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Interaction Effects of Mercury-Pesticide Combinations Towards a Cyanobacterium

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The proliferation of the numbers and quantities of environmental contaminants has elicited numerous studies on their potential ecological impact. Of particular importance is research into effects on non-target microorganisms, since these organisms are vital components of all biospheric nutrient cycles and are therefore unrivalled in their ecological significance. The two most commonly studied groups of environmental toxicants in microbiology are pesticides and heavy metals. Although these compounds have been the subject of numerous scientific reports, most have dealt only with individual pesticides or heavy metals and little data are available on toxicant combinations.

Toxicant interaction studies are vital to obtaining a valid *in situ* estimation of environmental impact. Pesticides, heavy metals, and other xenobiotics are rarely found alone in the environment, but are in association with other toxicants, their degradation products, solvents, and various naturally-occurring and synthetic organic and inorganic chemicals (Stratton and Corke 1982a). These complex mixtures can interact to create numerous synergistic and antagonistic responses that could alter an individual compound's toxicity pattern (Stratton and Corke 1982a,b; Stratton 1984). Although the importance of research involving toxicant mixtures is widely accepted (Environmental Studies Board 1981; National Research Council of Canada 1982), few data have been published on either pesticide or heavy metal interactions towards non-target microorganisms such as algae (Loeppky and Tweedy 1969; Stratton and Corke 1979; Wong and Beaver 1980; Wong et al. 1982), bacteria (Nayak and Rao 1982; Babich and Stotzky 1983), and fungi (Roslycky 1977). No data are available on heavy metal-pesticide interactions towards microorganisms.

The present study supplies interaction data for combinations of mercuric ion (supplied as mercuric chloride), atrazine (2-chloro-4-ethylamino-6-isopropylamino-1,3,5-triazine), and permethrin (3-phenoxybenzyl-(1RS)-*cis*,*trans*-3-(2,2-dichloro-vinyl)-2,2-dimethyl cyclopropanecarboxylate) when tested towards growth of the cyanobacterium (blue-green alga) *Anabaena inaequalis*. Mercury is one of the most important heavy metal pollutants (Rai et al. 1981) and has been widely used in toxicology research.. Atrazine

is the most heavily used pesticide in the United States (DeNoyelles et al. 1982) and its residues are widely distributed in terrestrial (Carey et al. 1979) and aquatic (Wu 1981) ecosystems. Permethrin is an important insecticide with expanding markets and is presently being evaluated for its environmental impact (Stratton and Corke 1982a,b; Stratton 1983). A. inaequalis has been used extensively in this laboratory in previous interaction studies (Stratton and Corke 1979, 1982a,b; Stratton 1983, 1984).

MATERIALS AND METHODS

The cyanobacterium Anabaena inaequalis was used as test organism and is fully described elsewhere (Stratton et al. 1979). All cultures were grown in a liquid nitrogen-free medium (Stratton et al. 1979) at a temperature of $22\pm2^{\circ}C$ and a light intensity of 7000 lux on a 12 h light-dark cycle.

The test chemicals used were mercuric ion, supplied as mercuric chloride (reagent grade; Fisher Scientific Co.), the pyrethroid insecticide permethrin (technical grade; Chipman Chemicals Ltd., Stoney Creek, Ontario, Canada; 86.6% pure; 40:60 mixture of the cis and trans isomers), and the s-triazine herbicide atrazine (technical grade; Ciba-Geigy Canada Ltd., Cambridge, Ontario, Canada; >95% pure). Stock solutions of the two pesticides were prepared in pesticide-grade acetone (Caledon Laboratories, Georgetown, Ontario, Canada). A total concentration of 0.1% (v/v) acetone was used in all bioassay experiments, as determined by the solvent-pesticide interaction analysis technique (Stratton et al. 1982). Mercuric chloride was dissolved in glass distilled water. All of the treatments were replicated five to ten times. All pesticide concentrations refer to the active ingredient, while the mercury levels refer to mercuric ion.

Growth of A. inaequalis was assessed by measuring the absorbance of cultures, with time, using a Bausch and Lomb Spectronic 88 spectrophotometer, a wavelength of 600 nm, and a pathlength of 1.8 cm, as described elsewhere (Stratton and Corke 1982a). Test tubes containing 9.5 ml of nitrogen-free medium and 0.1 ml of test chemical(s) were inoculated with 0.5 ml of an actively growing culture containing 6.5×10^{4} cells per ml, and incubated in racks inclined on a 45° angle. Optical densities (cell yield) were determined for 12 to 16 days and percent inhibition values calculated relative to growth in control systems (solvent added where applicable). Permethrin concentrations of 0.5 and 1.0 ppm (ug/ml), atrazine levels of 0.01 and 0.05 ppm, and a mercuric ion concentration of 0.004 ppm, were tested individually and in combination. The interaction data were analyzed as outlined below. For mercury-pesticide combinations, test chemicals were added in three ways: where both mercury and the pesticide were added simultaneously at day zero; where mercury was added at day zero and the pesticide 18 h later (day one); where the pesticide was added at day zero and mercury at day one.

Interaction data for mercury-pesticide combinations were analyzed by the multiplicative survival model (Gowing 1960; Morse 1978; Stratton et al. 1982). This involved mathematically calculating a theoretical expected additive interaction response of a mixture of toxicants using percent inhibition data obtained for each component compound tested individually. This expected inhibition was then statistically compared with the actual inhibition obtained experimentally for that mixture (Student's t test at P=0.05). Synergism and antagonism were defined as an actual experimental toxicity significantly greater or less than expected, respectively. An additive effect occurred when the actual and expected inhibitions did not differ significantly (Stratton et al. 1982).

RESULTS AND DISCUSSION

The effects of each toxicant tested individually towards growth of A. inaequalis are summarized in Table 1. These data were used to mathematically calculate expected additive interaction responses according to the multiplicative survival model, as discussed below. Mercury was the most toxic chemical tested, followed by atrazine and permethrin, respectively (Table 1). On a ppm basis, mercury was approximately 10 times more toxic than atrazine and 100 times more toxic than permethrin. Atrazine, in turn, was about 10 times more toxic than permethrin.

The levels of inhibition obtained for the individual test compounds are similar to those reported elsewhere. For example, mercury routinely causes significant growth inhibition of various freshwater and marine algae and cyanobacteria at concentrations between 0.001 and 0.008 ppm, or 1 and 8 ug/L (reviewed in Rai et al. 1981). Permethrin has previously been reported to have an EC_{50} of 0.07 ppm towards growth of the alga Skeletonema costatum (Walsh and Alexander 1980) and 1.6 to 5.0 ppm for cell yield and growth rate of Anabaena (Stratton and Corke 1982a). Atrazine has an EC_{50} of 0.1 to 0.5 ppm towards selected green algae (Zweig et al. 1963; Hollister and Walsh 1973; Stratton 1984) and 0.03 to 4.0 ppm towards various species of the blue-green alga Anabaena (Rohwer and Fluckiger 1979; Stratton 1984).

Table 1. Effects of interaction compounds tested individually towards A. inaequalis.

Compound Tested	Concn (ppm)	Percent Inhibition[1] Mean	S.D.
Mercury	0.004	33.7[a]	± 6.3
Permethrin	0.500	57.9	± 4.3
Permethrin	1.000	71.3	± 2.8
Atrazine	0.010	13.4	± 4.0
Atrazine	0.050	34.9[a]	± 5.8

[1] Percent inhibition values were calculated relative to growth in control systems (no toxicants; 0.1 percent v/v acetone where applicable). Those values followed by the same letter do not differ significantly at P=0.05.

Table 2. Interaction effects of mercury-pesticide combinations towards growth of A. inaequalis.

Pesticide interacted with Hg[1]	Pesticide concn (ppm)	Calculated expected inhibition[2]	Actual inhibition[3]		
			Hg day 0 Pest. day 0	Hg day 0 Pest. day 1	Hg day 1 Pest. day 0
Permethrin	0.50	72.1 ± 3.6	59.7 ± 6.5[5]	87.0 ± 2.6[6]	71.0 ± 8.3[4]
Permethrin	1.00	81.0 ± 2.4	70.6 ± 7.8[5]	85.6 ± 5.5[6]	78.1 ± 5.6[4]
Atrazine	0.01	42.6 ± 5.6	28.2 ± 3.2[5]	44.6 ± 7.4[4]	57.9 ± 4.3[6]
Atrazine	0.05	56.8 ± 5.2	48.9 ± 4.5[5]	61.1 ± 4.5[4]	72.4 ± 3.8[6]

[1]All combinations contained 0.004 ppm mercuric ion and the pesticide indicated. All control systems contained 1.0% (v/v) acetone, where applicable.

[2]Calculated using the formula of Gowing (1960) and the inhibition data contained in Table 1. Mean ± the standard deviation.

[3]Actual inhibition values (mean ± the standard deviation) obtained experimentally for each mercury-pesticide combination. Column headings refer to the order of addition of test compounds (Pest.=pesticide).

[4]Additive response: expected and actual inhibitions do not differ significantly (P=0.05).

[5]Antagonistic response: actual inhibition significantly lower than the expected inhibition (P=0.05).

[6]Synergistic response: actual inhibition significantly greater than the expected inhibition (P=0.05).

Interaction conclusions for combinations of mercury and permethrin or atrazine are summarized in Table 2. When mercury and either pesticide were added simultaneously at day zero, the mixture's components interacted antagonistically. When mercury was added at day zero and the pesticides at day one, mercury and permethrin interacted synergistically while mercury and atrazine interacted additively. When the heavy metal was added at day one and the pesticides at day zero, the reverse pattern was noted: permethrin interacted in an additive manner with mercury while atrazine interacted synergistically (Table 2). There were no consistent trends in the magnitude of the interaction responses (difference between the expected and actual inhibitions).

No other data are available for comparison purposes on the toxicity of heavy metal-pesticide combinations towards microorganisms. However, some data have been published for combinations containing either pesticides only or heavy metals only.

Most interaction studies deal with heavy metals and algae or blue-green algae and these data have been reviewed elsewhere (Rai et al. 1981; Singh and Pandey 1981). When mercury has been included in heavy metal combinations both synergistic and antagonistic responses have been reported, depending upon the test system employed and the other metals present. For example, mercury interacts synergistically with complex mixtures that include three or more other metals, such as copper, cadmium, zinc, nickel, and lead, when tested towards photosynthesis in various phytoplankton (Gachter 1976-cited in Rai et al. 1981; Stratton and Corke 1979; Wong et al. 1982). However, mercury and cadmium interact antagonistically when tested towards growth in Anabaena inaequalis, but synergistically when photosynthesis and nitrogenase activity are used as test criteria (Stratton and Corke 1979). Mercury-nickel combinations also elicit synergistic responses towards growth and nitrogenase activity in A. inaequalis (Stratton and Corke 1979). Heavy metal interactions have been explained on the basis of a competition for cellular binding sites (Stratton and Corke 1979) and the formation of stable metal-organic matter complexes (Singh and Pandey 1981).

Comparatively less research has been undertaken with pesticide combinations and phytoplankton (Stratton 1984). Mixtures of DDT and PCBs elicit a pronounced antagonism towards growth of the diatom Thalassiosira pseudonana (Mosser et al. 1974). Atrazine and metobromuron interact synergistically towards growth of Chlamydomonas reinhardtii (Loeppky and Tweedy 1969), while atrazine and permethrin combinations usually elict an additive response towards growth, phtosynthesis, and nitrogenase activity in A. inaequalis (Stratton 1983).

The response patterns observed here with mercury-pesticide combinations (Table 2) are complex and difficult to explain because of the lack of sufficient research data regarding interaction physiology. Mercuric ion has a tremendous capacity to solubilize cell membranes (Rai et al. 1981) and cause algal cell lysis

(Stratton et al. 1979). This would increase cell permeability, disrupt membrane transport systems, and interfere with membrane-related metabolic reactions (Stratton et al. 1979), such as electron transport in procaryotic cyanobacteria. Atrazine is a photosynthetic inhibitor that is readily adsorbed and accumulated by algae (Veber et al. 1981). Permethrin acts as a neurotoxin in insects but its mode of action in algae and other microorganisms is unknown. Therefore, the physiological mechanisms responsible for the data presented here (Table 2) can only be speculated upon.

The antagonistic interactions noted when all compounds were added at day zero indicate that one of the components is reducing the toxicity and/or uptake of the other. It is possible that mercury, due to its membrane effect, is interfering with the cellular uptake of the pesticides. The mixed synergistic and additive responses obtained when one of the compounds was added at day zero and the other at day one cannot be readily explained without further research. Synergisms occur when one compound enhances the toxicity and/or uptake of another, while an additive response indicates that the two components do not interact in any apparent way. The facts that the interaction patterns for permethrin and atrazine were dependent upon the order of toxicant addition and were opposite to one another are probably related to differences in their modes of action. Again, more research is required before these data can be fully explained.

Although the studies presented here deal with a pure culture of a cyanobacterium tested in vitro, and although it may be difficult to extrapolate these results to the natural environment, the data identify a significant deficiency in information regarding toxicant combinations. Heavy metal and pesticide contaminants can be expected in similar habitats and in order to accurately estimate the potential environmental impact of these compounds, further interaction studies must be undertaken. As well, more research is required in the area of interaction physiology before the effects of toxicant combinations can be fully understood.

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