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Immunoassays for Pesticide Detection

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IMMUNOASSAYS FOR PESTICIDE DETECTION

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Introduction

The occurrence of herbicides and other agricultural chemicals in groundwater continues to be a major concern in Iowa and across the country. Analytical capabilities have improved dramatically in the past twenty years, resulting in the ability to detect ever smaller quantities of chemicals (the vanishing zero). Detection of pesticides has been based primarily on conventional technology, such as gas or high-performance-liquid chromatographic methods. Although these methods are highly sensitive, they are also very expensive and time consuming. Immunoassay tests are alternative analytical methods that are also highly sensitive, but can be used by persons with little or no scientific background. The price of these tests also is within the range of most dealers and growers, approximately $150 for a kit that will analyze 10 to 15 samples. This paper will describe how immunoassays function and the potential areas where they might be used in agriculture.

Immunoassays are a biology-based analytical tool, rather than a mechanical test such as gas chromatography. Immunoassays are based upon the fundamental principle that antibodies produced in animals can recognize and attach to certain chemical configurations found on the surface of molecules. An antibody is a protein that is developed within an animal following the introduction of a foreign substance (antigen). Antibodies are highly specific and will bind only to the antigen, or very closely related compounds, that caused its formation. Binding of the antibody to the antigen allows the foreign substance to be removed from the animal before it can cause any damage. Antibodies commonly protect animals from disease-causing organisms or potentially toxic compounds. By combining an antibody specific to a pesticide with an appropriate indicator system, an immunoassay system can be developed that has the ability to detect concentrations of a pesticide as low as 1 ppb.

Development of pesticide immunoassay tests.

The first step in developing an immunoassay test is the production of antibodies to the desired pesticide. Small molecules such as pesticides normally are not capable of causing an immunological response, thus the pesticide is first attached to a larger protein that acts as a carrier molecule. The pesticide-carrier complex is then injected into an animal, normally rabbits, in order to cause the development of the desired antibody.

Separate antibodies specific for the carrier protein and for the pesticide will be developed. Blood is extracted from the animal and the appropriate antibody is extracted for use in the immunoassay test.

Following isolation of the desired antibody, it must be incorporated into a test that will result in a visible reaction in order to allow detection of the pesticide. Several methods may be used; the direct ELISA (enzyme-linked immunosorbent assay) test is the simplest and most commonly used method for pesticide detection. In an ELISA test, the pesticide is bound to an enzyme which catalyzes a reaction resulting in a colored product. When the sample to be tested and the pesticide/enzyme complex are placed in the presence of the antibody, the pesticide and pesticide/enzyme complex compete for binding sites on a limited number of antibodies. The intensity of color formation is dependent upon the concentration of the pesticide found in the sample. The test procedures are outlined in the attached figure.

Applications of immunoassay tests.

Potential applications of immunoassay tests in agriculture include testing for pesticide or antibiotic residues, diagnosing animal or plant diseases, and determining mycotoxin levels in grain or feed. ELISA tests have been developed for numerous herbicides, insecticides, and fungicides. A listing of these pesticides is provided in Table 1. Pesticide detection test kits that are currently available are targeted primarily for persons involved in research functions; however, it is likely that in the near future they will be marketed for the general public. The availability of these kits could allow dealers and growers to test fields for herbicide residues, therefore providing a more accurate estimate for potential carryover injury. The tests also could be used to analyze drinking water for the presence of agricultural chemicals.

Limitations of pesticide immunoassay tests.

Although immunoassay tests offer several advantages over traditional analytical tests, they are not without limitations. Most immunoassay test kits for pesticide detection are better suited for giving a yes or no answer on whether the pesticide is present, rather than determining the exact concentration in the sample. When used in combination with a colorimeter to determine small changes in color, they can provide fairly accurate estimates of actual concentrations.

Although immunoassay tests could offer growers a relatively inexpensive means of testing for the potential of herbicide carryover, there are problems that may limit this type use. Most herbicides bind tightly to soil colloids, and this can lead to problems in analyzing soils for herbicide residues. In order to get an accurate estimate of the quantity of chemical in the soil,

fairly rigorous extraction procedures must be used to remove the herbicide from the soil binding sites. These procedures normally require the use of strong solvents that are not readily available to the public, and also would pose a disposal problem for people using them. Because of this, immunoassay tests are better suited for testing water samples, rather than soil samples, for the presence of pesticides.

Conclusions

The immunoassay test offers a method to quickly and accurately determine the presence of a compound of interest. Tests have been developed for several uses in agriculture. Users of these tests must keep in mind that, although immunoassay tests are highly accurate, they can be misused. Collecting a representative sample is critical. Also, since the tests are used to detect very low concentrations of chemicals, it is critical to use sampling procedures that minimize the potential for contamination of the sample. Finally, proper storage of the kits is critical in order to maintain their accuracy.

Table 1. Immunoassays for Pesticide Residues

Herbicides

- Alachlor (Lasso)
- Atrazine
- Chlorosulfuron (Glean)
- Clomazone (Command)*
- Cyanazine (Bladex)
- 2,4-D
- 2,4,5-T
- Diclofop-methyl
- Imazaquin (Scepter)*
- Metolachlor (Dual)
- Paraquat
- Pichloram (Tordon)
- Simazine (Princep)
- Triclopyr (Garlon)

Insecticides

- Alicarb (Temik)
- Aldrin
- Carbofuron (Furadan)
- Chlordane
- Dieldrin
- Endosulfan
- Endrin
- Heptachlor
- Parathion

Fungicides

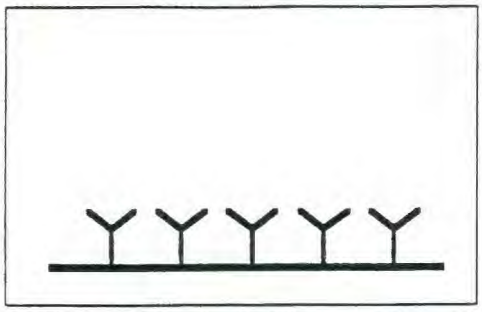
- Benomyl (Benlate)
- Metalaxyl (Ridomil, Apron)

*Under development

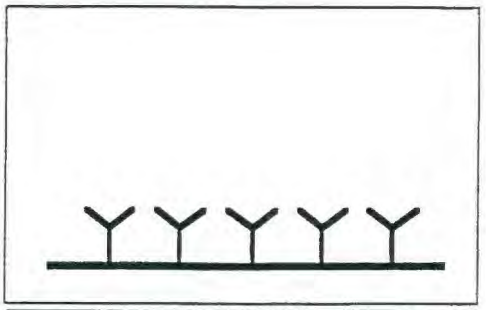
IMMUNOASSAY PESTICIDE DETECTION KITS

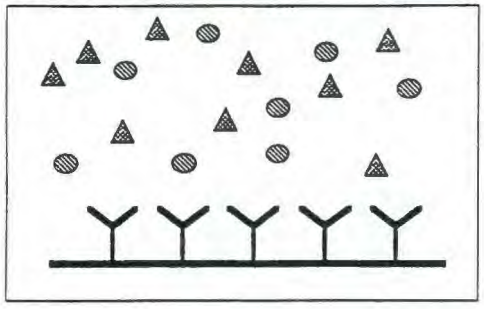
Sample containing pesticide residue

Sample with no pesticide residue

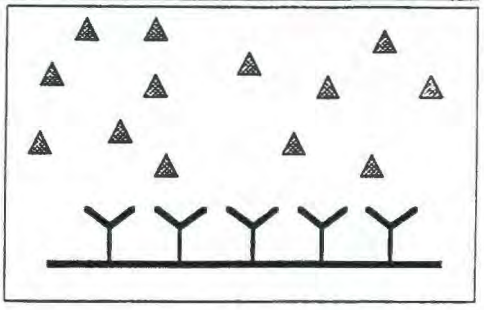


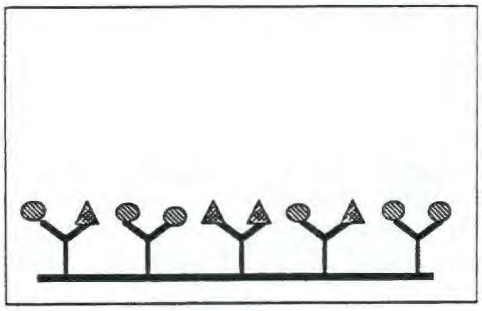
1. Antibodies are attached to plastic plate or walls of a test tube.



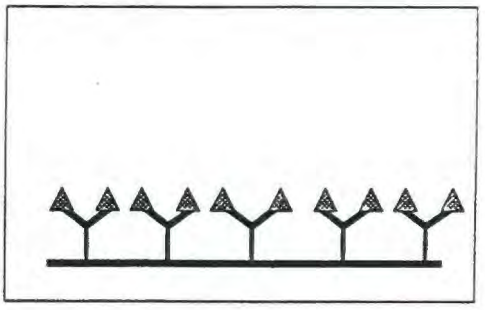


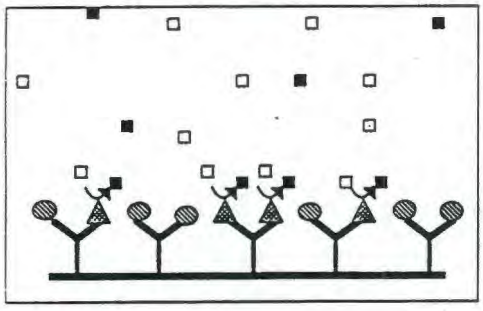
2. Sample and pesticide/ enzyme complex are placed in test tube. The pesticide and pesticide/enzyme complex compete for binding sites on antibodies.





3. Test tube is rinsed with water, leaving only those molecules that are bound to the antibodies.





4. Substrate for enzyme is added to the test tube. The bound pesticide/enzyme complex reacts with the substrate and produces a colored product. A dark color indicates low pesticide concentration in the sample.