acetone-pesticide combinations towards eucaryotic fungi (BURRELL & CORKE 1980) and procaryotic cyanobacteria (STRATTON et al. 1980). With these organisms, acetone interacts with test pesticides both additively, synergistically, and antagonistically, depending on the solvent concentration used. The selection of a solvent concentration that does not interact additively with the candidate pesticide will result in either a low or high calculated EC_{50} value, which can lead to erroneous conclusions regarding the toxicity of the test compound (STRATTON et al. 1980).

The purpose of this study was to determine whether this solvent-pesticide phenomenon also occurs with another major group of nontarget microorganisms - the eucaryotic, unicellular green algae. Acetone was used as the test solvent, as it is the solvent of choice in many microbial pesticide bioassay experiments

0007-4861/81/0027-0013 $01.00

(PARASHER et al. 1978). The pesticides used included the insecticide permethrin, which is being evaluated as a possible mosquito larvicide (HERALD et al. 1980), and the herbicide atrazine, whose residues can be found in most aquatic ecosystems (FRANK & SIRONS 1979).

MATERIALS AND METHODS

Test organisms used were the green algae Chlorella pyrenoidosa and Scenedesmus quadricauda, which were obtained from the Department of Botany and Genetics, University of Guelph, Guelph, Ontario, Canada. Cultures were grown in the medium described by STRATTON & CORKE (1979), supplemented with 1.5 g $NaNO_3$ L^{-1}. All experimental flasks were incubated at 22°C and a light intensity of 7000 lux on a 12 h light-dark cycle. Test chemicals used were the solvent acetone (glass distilled pesticide research grade) and the pesticides permethrin (technical grade, Chipman Chemicals Ltd., Stoney Creek, Ontario, Canada) and atrazine (technical grade, Ciba-Geigy of Canada Ltd., Cambridge, Ontario, Canada).

Photosynthesis was used as the test criterion and was quantitated by following the uptake of $^{14}CO_2$ from $NaH^{14}CO_3$, as previously outlined (STRATTON et al. 1980). The final 10 mL volume of mixture contained 1 x 10^5 cells/mL and 0.1 μCi (1 Ci = 37 GBq) of radioactivity/mL. Pesticide concentrations used were 100 mg L^{-1} permethrin for both algae and 0.3 mg L^{-1} atrazine for S. quadricauda and 0.5 mg L^{-1} atrazine for C. pyrenoidosa. Each concentration of pesticide was combined with acetone concentrations of 0.1, 0.2, 0.4, 0.6, 0.8 and 1.0% (v/v). Each pesticide was dissolved in acetone and added to test systems to give an acetone concentration of 0.1%. Additional acetone was added directly to reaction systems to yield the final concentration desired. Data were analyzed by the solvent-pesticide interaction method previously reported (BURRELL & CORKE 1980, STRATTON et al. 1980). Each treatment was replicated five to ten times and all significant differences were determined using Duncan's multiple range test ($P = 0.05$), as outlined by BLISS (1967).

RESULTS AND DISCUSSION

Acetone alone was not inhibitory to either S. quadricauda or C. pyrenoidosa. Photosynthetic activity in these cultures was stimulated above 0.2% acetone, and a stimulation of 30-40% was reached at 1.0% acetone.

This solvent is an organic polar compound that can extract molecules from cell membranes. It removes phospholipids from the membranes of animal cells (ASHWORTH et al. 1966) and may extract sterols from the cell membrane of fungi (BURRELL & CORKE 1980). Acetone also causes ultrastructural damage and a disintegration of the plasma membrane in C. pyrenoidosa (PARASHER et al. 1978) and Anabaena sp. (STRATTON et al. 1980). These observations suggest that acetone can alter the permeability of cells through the extraction of selected membrane components.

Stimulation of photosynthetic activity induced by acetone could be related to membrane damage. Increased membrane

Table 1. Acetone-pesticide interactions towards photosynthesis in green algae.*

Acetone	S. quadricauda		C. pyrenoidosa	
concn (% v/v)	permethrin	atrazine	permethrin	atrazine
0.1	7.2[a]	55.3[a]	-8.7[a]	75.3[a]
0.2	-3.2[a]	52.2[a]	4.5[a]	78.7[a]
0.4	1.6[a]	70.3[b]	6.9[a]	68.4[c]
0.6	6.3[a]	73.7[b]	-3.3[a]	80.7[a]
0.8	-2.6[a]	77.5[b]	-14.3[a]	87.3[b]
1.0	-0.1[a]	75.6[b]	7.9[a]	83.6[b]

*Table values (net pesticide effect) are mean per cent inhibition, calculated with reference to activity in those systems containing only the appropriate level of acetone. a--these values do not differ significantly (P = 0.05) from that calculated at 0.1% acetone and are considered as additive; b--these values are significantly greater than that calculated at 0.1% acetone and are considered as synergistic; c--these values are significantly lower than that calculated at 0.1% acetone and are considered as antagonistic.

permeability could result in an increase in the rate of CO_2 diffusion into the algal cells. This would result in a stimulation of photosynthetic activity, since CO_2 diffusion is the major rate limiting step in this process (ZELITCH 1975).

Data for the effects of acetone-pesticide combinations are outlined in Table 1. Acetone and permethrin interacted in an additive manner towards both algae at all levels of solvent assayed. Therefore, any concentration of acetone between 0.1 and 1.0% can be used in toxicity assays involving permethrin and these algae, providing that the data are calculated with reference to the appropriate solvent controls. These results are comparable to those obtained in a study of the effects of mixtures of acetone and permethrin towards photosynthesis and nitrogenase activity in the cyanobacterium Anabaena inaequalis (STRATTON et al. 1980).

Acetone and atrazine combined to produce a more complex series of responses with the two green algae (Table 1). With S. quadricauda, acetone and atrazine interacted additively at 0.1 and 0.2% acetone, and synergistically above 0.2%. With C. pyrenoidosa, an additive response was obtained with 0.1, 0.2, and 0.6% acetone. Antagonism was observed at 0.4% and synergism at 0.8 and 1.0% acetone. Consequently, an acetone concentration of 0.2% (v/v) should be used in bioassay systems employing these cultures to determine atrazine toxicity.

The reason why all concentrations of acetone do not give the same response when combined with pesticides in unknown. As outlined above, acetone may alter membrane structure and permeability. The responses obtained with combinations of acetone and pesticides

may be related to this phenomenon. Apparently a threshold level of solvent must be reached before the organism is altered in such a way as to become more or less sensitive to a second toxicant. Below this threshold level, acetone does not affect the toxicity of the test pesticide and an additive interaction is observed. Synergism could result from an acetone-induced disruption of membrane structure and transport systems. However, detailed studies are required before the cause of these responses can be elucidated.

In summary, the data presented here indicate that acetone can interact with pesticides additively, synergistically, and antagonistically, when tested towards photosynthesis in green algae. This, together with similar data reported for fungi (BURRELL & CORKE 1980) and cyanobacteria (STRATTON et al. 1980), indicates that the interaction between a solvent and pesticide can be an important source of error in all microbial toxicity bioassays. It is essential that the exact response of a test culture towards any solvent-pesticide combination be known prior to detailed toxicity experiments. This can be determined rapidly by using a method similar to that employed here.

Acknowledgements. Research was supported by the Natural Sciences and Engineering Research Council of Canada. The authors thank M. L. Hoye for technical assistance.

REFERENCES

ASHWORTH, C. T., J. S. LEONARD, E. H. EIGENBRODT, F. J. WRIGHTSMAN: J. Cell Biol. 31, 301 (1966).

BLISS, C. I.: Statistics in biology, vol. i. New York: McGraw-Hill 1967.

BURRELL, R. E.: MSc. Thesis, University of Guelph, Guelph, Ontario, Canada 1980.

BURRELL, R. E., and C. T. CORKE: Bull. Environm. Contam. Toxicol. 25, 554 (1980).

DALELA, R. C., S. K. BANSAL, A. K. GUPTA, S. R. VERMA: Water Air Soil Pollut. 11, 201 (1979).

FRANK, R., and G. J. SIRONS: Sci. Total Environ. 12, 223 (1979).

HERALD, F., J. L. CLARKE, F. W. KNAPP: Mosquito News 40, 380 (1980).

MAJEWSKI, H. S., J. F. KLAVERKAMP, D. P. SCOTT: Water Res. 12, 217 (1978).

PARASHER, C. D., M. OZEL, F. GEIKE: Chem. Biol. Interactions 20, 89 (1978).

STRATTON, G. W., and C. T. CORKE: Can. J. Microbiol. 25, 1094 (1979).

STRATTON, G. W., R. E. BURRELL, M. L. KURP, C. T. CORKE: Bull. Environ. Contam. Toxicol. 24, 562 (1980).

ZELITCH, I.: Environmental and biological control of photosynthesis. In: Environmental and biological control of photosynthesis. ed. by R. MARCELLE. The Hague: W. Junk 1975.

Accepted April 25, 1981