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# Polyclonal Antibody-Based ELISA for Triasulfuron

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Sulfonylurea herbicides (Levitt 1978) such as triasulfuron (Logran®), are widely used at very low application rates of 3-40 g/ha to control both broad-leaved and grass weeds in cereal crops. Under certain conditions, e.g. alkaline soils and low rainfall, these herbicides can persist long enough to affect the growth of subsequent crops (Beyer et al. 1987). The problem can be particularly serious when broad-leaved crops, such as rapeseed, sunflower, grain legumes or field peas, are sown in rotation with cereals (O'Sullivan 1982; Brewster and Appleby 1983; Foy and Mersie 1984; Peterson and Arnold 1985). This is a major factor limiting the use of high value rotation crops in many areas of the cereal belt in Australia. Growth of a sensitive crop can be severely reduced by triasulfuron levels as low as 100 pg/g in soil. These concentrations are below, or at the very limits of detection of the HPLC and bioassay methods currently being used (Zahnow 1982; Iwanzik and Egli 1989).

Enzyme immunoassays have been described for a number of herbicides and pesticides in recent years (Kelley et al. 1985; Jug et al. 1989; Schlaeppi et al. 1989; van Emon et al. 1989; Hall et al. 1990; Schlaeppi et al. 1992) and provide a versatile and sensitive alternative to traditional methods. Here we describe a sensitive, easy to operate, and inexpensive competitive inhibition ELISA for reliable detection of triasulfuron in soils.

## MATERIALS AND METHODS

The triasulfuron (mol. wt. 401 daltons) analytical standard and aminosulfonylurea analog used for

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immunisation were gifts from Ciba Geigy, Australia. Anti sheep IgG conjugated to biotin and streptavidin conjugated to alkaline phosphatase were obtained from Amersham. The phosphatase substrate, p-nitrophenyl phosphate, was obtained from Sigma. EIA plates were obtained from NUNC. Other chemicals were obtained either from E. Merck and Co. or from BDH, Australia. Sheep were obtained from and housed at the Victorian Institute of Animal Sciences, Attwood, Victoria.

Sheep were immunised i.m. with the aminotriasulfuron conjugated to bovine serum albumin (Wie and Hammock 1984). After the first injection, the immune response was boosted with the same amount of antigen, every 14 days until a good response was obtained, as detected in chequerboard ELISA. The polyclonal antiserum with the best sensitivity was then used for subsequent experiments. The polyclonal antiserum was specific for triasulfuron as the cross reactivity with related compounds (e.g. chlorsulfuron) was ≤10% of reactivity towards triasulfuron.

Two soil samples, gifts from Ms Andelys Jolley, Dept. of Agriculture, were used in this study. Dooen is a medium clay soil with a pH of 8.4 from the Wimmera Research Station, Victoria, while Rutherglen is a clay to sandy loam soil from the Rutherglen Research Institute, Victoria and has a pH of 5.8. Soils were air dried and ground to a fine powder. This powder was mixed 1:5 (w/v) with 0.2M ammonium bicarbonate buffer, pH 8.2 containing 0.01M calcium chloride as a flocculent, and physically shaken in a wrist-action shaker for 16 hr at room temperature. The supernatant obtained after centrifugation and filtration through a 0.45 µm filter, was neutralised and loaded onto an anti-triasulfuron immunoaffinity column prepared using the Pierce Reacti-Gel 6X and high titre sheep anti triasulfuron antibody (Ghildyal and Kariofillis, manuscript in preparation). Briefly, 20 mL of soil extract was loaded onto the 0.5 mL affinity gel. After washing thoroughly with phosphate buffered saline (50mM PBS, 50mM phosphate, pH 7.2, containing 0.15M sodium chloride), the bound herbicide was eluted in 2 mL of methanol. The eluate was dried, reconstituted with 2 mL PBS and used directly in the ELISA.

The EIA plates were coated with m-carboxysulfonylurea-ovalbumin conjugate (conjugate prepared by K. Ooi) in 0.1M sodium bicarbonate buffer, pH 9.6, at 10 ng/well overnight, at 4$^{0}$C. The wells were blocked with 3% ovalbumin in PBS-T (0.1M phosphate, 0.15M sodium chloride, pH 7.2 containing 0.05% Tween 20) for 3 hr at room temperature. Various dilutions of the standard (or the reconstituted sample) solution in 100 µL of 50mM PBS

were incubated with the sheep anti triasulfuron polyclonal antiserum for 30 min at room temperature and then added to the coated wells of the ELISA plate for 40 min at room temperature. All free antibody and that bound to free triasulfuron was washed off with PBS-T using an automatic microplate washer (Bio-Rad). The plate was incubated with goat anti sheep antibody conjugated to biotin for 90 min, followed by incubation with streptavidin conjugated to alkaline phosphatase for 45 min. Bound enzyme was visualised by addition of p-nitrophenyl phosphate (1 mg/mL substrate in 0.1M glycine-NaOH, pH 10, containing 1mM $MgCl_2$ and 1mM $ZnCl_2$). The reaction was stopped by adding 50 µL of 5M sodium hydroxide. The resultant colour was estimated at 405 nm using a Titertek Multiscan ELISA reader.

## RESULTS AND DISCUSSION

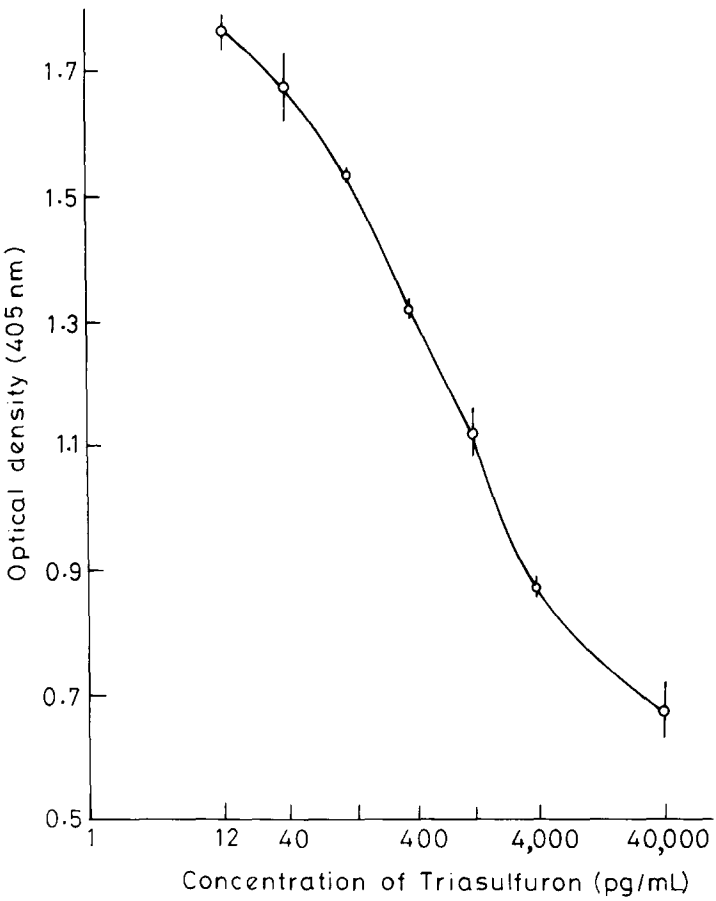
A standard curve was obtained using dilutions of the triasulfuron standard ranging from 40 ng to 4 pg in 50mM PBS in the ELISA detailed above. Fig. 1 depicts the curve obtained when absorbence values are plotted against pg triasulfuron per mL.

The curve is linear down to 40 pg, and reaches a plateau below 10 pg (data not shown). Thus, this curve can be used to reliably detect triasulfuron concentrations in solution as low as 40 pg/mL. As the final reconstituted sample is twice as concentrated as the aqueous soil extract, this implies that triasulfuron levels in soil as low as 20 pg/g can be detected with this standard curve given, no interference in the assay by soil coextractants.

Soil samples free of triasulfuron (soils from untreated fields, and tested by HPLC) were analysed by the above ELISA. Various amounts of standard triasulfuron was added to the soil in the initial ammonium bicarbonate buffer. The dried and reconstituted eluate from the affinity column was used directly as sample in the ELISA. The results obtained are given in Table I.

Higher clay content in the soil seems to lead to higher variation in the results. But, all results are within ±20% of expected herbicide values. The results are especially accurate at low levels of herbicide. Higher spike levels were not analysed as at those levels, variation of >20% in results will not have any pronounced effect on deciding the subsequent crops to be sown and also, the present techniques are quite capable of handling those levels.

Figure 1




ELISA standard curve.
Ovalbumin-carboxytriasulfuron conjugates were coated onto microtiter plates (10 ng/well). Sheep antiserum (1:51,000 diluted in PBS-T) was preincubated with various amounts of standard triasulfuron for 30 min and then allowed to react with the coated analog for 40 min. The amount of bound antibody was determined using an anti sheep IgG streptavidin biotin system conjugated to alkaline phosphatase as described in the text.

Triasulfuron is a widely used herbicide, effective at very low levels (application rate 10-40 g a.i./ha). Until now, the preferred method for estimating it has been HPLC. There are various drawbacks to this. It is time consuming; the farmer can expect to hear the result in a few weeks. It is not very accurate. It cannot reliably detect amounts below 200 pg/g. In contrast to HPLC, our

**TABLE I** Analysis of soils using competitive inhibition ELISA

| Soil | Spike level (pg/g) | Recovery (pg/g) | % Recovery* |
|---|---|---|---|
| Dooen | 0 | 35† | |
| | 100 | 144 | 108 |
| | 200 | 255 | 110 |
| | 317 | 411 | 118 |
| | 635 | 569 | 84 |
| Rutherglen | 0 | 80† | |
| | 100 | 183 | 103 |
| | 200 | 279 | 99 |
| | 317 | 400 | 101 |
| | 634 | 717 | 100 |

Soil samples were negative for triasulfuron as tested by HPLC. The readings reported are means of at least six different experiments.

* % recovery is calculated as: (recovery from spiked soil - value for unspiked soil)/(spike level) x 100.

† these false positive values are probably due to interference by coextractants.

ELISA is short; from dried soil sample to ELISA result takes 3 days. It is rugged and requires minimal training and equipment to operate. Our assay can potentially detect levels of the herbicide in soil as low as 20 pg/g. We could reliably detect 100 pg/g triasulfuron with the ELISA in soils studied. Thus it is an extremely promising method for easy, quick and reliable monitoring of triasulfuron levels in soil or water. Previously, an ELISA had been described by Schlaeppi et al. (1992) for detection of triasulfuron. This is monoclonal antibody based and the cleanup procedure involves handling of volatile solvents, and it is not as sensitive. As such, it is not suitable for field applications. The assay we have described here, on the other hand, is based on a polyclonal antibody, is more sensitive and reliable at low herbicide levels and the cleanup procedure does not involve handling of any organic solvents except methanol. Thus, at the moment, it is the simplest and most sensitive available, and holds promise for future use in the field, although a more extensive validation is required, using a wide variety of soils.

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**REFERENCES**

Beyer E.M., Brown H.M., Duffy, M.J. (1987) Sulfonylurea herbicide soil relations. Brit. Crop Prot. Conf.-Weeds. 6-5:531-540.

Brewster, B.D., Appleby, A.P. (1983) Response of wheat (Triticum aestivium) and rotation crops to chlorsulfuron. Weed Science 31:861-865.

Foy, C.L., Mersie, W. (1984). Chlorsulfuron selectivity and persistence in small grains. Proc. of the Southern Weed Sci. Soc. 37:108

Hall, J.C., Deschamps, J.A., McDermott, M.K. (1990) Immunoassays to detect and quantitate herbicides in the environment. Weed Technol. 4:226-234.

Iwanzik, W., Egli, H. (1989). Comparison of bioassay and chemical analysis for triasulfuron quantification in soil samples. Brighton Crop Prot. Conf. Weeds. 9A-6:1145-1150.

Jug, F., Meyer, H.H.D., Hamm, R.T. (1989). Development of sensitive enzyme linked immunosorbent assay for the fungicide fenpropimorph. J. Agric. Food Chem. 37:1183-1187.

Kelley, M.M., Zahnow, E.W., Peterson, W.C. and Tou, S.T. (1985). Chlorsulfuron determination in soil extracts by enzyme immunoassay. J. Agric. Chem. 33:962-965.

Levitt G. 1978 US Patent 4 127 405.

O'Sullivan, P.A. (1982). Response of various broadleaved weeds and tolerance of cereals to soil and foliar applications of DPX-4189. Can. J. Plant Sci. 62:715-724.

Peterson, M.A., Arnold, W.E. (1985). Response of rotational crops to soil residues of chlorsulfuron. Weed Sci., 34:131-136.

Schlaeppi, J-M, Fory, W., Ramsteiner, K. (1989). Hydroxy atrazine and atrazine determination in soil and water by enzyme linked immunosorbent assay using specific monoclonal antibodies. J. Agric. Food Chem. 37:1532-1538.

Schlaeppi, J-M, Meyer, W., Ramsteiner, K.A. (1992). Determination of triasulfuron in soil by monoclonal antibody based enzyme immunoassay. J. Agric. Food Chem. 40:1093-1098.

van Emon, J.M., Seiber, J.N., Hammock, B.D. (1989) Immunoassay techniques for pesticide analysis. In Analytical Methods for Pesticides and Plant Growth Regulators: Advanced Analytical Techniques. Ed: Sherma,J., Academic Press, New York. 17:217-263.

Wie, S.I., Hammock B.D. (1984). Comparison of coating and immunising antigen structure on the sensitivity and specificity of immunoassays for benzoilphenylurea insecticides. J. Agric. Food. Chem. 32:1294-1301.
Zahnow, E.W. (1982). Analysis of the herbicide chlorsulfuron in soil by liquid chromatography. J. Agric. Chem. 30:854-857.