Checking water for pesticide contamination

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Insect control in many Australian cotton cropping situations current requires multiple treatments with several pesticide groups: endosulfan (Thiodan), synthetic pyrethroids, organophosphates/ carbamates and in some cases chlorfluazuron in order to prevent damage to the crop by *Heliothis* and other insect species. While the industry has a real commitment to environmental protection, the use of several of these compounds is under close scrutiny from outside sources. If not managed properly, aerial application of pesticides can cause spray drift or pesticide dissipation through volatilization. Storm events soon after crop treatment can also mean that there is a risk of off-farm loss. Failure to retain endosulfan in tailwater on farm means that endosulfan and toxic metabolites can appreciably contaminate fish and wildlife. Other compounds such as pyrethroids and chlorfluazuron have high toxicities to fish and aquatic invertebrates respectively.

Currently, most pesticides are monitored using sophisticated laboratory instrumentation, such as gas-chromatography/ mass spectrometry or high-performance liquid chromatography. This equipment is reliable, but it is expensive and usually only located in capital cities, hundreds of kilometres away from the cotton-growing areas. If water samples are suspected of being contaminated, they must be shipped to these cities and await analysis. Apart from a delay of several days, the cost per analysis is usually several hundred dollars. Thus using these methods, monitoring cannot be as thorough as desirable.

In 1992 we started work on an alternative way of detecting pesticide

residues, based on the application of medical immunodiagnostic tests. This type of technology was introduced to the cotton industry last year, through the Lepton (R) test for *Heliothis* speciation. Immunoassays use antibodies that have been prepared in rabbits, mice or sheep to a particular pesticide. The pesticide molecules are too small to raise an immune response by themselves, so much of the art in developing a pesticide immunoassay consists of coupling a chemical analogue of the pesticide to a carrier, usually a protein that is foreign to the animal being immunized. The analogue must retain all of the characteristic features of the pesticide, but have a new chemical group in its structure that can act as a handle for coupling to the protein. Once coupled to the protein, the pesticide-protein "conjugate" is usually able to evoke antibodies, but for a useful test to be possible, antibodies must be evoked that can bind to the free pesticide, as it would appear in water and other environmental samples.

The key steps in development of an antibody test are as follows:

1.) Synthesis of a pesticide derivative, coupled to a suitable carrier protein for immunisation.

2.) Immumisation of rabbits and/or mice. Preparation and purification of antibodies.

3.) Development of initial immunoassay using pesticide standards. Check assay sensitivity and specificity.

4.) Assessment of assay performance with water and silt matrices in laboratory-spiked and field samples.

5.) Formatting of methods as prototype kits, stabilisation and stability trials on components and prototypes.

6.) Field trials of kits and training workshops in conjunction with users.

The high specificity and sensitivity of immunoassays enables the antibody to specifically "see" trace levels of pesticide molecules in a sample that may contain salts, silt particles fertilizer residues and humic materials. The pesticide tests use antibodies or enzymes that bind sensitively and specifically to target

pesticides in water and soil samples. Separate tests have been developed for endosulfan, organophosphate/ carbamates and pyrethroids. Two types of test kits have been developed: simple test kits for use by field workers such as growers and consultants to enable on-site analysis and a high-throughput test kit designed for low-cost analysis of samples in a small laboratory.

Using endosulfan as an example, the field tests take 15 minutes to perform, and are highly sensitivite (down to 0.1-0.2 parts-per-billion) and detect both endosulfan and its toxic sulfate metabolite. To put these figures in perspective, they are equivalent to about one drop in 5 to 10 backyard swimming pools or to 1/4 to 1/2 a second in a typical human lifetime. The diol breakdown product, which is less toxic to fish, is detected less sensitively. This degree of sensitivity is needed because of the levels at which some fish species are affected by some of the cotton pesticides. Testing a water sample in the tubes provided in the kits requires additions of chemicals from 3 dropper bottles (Table 1), and the result of the test is seen as different shades of a blue colour, depending on how much endosulfan was present in the sample. In addition to developing field tests for each of the compounds we are developing laboratory antibody test kits. These will enable simultaneous and sensitive analysis of dozens of samples at a time, with only limited equipment requirements.

We anticipate that the tests will be used to monitor:

1.) the proportion of runoff or floodwater that should be kept on farm after heavy rain,

2.) the efficiency of the spraying process (off-target application),

3.) accidental run-off from sprayed areas or container disposal into rivers and lagoons,

4.) whether fish kills are due to endosulfan, and if so, to act immediately to prevent further contamination of water bodies, and

5.) unapproved use of endosulfan outside the allowed time "window".

Table 1. Method for testing water using antibody kits

1. Using droppers provided, add 4 drops of each water sample to each tube. Always run a sample of pesticide-free water at the same time. Add 4 drops enzyme solution. Mix gently, wait 10 min.
2. Tip out tube contents (the enzyme and pesticide bind tightly to the tube wall). Wash tube by filling and emptying 5 times with tap water.
3. Add colour developer (4 drops each of Solution A and B). Wait 5 min. The tube contents will turn blue.
4. If pesticide is present in a sample, that tube will be a paler blue than the control tube ran with pesticide-free water.

Following test development, it is important to thoroughly check the performance of prototypes in the field. With endosulfan, this is being done in both the 1993/4 and 1994/5 summers in collaboration with other participants in the Cooperative Research Centre for Sustainable Cotton Production. The tests for endosulfan and other compounds will be used in part of a detailed project on the environmental fate of endosulfan on farms and nearby sites in the cotton-growing areas in the Namoi valley. In this project, 500 water, silt and soil samples will be collected each year for analysis. Gas Chromatographic analyses will be performed at 2-3 different labs (in NSW and QLD) and the data compared with the new immunoassay methods. We will also demonstrate the new tests to industry groups as opportunities arise. The final phase of the project will involve "technology transfer" to enable commercial kit production, for example in the case of endosulfan and organophosphates/carbamates by Millipore Australia. The cotton industry is already a responsible environmental manager. Availability of the new test kits will provide them with a unique opportunity to ensure that chemical pest control is performed in a sustainable and safe manner.