Estimation of Imazethapyr in Agricultural Water by Its Cross-Reactivity with either Imazaquin or Imazapyr ELISA Kits

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The cross-reactivity expressed by commercially available ELISA kits developed for two imidazolinone herbicides can be used for estimating the concentration of another compound within the chemical class in surface water and groundwater collected from agricultural fields. Imazaquin and imazapyr kits are used for estimating imazethapyr. Results on authentic samples indicate that dissolved constituents in the matrix can produce false positives with the imazaquin kit. Spiked HPLC water showed a pH dependence of the dose−response curve for imazethapyr when using the imazaquin kit. Practical concentration ranges for estimating imazethapyr are 1−125 μg/L with the imazapyr kit and 8−800 μg/L for the imazaquin kit.

Keywords: *Imazethapyr; imazaquin; imazapyr; residue estimation; ELISA; agricultural water*

The Management Systems Evaluation Area (MSEA) Program, a federal-interagency, state, academia, cooperative study of the impact of agricultural practices on water quality, was implemented in five midwestern states in 1991. To meet the needs of water quality monitoring, analytical method development for residues of both current and newly introduced herbicides was begun. Among the newest chemistry developed for weed control primarily in soybean and other leguminous crops is the imidazolinone family of herbicides (Shaner and O'Connor, 1991). Although procedures for soil, plant material, and tissue are published, rapid and inexpensive analytical methods for determining residues of most imidazolinone herbicides in water by conventional approaches are not readily available. Published methods have evolved from very tedious and laborious procedures of solvent extraction with derivitization GC, to very specialized capabilities in immunochemistry (Wong and Ahmed, 1992; Anis et al., 1993), to very rapid determinations utilizing liquid chromatography/mass spectrometry (Stout et al., 1996).

Environmental quality endpoints become important considerations in evaluating the acceptability of these products. The impacts of herbicide usage on both surface water and groundwater quality, both on-site and off, must be evaluated and the endpoints monitored to ensure that no preventable degradation of water resources occurs. In this paper we report our evaluation of an imazaquin ELISA and an imazapyr ELISA for screening agricultural water samples for residues of imazethapyr. Surface water and groundwater samples from a corn field receiving a single application of Pursuit are analyzed.

MATERIALS AND METHODS

ELISA. Both the Imazaquin 5.0 kit (Agri-Diagnostics, Moorestown, NJ) and the EnviroGard Imazapyr plate kit (Millipore Corp., Bedford, MA) are competitive binding immunoassay systems employing the multiple-well microtiter plate format. Each is designed to detect imidazolinones in environmental samples; both the imazaquin kit and the imazapyr kit are capable of determinations in water at 10 μg/L and in soil extracts at bioactive levels. For each, blanks, negative controls, and sets of standards were run simultaneously in duplicate according to each manufacturer's recommended procedure. The imazapyr kit expresses no cross-reactivity for imazaquin.

Water Samples. pH was determined with an Accumet model 915 pH meter equipped with a combination electrode (Fisher Scientific, Chicago, IL). Analyses were carried out with each kit on 24 authentic surface water and groundwater samples collected from actively farmed fields in southwestern Iowa. Samples were unfiltered and analyzed without concentration.

Analytical Standards. Standard solutions for imazethapyr were prepared from crystalline reference material provided by American Cyanamid. Calibration curves for imazapyr and imazaquin were developed from standards included with each kit.

RESULTS AND DISCUSSION

The very close structural similarities among imazapyr, imazaquin, and imazethapyr are shown in Figure 1. It would be expected that similar immune responses would occur, assuming that antibodies were developed from haptens in which the pyridylic acid moiety had been homologated.

A typical calibration plot (curve 1) for the imazaquin kit is shown in Figure 2a. At 650 nm with the absorbance of the negative control >2.0, absorbances measured for the series of standards ranging between 2.5 and 80 μg/L fall between 1.7 and 0.7 au, with a 50% B/B_0 of 12 μg/L. Curve 2 shows the dose−response cross-reactivity for imazethapyr using this kit. It shows good linearity over the concentration range of 8−800 μg/L with a 7 min optimum time for color development and with a 50% B/B_0 of 70 μg/L. Curves 3 and 4 illustrate the influence of elapsed time of color development on absorbance values for imazethapyr. Both also show good correlations, but smaller changes in absorbance per dose unit produce less precise determinations

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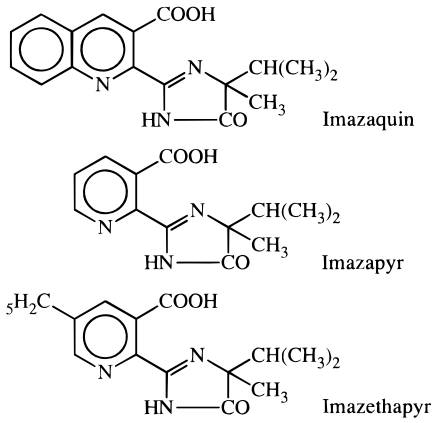

Figure 1. Structures of imazaquin, imazapyr, and imazethapyr.

over the entire range. This effect is typical of color overdevelopment.

Calibration of the imazapyr kit is shown in Figure 2b. Curve 5 illustrates the calibration plot obtained over the concentration range of 0−50 μg/L for imazapyr, with a 50% B/B_0 of 7 μg/L. With the photometer set at 650 nm, the maximum absorbance is 0.86 au. Prior to the addition of chromogen, this kit recommends a tap water wash of the microtiter cell, whereas the imazaquin kit calls for a cell wash using a dilute detergent solution. Substituting the detergent wash for the tap water wash improved the correlation over the concentration range by reducing the relative error and range of absorbance (curve 6). Attempts to increase absorbance values by addition of stop solution and measurement at 405 nm did not substantially increase the range of absorbance values. Similarly, extending color development time prior to the addition of stop solution had only a small influence on the measurement of maximum absorbance. The response of imazethapyr to this kit is illustrated in curve 7. Absorbance values >1.1 are achieved only after 50 min of color development. For imazethapyr estimation this indicates a narrower usable range, 1−125 μg/L, than that observed for the imazaquin kit, with a 50% B/B_0 for imazethapyr of 11 μg/L (Figure 2a).

The recomended statistical quality control test for parallelism (Robard, 1974) helped to establish that imazethapyr shows the same (or similar) dose−response calibration over a specified concentration range as imazaquin and imazapyr. Figure 3 shows the parallelism of response for imazethapyr in the imazaquin kit to be between 0.8 and 0.2 B/B_0. Similarly, over the narrower range of B/B_0 values between 0.95 and 0.65 the imazethapyr demonstrates parallelism with the imazapyr kit. The negative offset of the *y*-intercept for imazethapyr may be related to the absolute error associated with the range of absorbance values.

Agricultural runoff samples were collected in 1994 and 1995 from a southwestern Iowa corn field. The initial application of Pursuit on this field was in 1995, so that the 1994 samples served as a matrix blank. They were analyzed in parallel by both the imazapyr and imazaquin kits. False-positive results obtained for several of the 1994 samples suggest the matrix affected the competitive binding chemistry of the imazaquin kit. Conversely, the imazapyr kit did not furnish any false positives. Furthermore, neither kit produced detections for imazethapyr in any of the 1995 samples. Whereas

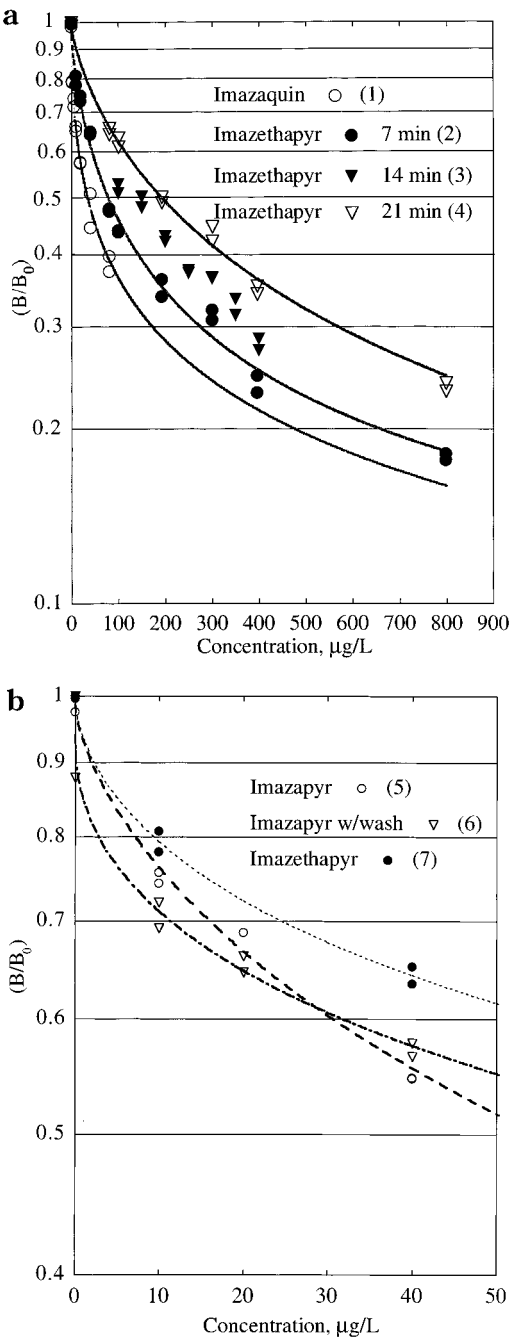

Figure 2. (a) Calibration plot showing imazaquin kit standard curve and imazethapyr cross-reactivity at 7, 14, and 21 min color development time intervals. (b) Calibration plot showing imazapyr kit standard curve (with/without detergent wash) and imazethapyr cross-reactivity with detergent wash prior to color development.

the standard blank and control were both at pH 7.0, the 1994 samples ranged between pH 8.0 and 8.8. This matrix effect in water samples and its influence on dose−response curves is known in general as a possible source of error in agrochemical immunoassays (Krotzky and Zeeh, 1995) and has been reported for both *sym*-triazine herbicides (Gascón et al., 1995) and organophosphate insecticides (Oubiña et al., 1996). The 1995 samples measured pH 7.6 and furnished no imazethapyr detects.

Calibration curves for imazethapyr were prepared from groundwater seepage spiked at 10 and 20 μg/L and

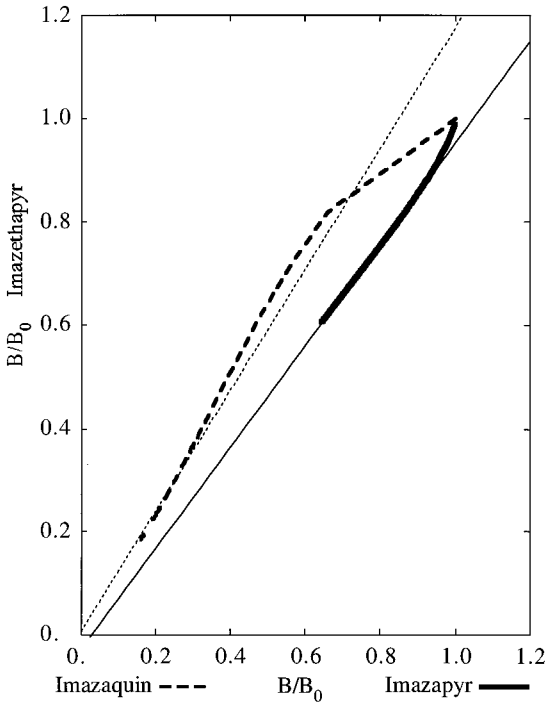

Figure 3. Results of parallelism test for imazethapyr against dose−response curves for both the imazaquin and imazapyr kits.

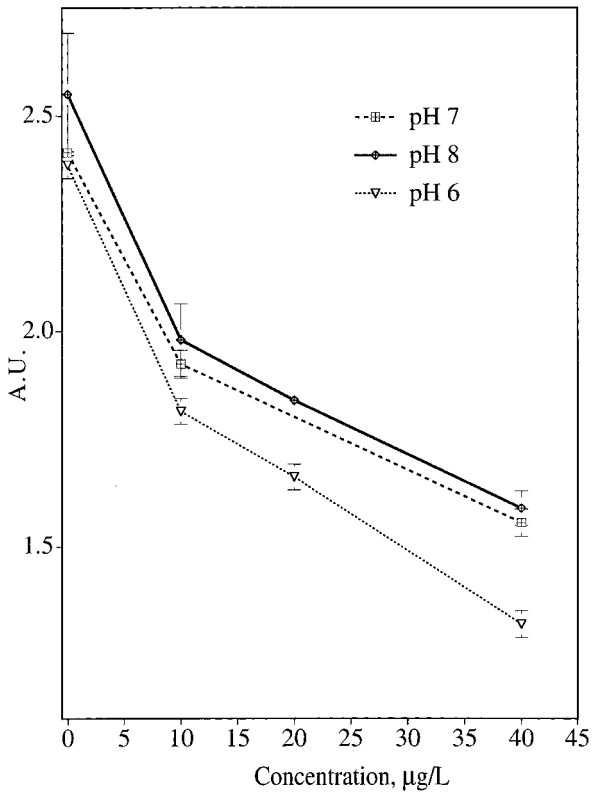

Figure 4. Influence of water sample pH on imazethapyr cross-reactivity with the imazaquin kit.

from surface runoff spiked at 10 μg/L. All samples and standards analyzed at pH 7.6 fell within the theoretical recovery ± the standard error. In contrast, authentic water samples at ambient pH of 7.16 furnished less than theoretical recoveries, indicating that imazethapyr competition with the imazaquin−enzyme conjugate for antibody sites diminishes as pH decreases.

To further investigate the effect of sample matrix upon the imazethapyr response, HPLC water was fortified with imazethapyr at 0, 10, 20, and 40 μg/L and adjusted to pH 6, 7, and 8 with dilute HCl or NaOH as needed. Figure 4 shows the effect of pH on the imazethapyr response. There is a slight difference between responses observed over the concentration range between pH 7 and 8. However, between pH 6 and 7, the imazethapyr response significantly decreases with increasing acidity, indicating that in laboratory-purified water, and probably in agricultural water samples, pH alone will influence the results of imazethapyr estimation using the imazaquin kit. At pH 6 standard deviation between replicates was very small.

The dissociation constant curve from potentiometric titration of dilute imazethapyr solution reveals three inflection points, only two of which are important from an environmental occurrence perspective. At pK_a = 2.1, the imino nitrogen of the imidazolinone ring is 50% protonated, and at pK_a = 3.9, the carboxylic acid group is 50% ionized. Thus, at soil pH of 6.0, 6.5, and 7.0, imazethapyr is 0.125, 0.0398, and 0.0125% dissociated, respectively. Therefore, at typical pH of root-zone pore water from agricultural soils, imazethapyr occurs dominantly as a zwitterion carrying no net electrical charge.

CONCLUSION

Imazethapyr concentration in agricultural water can be estimated by exploiting its cross-reactivity with the imazaquin kit over a wide range of concentrations, but it is sensitive to sample pH. The imazapyr kit is applicable over a narrower concentration range but is not influenced by sample pH. However, due to its lower limit of detection, the imazapyr kit is better suited for screening of real-world agricultural water samples than is the imazaquin kit with its somewhat broader dynamic range. The few samples testing the upper limit of either kit are probably better handled via dilution and reanalysis. The greater selectivity of the imazapyr kit also enhances its robustness for environmental monitoring.

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