Application of Immunoassays to Studies of the Environmental Fate of Endosulfan

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A comprehensive validation of three endosulfan immunoassays (two microwell assays and a tube assay) using field samples was conducted as part of a study on the environmental fate of endosulfan applied to Australian cotton fields. The validation included an initial examination of the relationship between tube and microwell immunoassays and then correlations between immunoassay data and gas−liquid chromatography (GLC) analyses for several thousand water (in a format with a detection limit of 0.2 μg L^{-1}) and soil samples and hundreds of aerial drift samples. In all cases, the immunoassay data proved to be closely correlated with GLC analyses, indicating that these immunoassays provide a reliable quantification of endosulfan. Validation of immunoassay methods against GLC by providing reliable correlations was an important result, but in this study immunoassay also was useful in the research program for improving protocols for sampling and analysis by GLC. This was possible because of the demonstrated advantages of immunoassay for greater speed and higher sample throughput with less complicated sample preparation, which allows many more samples to be analyzed and a more comprehensive study of field processes such as rain simulation. The ability of immunoassay to provide a summation of the three toxic forms of endosulfan (α, β, and sulfate) was exploited. It is concluded that this immunoassay for endosulfan is quantitative using soil, water, and aerial drift samples and that it would allow the possibility of decision making at field sites, improving environmental management of endosulfan residues.

Keywords: *Immunoassay; endosulfan; gas chromatography; application; dissipation studies; soil; water; drift*

INTRODUCTION

Endosulfan, a mixture of α- and β-isomers, is an organochlorine pesticide registered for use on a wide range of crops to control chewing and sucking insects, such as the Colorado potato beetle, flea beetle, cabbageworm, peach tree border, aphids, and leafhopper (Goebel *et al.*, 1982; Hoechst, 1990). The most important use of endosulfan in Australia and many other countries is to control *Helicoverpa* species, especially in cotton cropping (Fitt, 1994). While endosulfan has been regarded as a relatively environmentally safe pesticide, environmentally with a short half-life and little evidence of bioaccumulation, its acute toxicity to fish (24−96 h LC_{50} = 0.1−20 g/L; Fox and Matthiessen, 1982; Goebel *et al.*, 1982; Rao and Murty, 1982; Trim, 1987; Sunderam *et al.*, 1992) and the high application rate (750 g/ha, 2−5 kg/seasonal total for cotton in Australia; Shaw, 1994) pose a potential environmental threat. Endosulfan is degraded to two major degradation products by different mechanisms. Endosulfan diol is the major product found in water and is formed either by chemical (Cotham and Bidleman, 1989; Peterson and Batley, 1993), microbial, or photolytic hydrolysis (El Zorgani and Omer, 1974; Miles and Moy, 1979). Endosulfan sulfate is found predominantly in soil and in plants as a product of biotransformation. Its formation is not a detoxification mechanism since endosulfan sulfate exhibits toxicity to fish equal to those of the isomeric parent compounds (24−96 h LC_{50} is also 0.1−20 g/L; Goebel *et al.*, 1982; Sunderam *et al.*, 1992). This metabolite is more persistent than its isomeric parent compounds and thus also poses an environmental threat (Stewart and Cairns, 1974).

Increasing concern about the impact of endosulfan on the Australian riverine system has led to major research studies in recent years on the fate of endosulfan in aquatic systems and in soil. The NSW Department of Land and Water Conservation (Australia) has conducted an extensive water quality program in the central and northwest regions of New South Wales since 1990. Seasonal river contamination by endosulfan (both isomers) and endosulfan sulfate has been reported, with the latter being the major compound detected (Cooper, 1994). Contamination of river water is closely associated with the time of endosulfan application in these regions. Studies on the fate of endosulfan in soils of cotton fields in the northern region of New South Wales involving periodic sampling throughout the year, but not close to spraying dates, have indicated approximate half-lives for α-endosulfan, β-endosulfan, and endosulfan sulfate in these types of soil of 43, 76, and 100 days, respectively (Kimber *et al.*, 1995). Thus, endosulfan is

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S0021-8561(96)00915-6 CCC: $14.00

found to disappear from soil moderately rapidly, with little carry-over from one season to the next. These data also indicate that there is no long-term accumulation of toxic endosulfan residues in soil. However, there remains a need for close observation of the levels of endosulfan and endosulfan sulfate residues in water and soil during the cotton growing seasons to ensure that contamination does not occur outside the spraying area. Monitoring for possible contamination of endosulfan in water using on-site residue analyses would be essential, especially during the spraying seasons, for better management of contaminated irrigation water. In such field conditions, immunoassays provide a portable and cost-effective method of pesticide residue detection in soil and water, allowing a rapid assessment of the water quality.

Three prototype immunoassay methods, two laboratory tests (based on microwell plate assays) and one field test (based on use of polystyrene tubes), have been produced for an extensive validation of field samples and for use as an analytical tool in research on endosulfan dissipation (Lee *et al*., 1995). The field test consists of polystyrene tubes coated with antibody specific for α-endosulfan, β-endosulfan, and endosulfan sulfate, dropper bottles containing the immunoassay reagents, and vials of endosulfan standards. The assay requires only 15 min, using two short incubation steps, and the resulting color is read by a portable photometer. Thus, residue analysis at a field site of suspected contamination is possible. Two laboratory tests with greater precision are based on the same principles as the field test but use 96-microwell ELISA plates, require 90 min to perform, and utilize a microwell reader, but have the advantage of allowing much higher sample throughput. Thorough validation of immunoassay procedures is necessary before they can be accepted as routine analytical methods by analytical chemists. Currently the use of immunoassays for environmental analyses is limited in Australia, a fact that can be attributed partly to the lack of local validation based on field samples. Thus, the performance of these endosulfan prototype laboratory and field methods has been extensively evaluated in the field conditions. This paper describes the application of the endosulfan immunoassay in the context of validating the procedure by comparison with gas chromatographic analyses performed independently (i.e., in a separate laboratory). Further accounts of the field studies of which this validation formed part will be described elsewhere.

MATERIALS AND METHODS

Immunoassays. Three endosulfan immunoassay methods (Lee *et al*., 1995) were evaluated using endosulfan field dissipation studies: two laboratory assays (denoted microwell assays 1 and 2) and a field assay (denoted tube assay). The two microwell assays differed in their coating antibodies and enzyme conjugates (Lee *et al*., 1995). Microwell assay 1 was developed by utilizing antibodies raised from a hapten derivatized from 1-hydroxychlordene (hapten II) and an enzyme conjugated to a hapten prepared from 1,3,4,5,6,7,8,8-octachloro-3a,4,7,7a-tetrahydro-2-hydroxy-4,7-methanoindane (hapten III), which was a derivative of chlordane. While the two haptens contained the same hemisuccinate linker, they were different in the total number of chlorine atoms (i.e., haptens II and III consisted of six and eight chlorine atoms, respectively) and the presence of a double bond in the five-membered ring (hapten II). Microwell assay 2 was developed by utilizing antibodies generated from a hapten derived from endosulfan diol (hapten I) and an enzyme coupled to hapten II. Chemical structures for endosulfan, its derivatives, and these haptens are shown in Figure 1.

The two assays have similar sensitivities for endosulfan, with the limit of detection at 0.2 μg/L, but differ in their specificities for endosulfan sulfate and endosulfan diol (Lee *et al*., 1995). Microwell assay 1 was 10 times less sensitive to endosulfan diol than to the parent isomers (the percentage cross-reaction for endosulfan diol was 9% as compared to endosulfan) but was comparably sensitive to endosulfan sulfate (the percentage cross-reaction for endosulfan sulfate was 90% as compared to endosulfan). In contrast, microwell assay 2 was more sensitive to the diol metabolite (18% cross-reaction in microwell assay 2) but was less sensitive to endosulfan sulfate (65% cross-reaction in microwell assay 1). The tube assay was microwell assay 2 converted into a field assay format, containing the same reagents. The detection limit of this assay is also 0.2 μg/L. The specificity of the tube version is somewhat different from that of microwell 2, proving to be twice as sensitive for the β-isomer than for the α-isomer and 4−5 times less sensitive for the sulfate and diol metabolites.

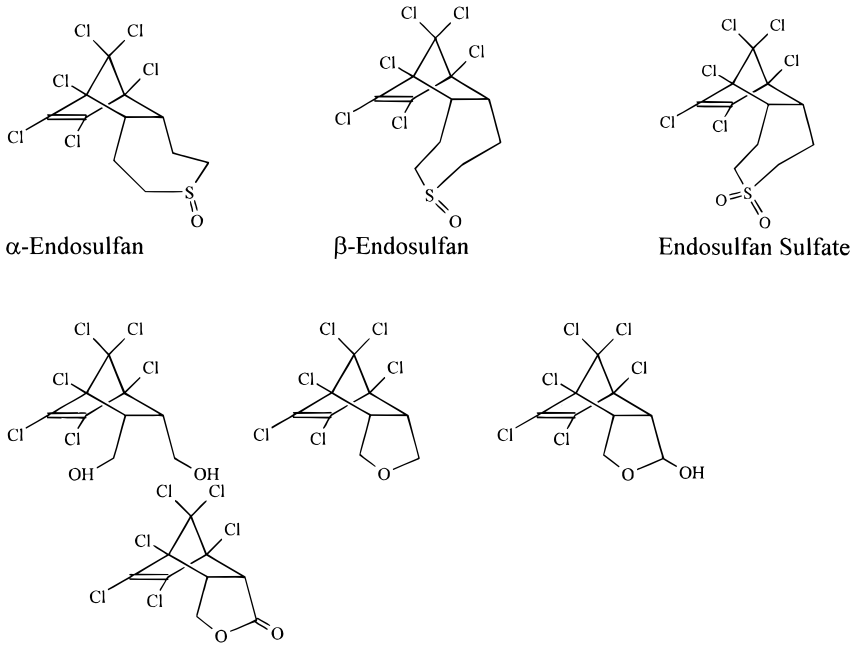
Water samples were analyzed by three assays: two microwell assays and the tube assay. Analyses were performed on the day of collection using the tube assay in a field laboratory and 2−5 days later using microwell assays 1 and 2 in a central laboratory. Samples were stored frozen between the two separate analyses, and they were brought to room temperature and mixed thoroughly before analysis. The degradation of endosulfan residues in soil was expected to be slower than in water (Peterson and Batley, 1993; Kimber *et al*., 1995), which allowed a transfer of soil samples to the central laboratory before analysis. Soil samples were analyzed by microwell assay no more than 1−2 weeks after field collection, following transport and storage at −20 °C until extracted. Water samples were analyzed directly (i.e., without sample preparation); soil samples were extracted with 90% methanol, and then the methanol extract was diluted 1/100 in water for immunoassays. Immunoassays were performed as described in Lee *et al*. (1995).

Gas−Liquid Chromatography (GLC) Analyses of Endosulfan Residues in Water. As soon as possible after collection with storage at 1−2 °C, water samples (500 mL) were filtered through a glass fiber filter paper (0.7 μm pore size) to separate water and sediment. The water portion was extracted with dichloromethane and hexane separately, and the sediment portion was extracted twice with boiling dichloromethane and acetone (1:1). The analyses were conducted using gas chromatography with mass spectrometry or with nitrogen−phosphorus or flame photometric detectors, using a 30 m DB-1 capillary column. This method provides recoveries of endosulfan >85%.

GLC Analyses of Endosulfan Residues in Soil. Soil (25 g) from samples stored at −20 °C was weighed into a 250 mL conical flask with ground-glass stopper and 150 mL of acetone/dichloromethane (3:1, v/v) was added (Kimber *et al*., 1995). The flask was shaken at 165 rpm overnight, and then the solvent was filtered through paper containing 2 g of anhydrous sodium sulfate. The filtrate (75 mL) was concentrated to 5 mL using a Kuderna-Danish apparatus and then chromatographed on neutral alumina (7 g), containing 7% (w/w) water. The column was eluted using 60 mL of hexane and then 60 mL of acetone/hexane (1:3). The first 10 mL of eluate was discarded and the remainder concentrated to 10 mL. Analysis was performed by GLC using split columns and electron capture detection with surrogate standards to correct residue analyses. This method provides >80% recovery of endosulfan and endosulfan sulfate (Kimber and Kennedy, unpublished data).

GLC Analyses of Endosulfan Drift. Filter paper strips on aluminum tables elevated 1 m above ground level were used to monitor application rates and drift of endosulfan applied in either ultralow volume (ULV, 250 g/L) or emulsifiable concentrate (EC, 350 g/L) formulation. Paper strips were rolled and placed in aluminum foil sealed glass jars as soon as possible after spraying and taken to −20 °C. Paper strips were extracted in the jars with nanograde acetone and diluted in water for immunoassay. Samples for GLC analysis were redissolved in hexane using a Turbo Vap concentrator (Zymark).

1. Endosulfan and Its Metabolites



2. Endosulfan Haptens

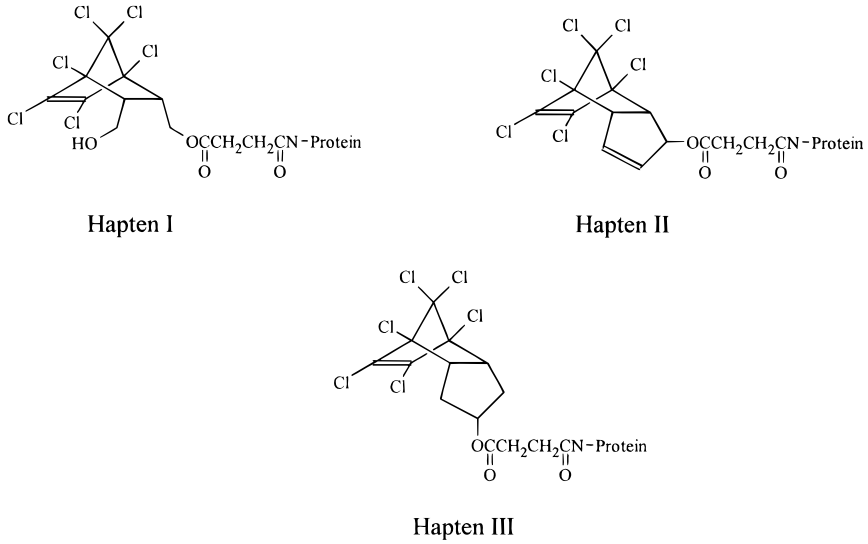


Figure 1. Molecular structures of α- and *β*-endosulfan isomers, their metabolites, and the three haptens used in antibody development.

Sampling of Endosulfan in Runoff Water. Samples of runoff water were taken from plots where rain was applied using a rainfall simulator, during a study of erosion and pesticide runoff. Rain was applied for 40 min at approximately 75 mm/h, with energy and drop sizes similar to natural, intense rainfall. The study was conducted on 1 m cotton furrows at Warren, NSW, in November 1994 when the cotton plants were small (<5% cover). The field plots allowed examination of the effect of wheat stubble cover (resulting in differing rates of soil erosion and pesticide load in runoff), times since pesticide application, and different application methods (ULV overall, EC 50% banded). Endosulfan was applied as ULV at 2.85 L/ha (680 g/ha active) or EC at 47.5 L/ha (166 g/ha active), 0.7−6 days before rain was applied. During rain, samples of runoff water (200 mL) from the plots were taken each 1−2 min for immunoassay. Samples (500 mL) also were taken less frequently for GLC analysis. The runoff samples contained 2−35 g/L of sediment, depending on the plant cover on the soil surface. Samples of irrigation water (used as the "rainwater" in the simulator), containing little sediment, also were analyzed by both methods. All samples were placed in insulated boxes with ice immediately after collection. Immunoassay samples were taken to a nearby laboratory and analyzed within 6 h of sampling. Samples for GLC were transferred to the laboratory by air courier (at 4 °C) and extracted for GLC within 36 h of sampling. This provided 44 pairs of immunoassay and GLC samples for assay validation (39 runoff, 5 irrigation).

Sampling Design for Dissipation of Endosulfan in Soil. This joint study was conducted to establish dissipation rates in the field. Field 21 on the Auscott farm (Narrabri, NSW, Australia) was previously used for growing cotton in the

1990−1991 and 1991−1992 seasons. The field was used for wheat cropping in the winter of 1992 and lay fallow during the 1992−1993 cotton season, with new beds being formed in 1993. Endosulfan was applied only in the 1991−1992 season at an operation rate of 3.0 L/ha of ULV (720−750 g/ha of α- and β-endosulfan in 7:3 isomer ratio) and 2.1 L/ha of EC (735 g/ha of α- and β-endosulfan in 7:3 isomer ratio); thus, no endosulfan had been sprayed on the field for almost 2 years. Field 21 on the Auscott property at Narrabri was marked into 90 square strata of approximately 80−90 m sides (Kennedy *et al.*, 1994). A subsampling design for the strata was selected to allow representative values and spatial variation to be assessed statistically. Twenty soil cores of 5 cm depth were collected at regular intervals on a V-intersection of the selected strata. These were bulked and vigorously mixed, and a subsample was taken in a glass jar with an aluminum foil lined plastic cap and stored at −20 °C until analysis. Three kinds of composite samples were collected: the top soil of beds adjacent to cotton plants, from the edges of irrigation furrows, (where soil is more prone to erosion), and from the base of furrows (where eroded soil may accumulate). Soil samples were collected from strata on field 21 11 days prior to the first spray of endosulfan to determine the carry-over of endosulfan residues from the previous years. The endosulfan residues in field 21 were very low and did not exceed 0.044 mg/kg, averaged over the top 5 cm of soil. Three aerial sprays of endosulfan (Thiodan ULV) at 3 L/ha were performed on three occasions approximately 10 days apart (December 13, 1993; December 23, 1993; and January 4, 1994). The aerial sprays were carried out in the very early morning (between 12:00 and 4:00 a.m.) partly to avoid the immediate volatilization caused by high day temperatures. A field site on field 4 on Auscott, Warren, northern NSW, also was subjected to soil sampling in 1994−1995, using a stratified design of 18 blocks. Similar background endosulfan residue also was found here.

RESULTS AND DISCUSSION

Relationship between Data Obtained Using Tube Assay and Microwell Assays. The water samples were analyzed by tube immunoassay on the day of collection and reanalyzed by microwell assays 2−5 days after the collection. The two microwell assays, utilizing heterologous systems (differing in their coating antibodies and enzyme conjugates), showed different specificities for endosulfan and endosulfan degradation products (Lee *et al.*, 1995). However, a comparison of data for 82 water samples by immunoassays revealed that they were closely related. Regression equations determined for two immunoassays are shown in Figure 2. The regression indicated that the analysis of the tube assay was slightly better correlated with the analysis of microwell assay 2 than with the analysis of microwell assay 1. This result was expected since the same antibody was utilized in the tube assay and microwell assay 2. A comparison of the two microwell assays also indicated that the data were correlated well with each other. The regression equation of the best fit line was obtained on log−log scale since the data spread out over the wide range and was log (microwell assay 2, μg/L) = 0.87 × log (microwell assay 1, μg/L) − log 0.24 (μg/L) (n = 92, r = 0.90, P < 0.001).

Soil samples were analyzed using microwell assays only. Analyses of the endosulfan residues (total of endosulfan and endosulfan sulfate) by the two microwell assays varied with sample collection. The analyses were closely related at early stages of sampling, but differences became greater at later stages, as endosulfan isomers declined and endosulfan sulfate in the soil increased. The regression equation determined from data for 104 soil samples (collected 1−3 days after the first application of endosulfan, in 1993−1994 season)

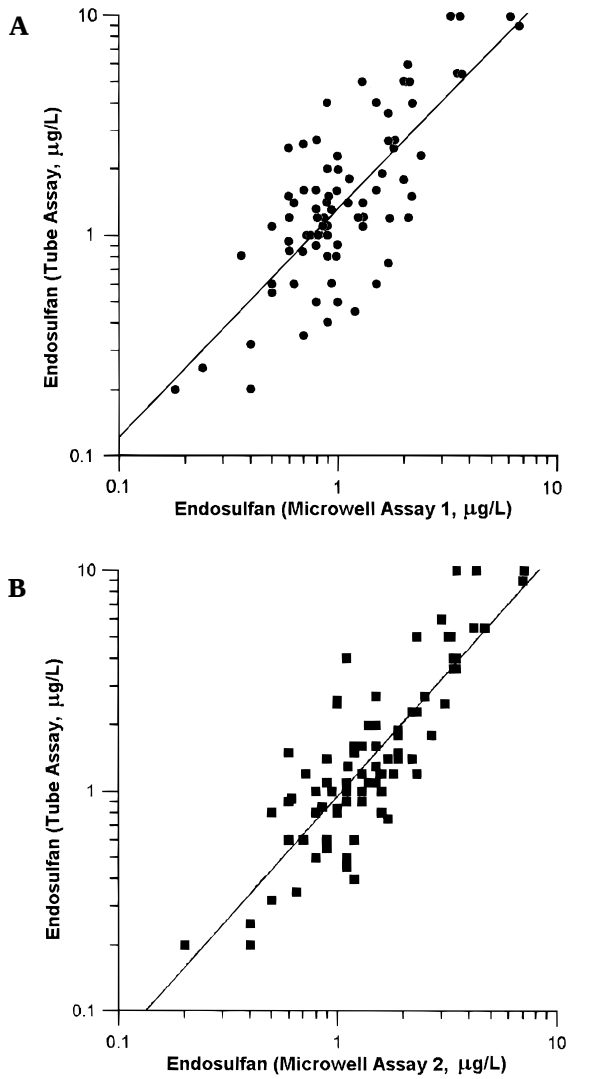


Figure 2. Relationship between the tube assay and (A) microwell assay 1 and (B) microwell assay 2 for endosulfan residues in water samples collected from trays (for volatilization experiment). The regression equations are as follows: log (tube assay, μg/L) = 1.05 × log (microwell assay 1, μg/L) + log 0.28 (μg/L) (n = 82, r = 0.77, P < 0.001) and log (tube assay, μg/L) = 1.11 × log (microwell assay 2, μg/L) − log 0.05 (μg/L) (n = 82, r = 0.84, P < 0.001).

showed greater estimations with microwell assay 1 (Figure 3). The difference can be explained by the different specificities of the two assays for α-endosulfan, β-endosulfan, and endosulfan sulfate and changes in the relative proportions of the two isomers and the sulfate. The lower estimation of endosulfan residues by microwell assay 2 would be expected as it detects endosulfan sulfate with less sensitivity (65% cross-reaction) than the parent isomers (90% cross-reaction; Lee *et al.*, 1995).

The concurrent detection of three compounds for analyzing the total burden of toxic residues is a particular feature of these immunoassays. Quantitative analysis of mixed analyte samples depends both on the specificity of the immunoassay and on the concentration of individual compounds in the sample. As can be seen in these results, analytical data can differ between two immunoassays for the same samples when they contain different relative concentrations of the analytes. This may result in either overestimation or underestimation of the actual residues in the soil. Thus, special consideration is required to select an appropriate immunoassay from the antibodies available. Extensive validation

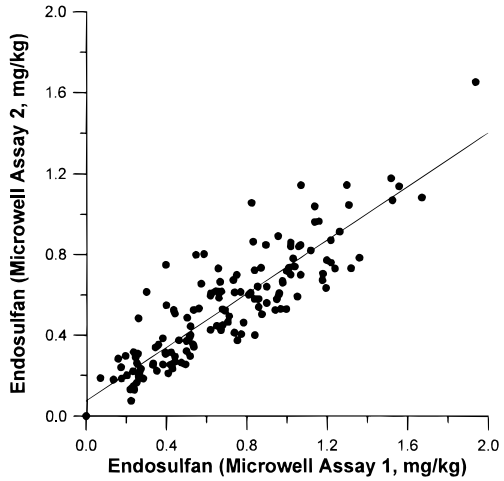


Figure 3. Relationship between data obtained using the two microwell assays for endosulfan residues in soil samples collected from field 21 on Auscott, Narrabri. The regression equation is as follows: microwell assay 2 (mg/kg) = 0.67 × microwell assay 1 (mg/kg) + 0.07 (mg/kg) (n = 104, r = 0.89, P < 0.001).

of the immunoassay with field samples also is necessary to assess its performance prior to routine use in quantification of residues in water and soil.

The precision of the assay was assessed by the variations of the IC_{50} of the standard curves prepared in purified water, field water, and soil extract for microwell assays 1 and 2 and the tube assay (Figure 4). A one-way analysis of variance on each set of three types of samples for microwell assays was also performed, showing that they were not significantly different (P < 0.05; F = 0.140 and 0.205 for microwell assays 1 and 2, respectively). There were no significant differences in the mean IC_{50} values for standard curves in purified water, field water, and soil extract, suggesting that matrix effects were minimal. However, the deviations of the IC_{50} values from the mean were slightly greater for field water and soil extracts (Figure 4).

Correlation between Gas Chromatographic Analysis and Immunoassay Data. Analyses using GLC and immunoassay were performed independently at different laboratories and without the possibility of foreknowledge by the personnel involved of the data from the other analytical method. Also, the procedures used for each method, from sampling in the field, to method of extraction, to the analysis itself, differed in numerous ways. Unlike immunoassay, where samples were all analyzed within a month from the collection, GLC analyses were completed over several months. Thus, the comparison between gas chromatography and immunoassay presented here cannot be considered as simultaneous comparisons of analyses by the two methods on extracts of the same samples by the same laboratory personnel. In general, field samples were subsampled for immunoassays and then processed for analysis separately. Despite this temporal and physical separation of analytical procedures, good correlations between the separate data obtained by immunoassay and GLC were obtained as shown below—an outcome that should be considered all the more impressive given that this would be expected to increase variation.

Immunoassay data (analyzed using microwell assay 1) showed a good correlation with gas chromatographic analyses for runoff water samples collected for the rain simulator studies, with a regression coefficient of 0.91 (Figure 5). Samples for GLC analysis were cooled and

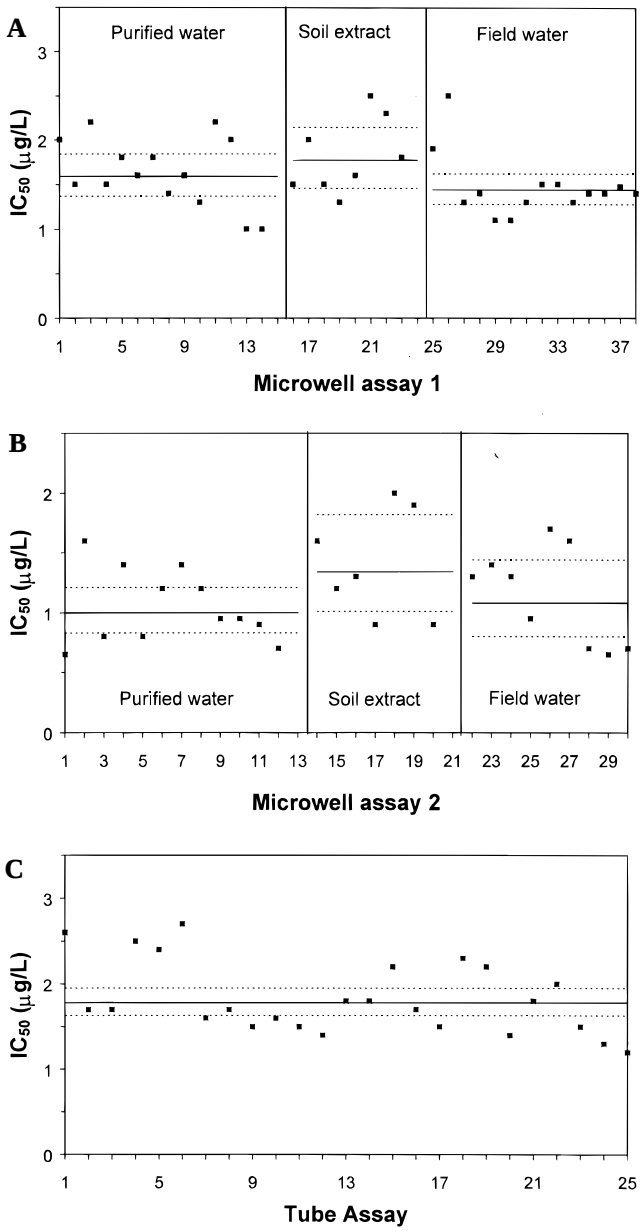


Figure 4. Precision of the assays; variation of IC_{50} of the standard curves prepared in (A) purified water, soil extract, and field water using microwell assay 1; (B) purified water, soil extract, and field water using microwell assay 2; and (C) purified water using the tube assay in sequential assays performed on different days. The geometric means and 95% confidence limits of the data are indicated as solid and dotted lines, respectively.

promptly transported by air courier to a central analytical laboratory, where they were extracted within 24 h of collection to minimize endosulfan loss by hydrolysis and volatilization. Immunoassay analysis was performed in laboratories only 30 min travel from the field site, providing analysis of water and extraction of sediment within 1−3 h of collection.

Due to the nature of these water samples (containing a large quantity of eroded soil), water was separated into solution and solid components by centrifugation for separate analysis by immunoassay. The total concentration of endosulfan in these samples was calculated by adding the endosulfan concentrations of the two components, and the correlations of ELISA and GLC/ECD were determined using these data. For immunoassay, samples were centrifuged on the bench centrifuge at ~300g for 5 min, and the supernatant was

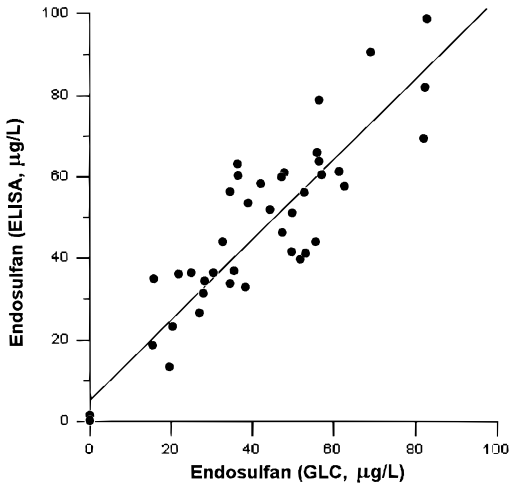


Figure 5. Relationship between GLC-ECD and microwell assay 1 data for water samples. The regression equation is as follows: microwell assay 1 (μg/L) = 0.98 × GLC-ECD (μg/L) + 5.7 (μg/L) (n = 44, r = 0.91, P < 0.001).

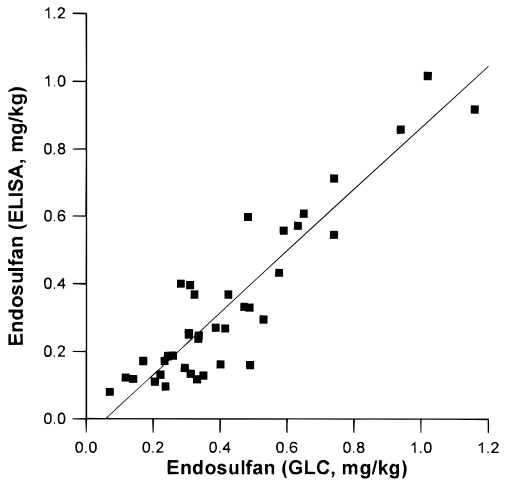


Figure 6. Relationship between GLC-ECD and immunoassay data for soil samples. The regression equation is as follows: ELISA (microwell assay 1, mg/kg) = 0.92 × GLC-ECD (mg/kg) − 0.05 (mg/kg) (n = 40, r = 0.94, P < 0.001).

removed for analysis. The sediment was extracted in methanol overnight, and then an aliquot of extract was diluted in water for immunoassay.

Any discrepancies between gas chromatography and immunoassays could result from a combination of factors, including possible inefficiencies of the sample preparation procedure for GLC analysis, poor sample handling, and losses during transport and storage. Lower extraction efficiencies of liquid−liquid extraction also have been reported for samples with high sediment content and aged samples (Carlberg and Martinsen 1982; Driscoll *et al.*, 1991). This implies liquid−liquid extraction may not sufficiently partition adsorbed residues into solvent layers. Adsorption of endosulfan onto sediment and particulates is also likely and has been shown to occur in field samples (Peterson and Batley, 1993).

A comparison of gas chromatography and immunoassay for 44 soil samples collected between December 13, 1993, and December 16, 1993, in field 21 on the Auscott property at Narrabri showed a reasonable correlation, but with greater values estimated again by immunoassay. Loss from degradation and volatilization of endosulfan in soil is expected to be minimized when frozen, as supported by the comparison of correlations between GLC and immunoassay data for water and soil, showing better correlation for soil (heterogeneous sample) than for water (homogeneous sample). The regression equations determined for 44 soil samples collected between December 13, 1993, and December 16, 1993, were as follows: for microwell assay 1, microwell assay 1 (mg/kg) = 1.29 × GLC-ECD (mg/kg) − 0.04 (mg/kg) (n = 44, r = 0.84, P < 0.001); and for microwell assay 2, microwell assay 2 (mg/kg) = 1.1 × GLC-ECD (mg/kg) − 0.01 (mg/kg) (n = 44, r = 0.86, P < 0.001). Analyses for different sets of soil samples collected after these dates, however, showed poorer correlation of GLC and immunoassay data. Some batches of soil samples analyzed by gas chromatography provided lower estimates than expected from the previous studies, probably due to losses during storage and handling. Similar studies conducted in the 1994−1995 season at another site showed much better correlation between gas chromatography and immunoassay (microwell assay 1) for 40 soil samples (Figure 6). A good agreement with regression coefficients >0.98 was also shown between

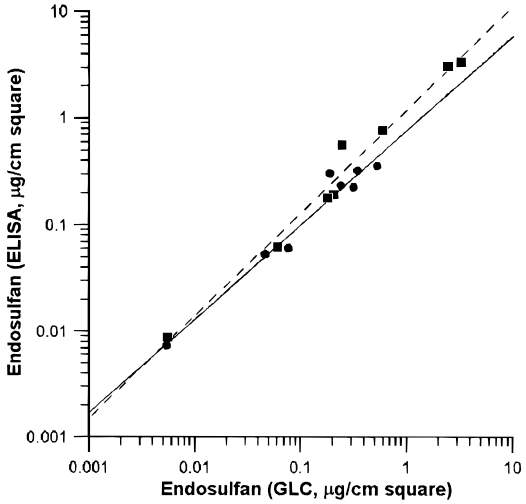


Figure 7. Relationship between GLC-ECD and immunoassay data for drift endosulfan residues on filter paper. The regression equations are as follows: for emulsifiable concentrate (dashed line), log ELISA (microwell assay 1, μg/cm^2) = 0.97 × log GLC-ECD (μg/cm^2) + log 0.18 (μg/cm^2) (n = 8, r = 0.99, P < 0.001); and for ultralow volume (solid line), log ELISA (microwell assay 1, μg/cm^2) = 0.89 × log GLC-ECD (μg/cm^2) − log 0.27 (μg/cm^2) (n = 8, r = 0.98, P < 0.001).

immunoassay and GLC analyses for endosulfan residues collected on filter papers for the drift study (Figure 7).

Both gas chromatography and immunoassays were evaluated using spiked samples and found to provide sufficient recoveries. Spike and recovery studies validating the performance of the immunoassays indicated that the analysis of endosulfan residues in soil correlated well with the spiked concentrations (Lee *et al.*, 1995). The extraction method used for soil samples provides recoveries >80% of endosulfan (Kimber and Kennedy, unpublished data). However, there remains the possibility that the extractions of "aged" endosulfan residues by either method may not be as effective as shown with the freshly spiked samples (Kloskowski *et al.*, 1986; Monteiro *et al.*, 1989; Pignatello *et al.* 1993). Also, the use of different endosulfan standards by laboratories performing the two analytical methods also must be considered from these results. It is reasonable to conclude that with due care, the immunoassays (tube assay and microwell assay 1) would be very beneficial

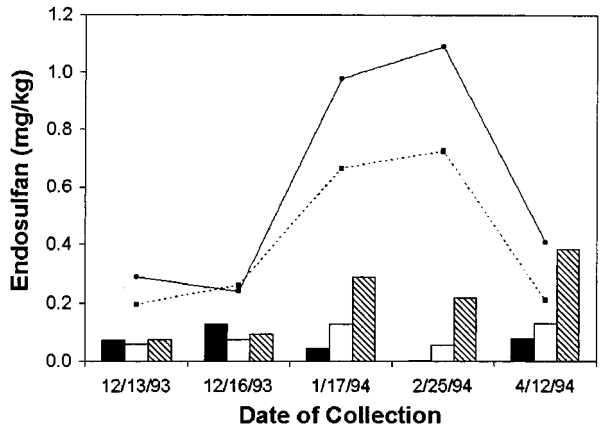


Figure 8. Comparison of analyses of microwell assay 1 (solid line) and microwell assay 2 (dotted line) for endosulfan residues in edge soil samples at different times with the concentrations of α-endosulfan (black bar), β-endosulfan (empty bar), and endosulfan sulfate (striped bar) determined by GLC-ECD.

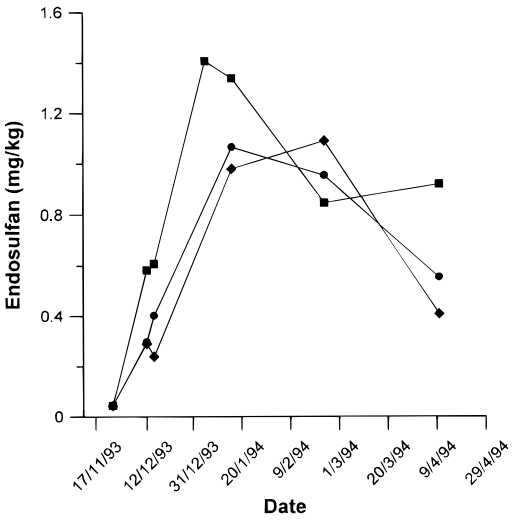


Figure 9. Endosulfan residues in soil during and after three applications of endosulfan analyzed by microwell assay 1: soil collected from the top of beds (■); soil collected from the furrows (●); soil collected from the edges of beds (◆).

as an analytical tool for quantification of total toxic burden of endosulfan in water and soil. The high throughput capacity of immunoassays enables a more comprehensive collection of data in endosulfan dissipation studies, with the tube assay being able to perform on-site analysis for better on-farm management of contaminated water.

The two microwell assays for endosulfan in soil were compared with gas chromatographic measurements of α-endosulfan, β-endosulfan, and endosulfan sulfate (Figure 8). Soil samples were collected at various times following the application of endosulfan, and the gradual conversion of endosulfan to endosulfan sulfate in soil was observed in analyses by gas chromatography, through changes to the relative concentrations of α-endosulfan, β-endosulfan, and endosulfan sulfate at each collection. The two microwell assays closely correlated with each other for soil samples collected 1−3 days after the application as the residues at this time were constituted primarily of α-endosulfan and β-endosulfan. As the concentration of endosulfan sulfate built up in the soil, the endosulfan residues analyzed by microwell assay 2 were lower than those analyzed by the microwell assay 1, reflecting the lower relative sensitivity of this microwell assay for endosulfan sulfate (Lee *et al.*, 1995). These results were suggestive of a possible underestimation of microwell assay 2 for the soil burden of endosulfan and endosulfan sulfate residues.

Dissipation of Endosulfan in Soil under Field Conditions. Previous studies conducted in similar cotton fields had shown that the endosulfan residues were retained mainly in the top 5 cm of soil (Kimber *et al.*, 1995); thus, a maximum residue of 1.5 mg/kg at each endosulfan ULV spray (using the recommended rate) can be expected in the surface soil of average bulk density 1.5. Accumulation of residues was seen after each endosulfan spray, and a maximum of 1.4 mg/kg was detected in the bed top samples after three aerial sprayings, with about 1.0 mg/kg total endosulfan residues found in edge and furrow samples (Figure 9). These concentrations were much lower than the predicted total concentration of 4.4 mg/kg for three sprays of endosulfan. There were consistently high temperatures with a moderate humidity during the spraying season in this region (the average daily maximum temperature and humidity were 25.5 ± 2.7 °C and 46 ± 11%, respectively), suggesting that much of the applied endosulfan was rapidly lost by volatilization or chemical degradation (Igue *et al.*, 1972).

The concentration of endosulfan declined rapidly to 0.4, 0.6, and 0.9 mg/kg for edge, furrow, and top samples, respectively, 4 months after the last spray (Figure 9). These results correlated well with similar studies conducted in the Australian cotton areas in northern New South Wales (Kimber *et al.*, 1995) and studies conducted overseas (Goebel *et al.*, 1982; Stewart and Cairns, 1974), which suggests that endosulfan is unlikely to accumulate in soil over long periods of use and disappears fairly rapidly from soil. The concentrations of endosulfan in soil from the edges and bottom of furrows were invariably lower than that from the top of beds. This is not likely to be a result of soil erosion during irrigation and rainfall as no irrigation and no rainfall occurred between the first spray and the two soil samples collected following that spray. Explanations for variation include changes in bulk density (resulting from compaction), such as that caused by tractor wheels, resulting in more weight of soil in each core and providing greater dilution of endosulfan residues in the composite samples, or the wind direction at spraying. Analysis of these studies will be published in detail elsewhere.

Drift Analyses. These immunoassays were applied extensively for monitoring drift, using a range of collecting surfaces such as copper wires, nylon gauze, and filter paper on flat surfaces. The results of one drift trial in which ULV and EC formulations were applied simultaneously by two aircraft flying in parallel to ensure consistency of meteorological conditions are shown in Figure 10. The contrasting pattern of efficiency of collection of the different sized droplets on a flat surface of filter paper was shown. Most of the larger droplets of the EC formulation were collected on the flat surface only a short distance from the aircraft centerline (25 m), while the smaller droplets of the ULV formulation were still detected at 100 m distant from the centerline. Horizontal surfaces are not regarded as efficient in droplet collection in drift downwind.

Runoff Studies. Immunoassays were used to advantage in runoff studies in which runoff water samples are taken during furrow irrigation, using natural and simulated rainfall. The time pattern of endosulfan

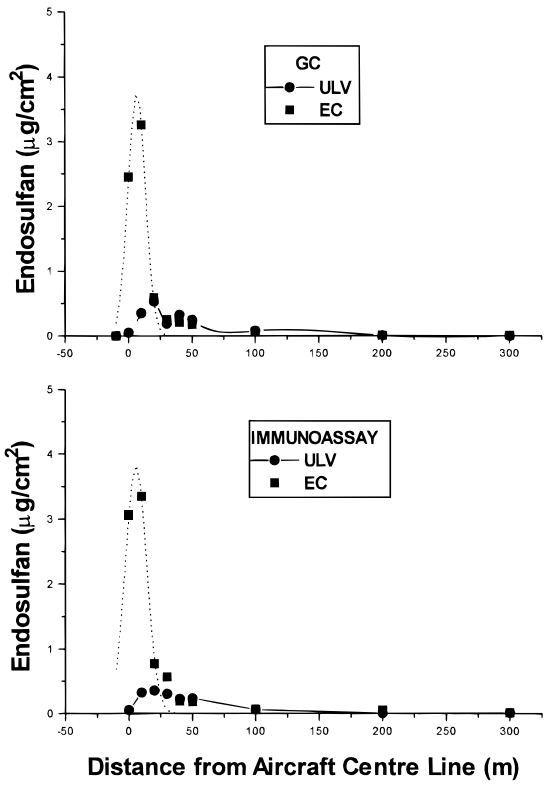


Figure 10. Endosulfan in aerial drift samples collected on flat filter papers (ca. 8 × 25 cm) attached on rows of aluminum tables 1 m above ground level. The good correlation between GLC and immunoassay for separate trials using ultralow volume (ULV) (●, dotted line) and emulsifiable concentrate (EC) (■, solid line) formulations is shown.

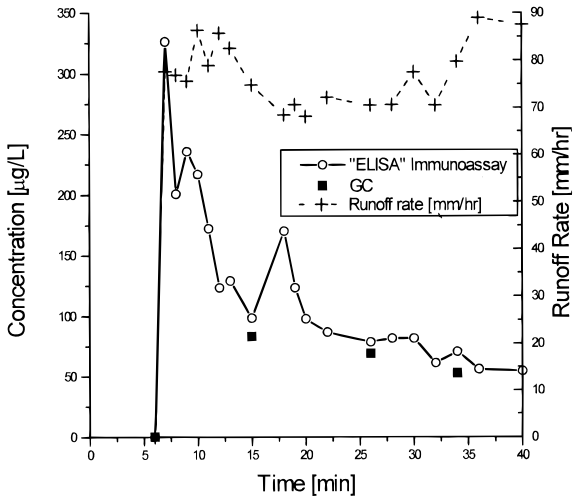


Figure 11. Immunoassay of endosulfan in rain simulator runoff (mm/h) (+) showing analysis by immunoassay (○) vs GLC (■). Because of the cost, relatively few samples can be analyzed by GLC. The correlation plot between the data obtained from the two methods for numerous trials conducted as above was shown in Figure 5.

concentration in runoff and runoff rate from one rainfall simulator trial is shown in Figure 11. Typically, the endosulfan concentration is highest during the early period of increasing runoff and decreases while runoff rate remains high or increases. Thus, pesticide transport rate (mass per unit time) is more uniform over time than is its concentration, with concentration varying by a factor of about 6 for the data shown in the figure. Nineteen samples were analyzed in this particular experimental plot by immunoassay, whereas high cost limits the information from GLC analyses (4 samples). Some 10−20 samples are obviously needed to obtain an accurate description of the time pattern and the total mass of pesticide transported, much more readily achieved using immunoassay.

CONCLUSION

Extensive validation of three endosulfan prototype methods (two microwell assays and one tube assay) were conducted in collaborative research organized by the Australian Cooperative Research Centre (CRC) for Sustainable Cotton Production. In the 1993−1994 cotton-growing season, approximately 400 soil and water samples were analyzed using both assays, as well as by GLC. Samples were tested on the day of collection using the tube assay and then results confirmed with the microwell assays several days later. Analyses of water samples by microwell assays and the tube assay were comparable, showing both methods can provide quantitation of endosulfan in water. The difference in results between the two microwell assays for analysis of soil samples was related to the specificity of these assays, showing that selection of a suitable immunoassay was crucial for reliable data. The most suitable immunoassay, employed extensively in subsequent work in this program, was chosen to be microwell assay 1, and this has been used in both the 1994−1995 and 1995−1996 seasons.

The earliest attempts at validation using comparisons of independent analyses by immunoassay and GLC showed poor correlations, due to poor sample handling, long transport of samples, and extended storage period. As a result, procedures for sample handling and storage with more prompt deposit of samples to the central analytical laboratory for immediate extraction and GLC analysis were improved, and very good correlations between GLC and immunoassay were then found for both field water and soil samples. Thus, immunoassay provided information that improved the adequacy of sampling and storage procedures. While some differences between methods would be expected for analysis of residues [i.e., extensive sample preparation (extraction and concentration) is needed for GLC, whereas samples are analyzed either by immunoassay directly or with simple extraction], these results suggested that quality assurance and validation of both methods are crucial to obtain reliable correlations. Having both methods available enables a better outcome for both, since immunoassay is rapid and corrective measures can be taken to improve the reliability of data.

However, the advantages of immunoassay, including speed, higher sample throughput with less complicated sample preparation, and the possibility of minimizing GLC analysis of negatives, have been amply demonstrated in these trials. More samples were able to be analyzed to obtain a more comprehensive data set; direct on-site decision-making for improved experimental design in the field is possible. Although less storage space is required for immunoassay samples, soil samples ≥10 g are still required to avoid sampling error. An added advantage of immunoassay has been observed with rain simulator studies, in which identical water samples have been used for simultaneous analysis of four different pesticides with little sample preparation required. In addition, the immunoassay allowed more samples to be analyzed, providing more comprehensive data at a lower overall cost.

The immunoassay results indicated that endosulfan did not accumulate but disappeared relatively rapidly

from soil, with endosulfan residues detected not exceeding 1.4 mg/kg. These results were supportive of similar studies conducted in this region using gas chromatography. Additional studies using immunoassay also support the conclusion that applied endosulfan can be partly lost through air drift and volatilization. While it was not possible to determine the actual amount volatilized with these experimental designs, the amount of endosulfan detected in the shallow (5 cm) water trays in fields nearby those sprayed with endosulfan was within the range toxic to fish and well exceeded the maximum residue level recommended by the Australian and New Zealand Environment and Conservation Council (ANZECC, 1992) for aquatic environments.

ACKNOWLEDGMENT

We are grateful to Bruce Simpson, Philip Hargreaves (Department of Natural Resources, Queensland), and Nazir Ahmad (Biological and Chemical Research Institute, NSW Agriculture) for the gas chromatographic analyses of water samples and to Lyndal Hugo, Sylvia Coleman, and Francisco Sánchez-Bayo for sample and data collection.

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Received for review December 2, 1996. Accepted June 13, 1997.[⊗] We acknowledge the financial support of the Cotton Research and Development Corp. of Australia, the Cooperative Research Centre for Sustainable Cotton Production, and the Land and Water Resources Research and Development Corp.

JF960915Q

[⊗] Abstract published in *Advance ACS Abstracts,* July 15, 1997.