SESSION 3D

NOVEL SOLUTIONS TO PROBLEMS ASSOCIATED WITH THE SAMPLING AND ANALYSIS OF PESTICIDE RESIDUE SAMPLES

SESSION ORGANISER MR M. EARL

POSTERS 3D-1 to 3D-4

DEVELOPMENT OF SIMPLE AND RAPID IMMUNOASSAY SYSTEMS FOR ANALYSIS OF PESTICIDES

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ABSTRACT

Immunoassay technology provides a relatively simple and economical analytical tool that when used appropriately in combination with classical analytical methods can improve the information base upon which agricultural or environmental management decisions are made. Agri-Diagnostics Associates has developed immunoassays for alachlor and triazine analysis in both a simple field usable format and a laboratory format for higher sample throughput. A simple, single use immunoassay format consisting of an absorbant flow through immunoassay device and a small handheld, battery operated reflectometer for quantitation of assay results has been developed for field use. The 96 well microtiter plate format was employed for laboratory use and is ideally suited for water monitoring programs and other applications where multiple samples are analyzed and quantitative results are desired. Agricultural ground and surface water samples were analyzed for alachlor and triazines using the multiwell immunoassays and standard GC methods. Excellent correlations were observed between the two techniques.

INTRODUCTION

The accurate and precise analysis of pesticides is a critical requirement for the registration and use of pesticides throughout the world. Parent molecules, key metabolites and chemical breakdown products must be identified and studied in well designed laboratory and field research trials. Environmentally sound management practices rely on significant amounts of information about the levels and movements of pests, pathogens and specific chemical treatments within the environment. The methods available for such analysis have become extremely sophisticated and sensitive in response to the need to detect lower and lower levels of contaminants in crops, water, soil, and farm animals.

Despite the tremendous sophistication of pesticide residue and environmental chemical analysis, there remain a number of serious limitations to certain aspects of classical analysis. A number of those limitations can be addressed through the application of immunoassay technology to residue analysis (Hammock and Mumma, 1980; Harrison et al., 1988; Vanderlaan et al., 1988). Immunoassays rely on highly specific antibody proteins and relatively simple analytical apparatus to detect and quantify a wide variety of target materials in a broad range of analytical matrices. Since the antibody reagents are specific for the analyte of interest, immunoassays can generally be performed with relatively crude sample preparations. Reduced sample preparation, simple assay procedures, high throughput capabilities, and relatively inexpensive automation make immunoassay procedures much less expensive on a per sample basis than conventional methods. These assays are usually rapid, taking from several minutes to several hours to complete and generally have limits of detection in the low pg/ml to low ng/ml range.

ANALYSIS OF ALACHLOR BY IMMUNOASSAY

The herbicide alachlor is one of the most widely used pesticides in North America. It is used primarily to control grassy weeds in corn and soybeans and is sometimes found as

a groundwater contaminant (Chesters et al., 1989). Some laboratory testing services routinely analyze water samples for alachlor residues using chromatographic methods. The availablility of an appropriate immunoassay would reduce the turnaround time and costs incurred in current residue analyses. Development of a laboratory immunoassay for Alachlor analysis was first described by Wratten and Feng (1989). Development of a standardized and stabilized immunoassay system for analysis of alachlor in surface and groundwater was subsequently undertaken.

A standardized and stabilized multiwell immunoassay kit for measuring levels of the herbicide alachlor in water samples has been developed. The assay has been designed as a quantitative screen to be used for analyzing water samples for the presence of alachlor at levels at or above the 2 ppb Maximum Contaminant Level (MCL). The immunoassay can be completed in about 30 minutes and could enable analysis of up to several hundred water samples per day. The assay demonstrates excellent quantitation of alachlor between concentrations of 1ppb and 50 ppb in incurred water samples. Cross-reactivity of this assay with two other commercially available acetanalide herbicides, metolachlor and butachlor, is very low (0.5% and 4.0% respectively).

A sample validation study was completed in which 200 water samples were analyzed using the standardized immunoassay kit and by the established GC-MS method. Excellent correlation was observed between the immunoassay results and the GS-MS analyses. The immunoassay correctly identified all 28 water samples that had alachlor levels of 2.0 ppb or higher by GC-MS analysis.

The standard immunoassay protocol used for this validation study utilizes a simple solid phase extraction sample preparation step to minimize the chance of adverse sample matrix effects introduced by environmental and agricultural samples. The same set of 200 samples was also analyzed without any sample preparation. Very little difference was seen in the results obtained with and without sample preparation illustrating the resistance of the immunoassay system to sample matrix effects.

The alachlor assay described is a direct competitive immunoassay utilizing a horseradish peroxidase-alachlor conjugate and multiwell plates coated with affinity purified polyclonal sheep anti-alachlor antibodies. The following protocols were used for sample clean-up and immunoassay respectively:

Sample preparation using C-18 solid phase extraction column

1. Assemble C-18 columns on a vacuum manifold. Add 1.0 ml methanol to each column and apply vacuum until column reservoir is empty. Immediately add 1.0 ml distilled water and apply vacuum until column reservoir is empty.
2. Apply 10.0 ml water sample to column and apply vacuum until sample has completely washed through.
3. Apply 1.0 ml distilled water and apply vacuum until washed through.
4. Place clean collection tubes in manifold. Apply 1.00 ml methanol to each column and apply vacuum until methanol has completely washed through.
5. Remove extract tubes from manifold and vortex.

Alachlor direct competitive immunoassay procedure

1. Dilute methanol extract 1:10 in working concentration of assay buffer by adding 100 µl extract to 900 µl buffer. Vortex.
2. Add 50 µl of diluted sample or alachlor standard prepared in 10% methanol-90% assay buffer to each well. Run each sample in triplicate.
3. Add 50 µl of Alachlor-HRP Conjugate to each well using an Eppendorf repeating pipettor.
4. Incubate for 10 minutes at room temperature on plate shaker at setting 9.

5. Wash wells five times with wash solution. Invert and shake excess moisture from wells.
6. Add 100 µl of substrate to each well using an Eppendorf repeating pipettor.
7. Incubate at room temperature for 10 minutes on the shaker.
8. Add 50 µl of stop solution to each well and mix on the shaker for 10 seconds.
9. Read the absorbance of the wells at 650 nm.

Alachlor validation study

The standard curve shows 15% inhibition at 0.5 ppb and 32% inhibition at 1.5 ppb and 50% inhibition at 4.0ppb (Figure 1). The assay demonstrates excellent quantitation of alachlor between concentrations of 1ppb and 50 ppb in incurred water samples.

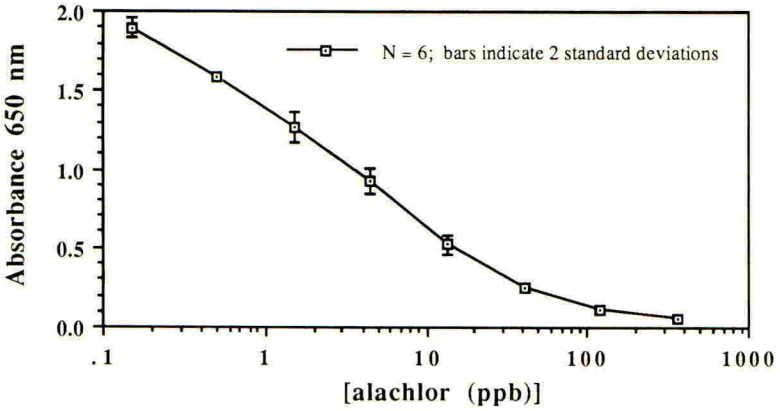

Figure 1. Dose response curve for alachlor multiwell assay

Cross-reactivity was determined for metolachlor, butachlor, and six alachlor metabolites. Results are shown in Table 1. Alachlor methylsulfide is a minor plant metabolite which was selected because its structure resembles that of the immunogen and would be the most likely to show cross-reactivity. Alachlor mercapturate is an animal metabolite not likely to be present in groundwater. The remaining four metabolites are those most commonly found in soil, and would be the most likely to be encountered in groundwater.

TABLE 1. Cross-reactivity of alachlor related herbicides and metabolites

Compound	IC_{50} (ppb)	% Cross-reactivity[1]
Alachlor	4.0	100.0%
Butachlor	100.0	4.0%
Metolachlor	750.0	0.5%
Alachlor methylsulfide	4.0	100.0%
Alachlor mercapturate	6.9	58.0%
Alachlor sulfinyl acetic acid	105.0	3.7%
Alachlor sulfonic acid	1200.0	0.3%
Alachor, sodium oxanilate	2100.0	0.2%
Alachlor, sodium oxanilate, desmethoxymethyl	>32,000.0	<0.01%

[1]%Cross-reactivity = IC50 alachlor/IC50 test compound x 100

The multiwell immunoassay was used to measure alachlor levels in surface and groundwater samples collected from agricultural areas in the midwest United States. The values obtained by immunoassay were correlated to the GC/MS values determined by the standard method of analysis (Figure 2).

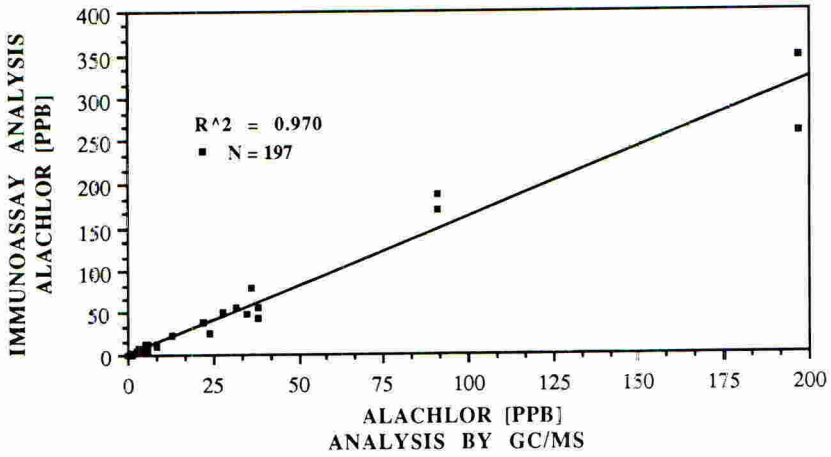

Figure 2. Correlation between results of alachlor analyses performed by immunoassay and GC/MS

Excellent correlation ($R^2 = 0.970$) was observed between the two methods. Comparison of the data generated with and without without sample preparation showed an $R^2 = 0.976$ with no significant outliers and a slope of 1.03. The immunoassay results obtained both with and without prior sample preparation compared very well to the GC-MS analyses, $R^2 = 0.970$ and $R^2 = 0.980$, respectively. This indicates that for this group of samples, no significant matrix effects were seen as the use of a sample preparation step did not alter the results obtained by immunoassay. A systematic bias of about 1.4 was observed throughout the study. Alachlor concentrations in the samples were generally about 37% higher as measured by immunoassay compared to the GC/MS results. This could reflect differences in the standards used for quantitation since the GC uses alachlor standards made in solvent whilst the immunoassay requires aqueous standards.

The alachlor immunoassay described is useful for quantitating alachlor in agricultural water samples at concentrations of 1 ppb and above and provides an effective means of screening water samples at the MCL level of 2.0 ppb alachlor. The immunoassay produced no false negatives at the 2.0ppb screening level when compared to GC/MS results. Excellent correlation was seen between GC-MS and immunoassay results. The immunoassay results obtained for samples assayed without prior sample preparation correlate well with results for samples subjected to sample cleanup for the 200 water samples analyzed in this study.

ANALYSIS OF TRIAZINES BY IMMUNOASSAY

A standardized multiwell immunoassay system was developed for analysis of surface and groundwater samples for the presence of triazine herbicides. The assay is a direct competitive immunoassay utilizing a triazine hapten-horseradish peroxidase conjugate and 96 well microtiter plates coated with monoclonal antibody that has specificity for the major

commercial triazine herbicides (atrazine, simazine, cyanazine, and prometon). The immunoassay can be completed in 30 minutes and could allow for analysis of several hundred water samples per day. Water samples can be assayed directly or a simple solid phase extraction step has been developed for use with the assay to minimize the possibility of sample matrix interference. The immunoassay has been designed as a quantitative screen for analyzing water samples for the presence of triazine herbicides at levels at or above the 1.0ppb and has excellent quantitation in the range of 0.5 to 8.0 ppb. Samples containing greater than 8ppb triazine can be diluted prior to immunoassay for quantitation.

The triazine sample preparation and immunoassay protocols are the same as previously described for alachlor. The standard curve shown in Figure 3 illustrates excellent quantitation between 0.5ppb and 8ppb atrazine. A more sensitive assay with detection at the 0.1ppb level also shown in Figure 4 has been developed but not yet validated.

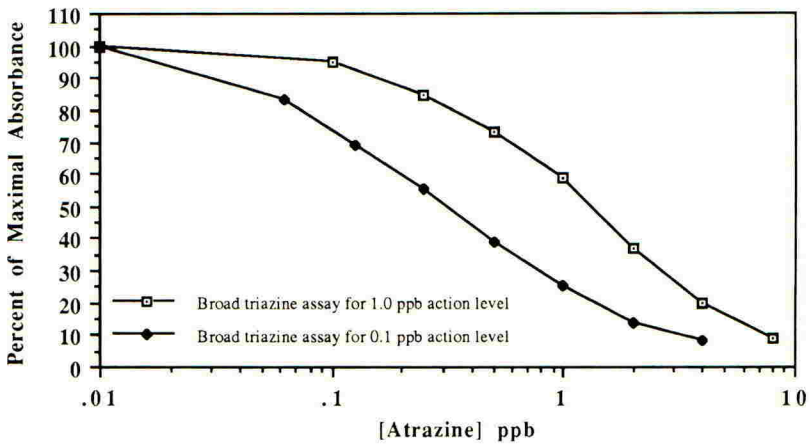

Figure 3. Dose response curves for triazine multiwell immunoassays. The validation work described in this study utilized the assay set for a 1.0ppb action level.

The cross-reactivity to other major triazines is shown in Table 2. The broad triazine specificity of the antibody used in this assay make it well suited as a water monitoring screen.

TABLE 2. Cross-reactivity of atrazine related herbicides and metabolites

Compound	IC_{50} (ppb)	% Cross-reactivity[1]
Atrazine	1.4	100.0%
Simazine	2.3	62.0%
Cyanazine	1.7	87.0%
Propazine	9.0	16.0%
Prometryne	3.0	47.0%
Hydroxy-atrazine	17.0	8.0%
Hydroxy-Simazine	3.1	47.0%
Des-isopropyl-atrazine	120.0	1.0%
Des-ethyl atrazine	120.0	1.0%
Di-dealkyl atrazine	>1,000.0	<1.0%

[1]%Cross-reactivity = IC50 alachlor/IC50 test compound x 100

A validation study was undertaken in which 216 ground and surface water samples were analyzed by immunoassay and the standard GC method. Comparison of the results obtained by the two methods showed excellent correlation. When water samples were analyzed in the immunoassay directly without using any sample clean-up, correlations with GC values of R^2=0.98 were observed (Figure 4). The same samples analyzed by immunoassay following a C-18 clean-up showed similar correlation to the GC results (R^2=0.97). A systematic bias of 0.84 was observed throughout the study. Thus the immunoassay values were approximately 16% lower than the corresponding GC values. One water sample consistently indicated a very high level of triazine by the immunoassay but had been reported as negative by the GC. Further analysis of the GC data indicated the presence of a high level of Cyanazine that was being detected by the immunoassay.

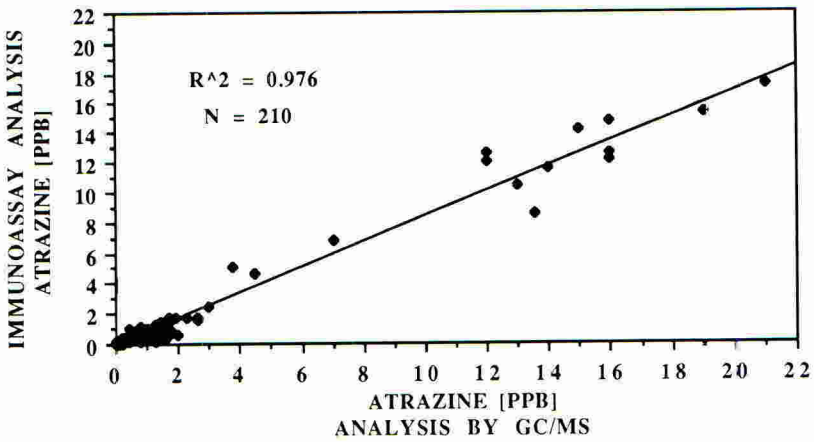

Figure 4. Correlation between results of triazine assays performed by immunoassay and GC

This evaluation indicates that the triazine immunoassay can effectively quantitate atrazine as well as other triazines in agricultural water samples at concentrations above 0.5ppb. The assay provides a reliable and reproducible quantitative screen for triazine herbicides at the 1ppb level which is the maximum contaminant level (MCL) in many areas. Analysis by immunoassay without using any prior sample clean-up produced results very similar to that obtained when a C-18 column clean-up was employed indicating no matrix interference. The possibility still remains that certain samples could eventually be encountered that cause matrix interference and in that event the simple solid phase column clean-up provides a way to deal with those samples.

FIELD USABLE IMMUNOASSAY FORMAT

Rapid on-site immunoassay systems for monitoring the levels of a variety of plant pathogens (Miller et al., 1988) have been developed by Agri-Diagnostics Associates. The information provided by these tests aids in the selection and timing of the appropriate chemical treatments to be used in an agricultural management program. These same types of immunodiagnostic systems are now being applied to the rapid on-site analysis and quantitation of agricultural and environmental chemicals (Rittenburg et al., 1991).

The assay system consists of a plastic device with a hydrophilic absorbant core and surface and a small handheld reflectometer for quantitation of the assay results. The

microporous surface of the device is treated with a hydrophobic mask to leave two circular zones exposed in which antibody or antigen bound to latex can be entrapped. The competitive immunoassay is carried out by sequentially adding sample, enzyme-labeled reagent, and enzyme substrate to the surface of the device using dropper bottles. Each solution is absorbed into the device passing through the surface zone of immobilized antibody or antigen. The immunoassay can be completed within 10 minutes and results in a visually observable color endpoint. Each assay device contains a reference zone that is used for comparison to the sample zone. The assay device couples to the handheld dual beam reflectometer which compares the color intensity of the sample zone to that of the reference zone. The results can be displayed as percent inhibition, actual analyte concentration, or as any value extrapolated from a pre-programmed curve. The meter memory can store up to 84 readings with an optional 4 digit label to identify each reading. An Rs 232 interface enables contents of the memory to be copied directly to a printer or computer. The use of the reflectometer removes the subjectivity from operator interpretation and provides data for documentation.

The same reagents used in the alachlor and triazine multiwell immunoassay kits have been formatted into the on-site system. The dose response curve shown in Figure 5 was generated for atrazine using the on-site assay system.

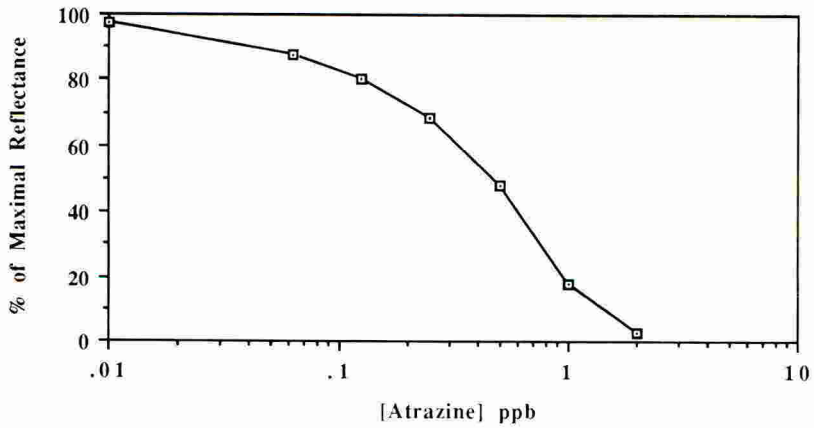

Figure 5. Dose response curve for triazine on-site immunoassay

The assay is simple to perform, can be completed in less than 10 minutes, and provides an extremely sensitive system with detection at around the 0.1ppb level. Once fully validated, this system will provide a simple method for sensitive field analysis of atrazine, alachlor, and other agricultural and environmental chemicals.

SUMMARY

Quantitative immunoassay kits were developed for analysis of alachlor and triazine herbicides in surface and groundwater. Validation studies were performed in which several hundred environmental samples were analyzed by the 96 well microtiter plate immunoassay and by the standard GC methods. Analytical results obtained using the immunoassays correlated very well with the results of the GC analyses. Although a simple solid phase extraction method was developed to clean-up the samples prior to immunoassay, no difference in results were observed when water samples were assayed directly. The immunoassay can be completed in about 30 minutes and enables several hundred water samples to be easily analyzed per day. Use of the immunoassay as an up front quantitative screen can greatly increase the throughput and reduce the expense of monitoring programs.

The standard analytical method can then be used to confirm positive samples flagged by the immunoassay.

A rapid on-site immunoassay system with a versatile and easy to use handheld meter has also been developed. This assay format provides an objective means of screening for levels of agricultural and environmental chemicals at a remote site. Immunoassay technology provides a cost effective way to obtain the timely information needed in many crop management and environmental monitoring programs.

ACKNOWLEDGEMENTS

The authors thank Monsanto Corporation for providing water samples, standards and GC/MS data and for their helpful advice pertaining to validation of the alachlor immunoassay. We are grateful to Beak Consultants Ltd for providing water samples and GC data used in the validation of the triazine assay. The authors also acknowledge the excellent technical contributions by Bharat Kikani, Jeanette Kosinski and Celeste Twamley during assay development and validation.

REFERENCES

Chester, G.; Simsiman, G.; Levy, J.; Alhajjar, B.; Fathulla, R.; Harkin, J., (1989) Environmental Fate of Alachlor and Metolachlor, Rev. of Environ. Contam. and Toxicol. **110**, 1-73.

Hammock, B.D.; Mumma, R.O. (1980). Potential of Immunochemical Technology for Pesticide Residue Analysis. In Pesticide Analytical Methodology; Zweig, G., Ed.; ACS Symposium Series No. 136; American Chemical Society: Washington, D.C.; pp 321-352.

Harrison, R.O.; Gee, S.J.; Hammock, B.D., (1988) Immunochemical Methods of Pesticide Residue Analysis. In Biotechnology for Crop Protection; Hedin, P. A.; Menn, J.J.; Hollingworth, R.M., Eds; ACS Symposium Series No. 379; American Chemical Society: Washington, D.C.; pp 316-330.

Miller, S.A., Rittenburg, J. H., Petersen, F. P., and Grothaus, G. D. (1988). Application of Rapid, Field Usable Immunoassays for the Diagnosis and Monitoring of Fungal Pathogens in Plants. Brighton Crop Protection Conf. Proc. 795-803; Lavenham Press Limited, Lavenham U.K. Publ.

Rittenburg, J. H.; Grothaus, G.D.; Fitzpatrick, D.F.; Lankow, R.K. (1991) Rapid On-Site Immunoassay Systems. In Immunoassays for Trace Chemical Analysis;

Vanderlaan, M.; Stanker, L.; Watkins, B.; Roberts, D.; Eds; ACS Symposium Series No. 451; American Chemical Society: Washington, D.C., pp 28-39

Vanderlaan, M.; Watkins, B.; Stanker. (1988) Environmental Monitoring by Immunoassay, Environ. Sci. Technol., **22**, (3), pp 247-254.

Wratten, S. J.; Feng, P. C. (1990). Pesticide Analysis by Immunoassay. In Development and Application of Immunoassay for Food Analysis; Rittenburg, J. H., Ed.; Elsevier Applied Science Publishers, London., pp 201-220.

AN INVESTIGATION INTO ALTERNATIVE SAMPLING AND STORAGE TECHNIQUES FOR VOLATILE PESTICIDES IN AGRICULTURAL SOILS

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ABSTRACT

A preliminary study was undertaken to evaluate alternatives to field extraction of soil for volatile pesticides. A series of storage conditions were investigated on soil samples taken upto 14 days after treatment. Elimination of air movement by surrounding the soil with aluminium foil and/or rapid chilling/freezing produced results comparable with 'field' extraction. Loss from soil in acetate core liners stored at ambient was observed. Further studies to elucidate the mechanism of loss are required.

INTRODUCTION

Field dissipation studies are conducted in many European countries to aid our understanding of the fate of pesticides in soil and to satisfy regulatory requirements. Volatile pesticides present specific problems, particularly at early sampling times, when possible loss of active between sampling and analysis can adversely affect calculation of the half-life.

A preliminary study was undertaken to find a viable alternative to solvent extraction in the field. A radiolabelled volatile herbicide was applied pre-emergence to soil. Core samples were taken and sealed in aluminium foil, either frozen in the field or stored overnight before freezing. Further samples were extracted immediately after sampling. Recovery data were compared for these three techniques.

An alternative system utilising acetate core liners for storage was also evaluated.

EXPERIMENTAL

The study consisted of two experiments:

Study A

This was initiated to evaluated storage techniques against solvent extraction in the 'field'.

A series of 18 plastic pots (95 mm diameter x 100 mm depth) were filled with a sieved (4 mm) sandy clay loam soil (Table 1) and compacted under a uniform pressure of approximately 0.1kg/cm^2 for 30 seconds.

TABLE 1 : Soil characterisation data typical of test soil

Particle Size Distribution (mm)						
1-2	0.5-1	0.25-0.5	0.106-0.25	0.053-0.016	0.002-0.053	<0.002
2	6	19	16	5	22	30

pH	7.70
Organic Matter	4.5%
Cation Exchange Capacity	26 me/100g
Biomass	17 mg/100g

A formulation containing additional radiolabelled material was diluted with water to give a spray concentration equivalent to field rate (nominally 1440g ai/ha). The soil was treated with a total of 250μl of solution. The surface was divided into four and an equal volume of solution was applied (using a microlitre syringe) evenly over the surface of each quarter. The herbicide was incorporated into the the first 1-2 cm of the soil by using a spatula to 'harrow' the soil.

The pots were housed outside in the Lysimeter complex at Letcombe and sampled at 0, 7 and 14 day intervals.

At each time interval 6 pots were removed and sampled using a 50mm dia x 100mm acetate liner.

A pair of soil cores were extracted immediately after sampling by placing the soil directly into a preweighed jar containing 200ml acetonitrile/water (99/1). The weight of soil was calculated. The soil was extracted by shaking for 15 mins, the mixture was centrifuged at 2000 rpm for 10 mins and a 5ml aliquot taken for counting.

The remaining 4 cores were split into 2 replicates, each core was removed from the liner and sealed in aluminium foil and placed in a bag. One pair of duplicates was stored at ambient and the other placed in a cold box containing dry-ice. Both sets of replicates were left overnight and placed in the freezer (<-16°C) prior to analysis.

All soil cores were taken out of the freezer and allowed to stand at ambient for 4 hours to mimic the maximum time that would be taken for sieving. The soil was weighed, split into 2 portions and analysed with solvent at a pro rata of 90g soil/100ml solvent.

The remaining soil from the pots was stored in situ in the freezer (<-16°C) and analysed later in a similar manner to the cores from the storage trials.

Total radioactivity was determined by scintillation counting.

Study B

A preliminary evaluation of the liners as a short term storage container was undertaken.

A further 450g of soil was spiked and equilibriated for 2 hours and then thoroughly mixed. 21g aliquots of soil were removed and the radioactivity determine by solvent extraction.

Further 21g aliquots of the remaining soil were added to a series of 25 mm diameter acetate liners containing a 10 cm core of soil. The addition of 21g to a 150mm long liner containing 100mm of soil gave a resultant air gap of <5mm, a 300mm long liner gave an air gap of 100-150 mm. All cores were capped and left at ambient for 6 hours in a fume cupboard prior to analysis.

RESULTS AND DISCUSSION

Study A

The soil core results from Study A showed the inherent variability associated with the incorporation (Table 2). Analysis of the remaining soil in the pots accounted for the apparent variation. In terms of the overall percentage recovery, acceptable reproducibility was observed.

TABLE 2 : Percentage of nominal concentration in relation to storage procedure and duration after application

Duration Days Days	Storage Prior to Analyses	As percentage of nomimal Core Value	Remaining Soil	Overall Percentage
0	none	92.5	101.0*#	100.6#
	ambient	104.7	93.4*	96.6
	frozen	105.5	95.0*	97.9
7	none	92.9	83.1**	85.8
	ambient	90.4	75.6**	79.7
	frozen	85.7	75.7**	78.5
14	none	78.3	58.2**	63.4
	ambient	96.0	52.3**	63.7
	frozen	67.9	63.9**	65.0

* Stored in pots under identical conditions to cores then placed in freezer for 6 days prior to analysis.

** Stored in freezer for 4 days prior to analysis.

\# Based on single replicate. The second replicate (151.7%) was excluded because it was attributed to contamination.

Previous work has shown that degradation in soil would not be expected during the period of this study. The stability of this herbicide under the frozen (<-16°C) storage conditions was confirmed by the lack of significant loss of radioactivity during the storage period (6 days) prior to the analysis of these samples.

Study B

In the second experiment; the storage of soil samples in acetate liners at ambient for 6 hours with an air gap above the soil resulted in 13.1 to 24.5% loss (Table 3).

TABLE 3 : Loss from acetate liners

Soil storage Conditions	percentage of Nominal (based on 5 replicates)
Immediate analysis	99.1
Ambient 6 hrs with <5mm air gap	86.9
Ambient 6 hrs with 100-150mm air gap	76.5

The degree of loss appeared to be related to the air gap above the soil. Adsorption to the surface or diffusion through the liner or the cap could not be evaluated in this study due to the limitation in the specific activity of the herbicide.

CONCLUSION

The results from Study A indicated that if soil is sealed in aluminium foil and transported to a freezer facility in a cold box with a suitable coolent losses can be miminised and results comparable with field extraction can be obtained.

The use of acetate liners to collect and store soil cores has advantages in terms of sample integrity and in dividing the sample into seperate horizons. The initial results obtained from Study B indicate that under non ideal storage conditions, ie ambient temperature with free air movement, the presence of an air gap could result in loss of residue.

SAMPLING AND ANALYSIS TECHNIQUES TO STUDY ENVIRONMENTAL FATE OF PESTICIDES

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ABSTRACT

This poster describes the evaluation and use of a range of techniques which are proving valuable in our studies of the fate of pesticides in terrestrial and aquatic environments:

- Ceramic cup "Suction samplers" to sample soil pore water and piezometers for sampling shallow groundwater.
- Various sampling procedures for soil, water and sediment to assess pesticide movement in surface run-off.
- The potential of immunoassay (ELISA) for screening soil and water for pesticide content.
- Use of deuterated standards in conjunction with GC-MS as a quality control to monitor the integrity of low pesticide concentrations in field water samples during transport, storage and analysis.
- The utility of field and trip blanks in environmental monitoring studies.

INTRODUCTION

To investigate the environmental behaviour of pesticides for agricultural use, it is necessary to undertake environmental fate and monitoring studies. To ensure such studies provide valid and relevant data, a number of newer analytical techniques are being developed and applied. These range from procedures to sample in ways not previously practicable (e.g. in situ soil pore water) to techniques like immunoassay for screening large numbers of environmental samples prior to confirmatory chemical analysis in selected cases. Included are the introduction of field and laboratory quality control (QC) checks through use of various blanks and deuterated internal standards to ensure the integrity of water samples for "low-level" pesticide analysis. All the above techniques are proving increasingly valuable in our studies of the fate of pesticides in terrestrial and aquatic environments and examples of their development and use are the subject of this poster.

SOIL SUCTION SAMPLERS

In a typical soil persistence study, a pesticide is applied to the soil in the first year and soil samples (0-15, 15-30 cm) taken and analysed at intervals during the next twelve months. The same application and sampling regime is repeated during the second and third years. At the end of the third year, samples down to 120 cm are taken (15 cm intervals to 60 cm and 30 cm intervals below that). These samples are analysed for parent compound and metabolites to check in more detail for evidence of mobility

and potential for leaching into groundwater. In order to obtain an earlier insight into the mobility of a pesticide under field conditions, a set of soil water suction samplers have been installed by the Soil Survey and Land Research Centre (Carter, 1991). The samplers have been emplaced in augered holes lined at the bottom with silica flour at depths of 0.75 m and 1.5 m in each of three plots (6 m x 12 m) within an experimental site at Reculver, Kent.

Sampling should be carried out when the soil moisture deficit is <40 mm, which usually limits sampling to the autumn and winter months (Clark et al., 1990). Samples are taken every four weeks during this period, or after trigger events (i.e. 10 mm rainfall in a single day or >20 mm over three consecutive days). Soil moisture deficit data are obtained from the Meteorological Office, Bracknell, who supply information for 40 km^2 areas of Great Britain as a MORECS (Meteorological Office Rainfall and Evaporation Calculation System) report.

Sampling is effected by creating a vacuum within the sampler so that water in the vicinity of the silica flour and surrounding soil moves into the sampler through the ceramic cup. Vacuum is again applied to draw water from inside the sampler into an amber glass collection bottle. Experience has shown that soil suction samplers can yield samples similar in volume to those normally taken for surface or groundwater sampling (ca. 1 litre).

As well as providing an early indication of leaching of pesticides and their metabolites, it has been demonstrated (Clark et al., 1990) that analysis of soil pore water provides a more sensitive approach for monitoring leaching than analysis of whole soil. On our Reculver site we have applied test compounds to study their downward movement. The experience gained from this study will allow the samplers to be applied to the study of new candidate pesticides.

PIEZOMETERS

To examine the environmental fate and movement of a pesticide and its metabolites into groundwater it is important to use a study area in which a suitable number of wells/boreholes are located. In shallow aquifer systems (to ca. 3 m deep), piezometers can be easily installed in place of expensive boreholes for sampling the upper groundwater directly beneath treated fields. A typical piezometer installation is depicted in Figure 1.

To collect the sample, groundwater is pumped to waste until it is as clear as possible of suspended particulates. A glass collection bottle connected directly to the teflon tube is used to collect the sample water.

In one of our studies, piezometers have been installed by the Water Research Centre (WRc) to supplement sampling from permanent sources. Analytical results for pesticides in piezometer samples were similar to those for samples taken from adjacent wells (i.e. <0.05 $\mu g/l$). The low levels gave confidence that the installation technique did not result in contamination of the surrounding groundwater.

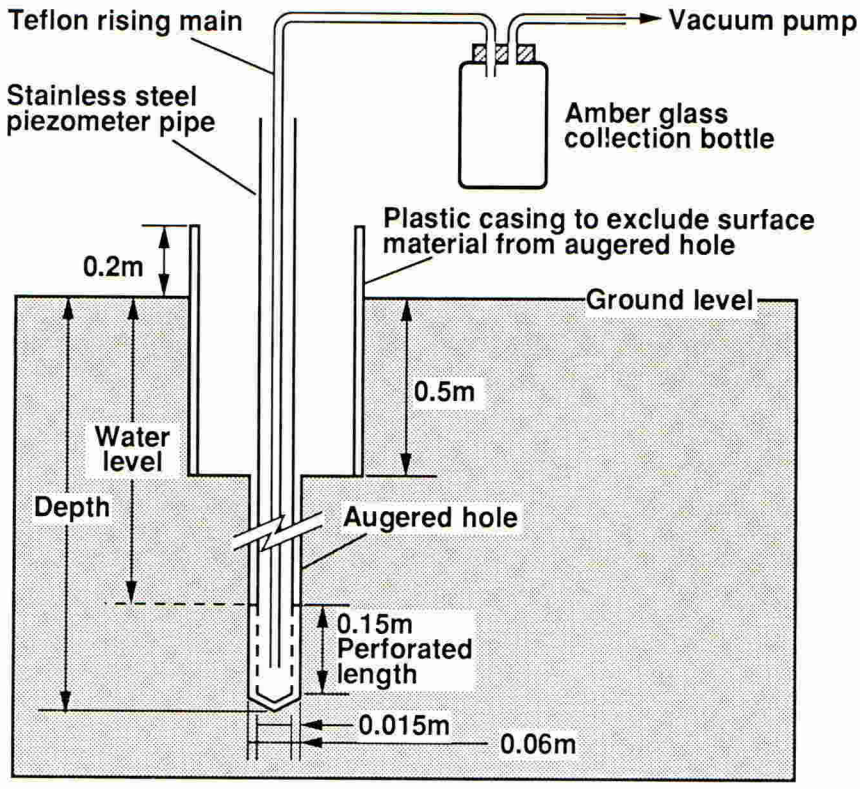


FIGURE 1. Piezometer sampling apparatus.

SAMPLING PROCEDURES IN RUN-OFF STUDIES

When monitoring surface run-off for pesticides, it is necessary to examine various matrices: run-off water, water from collecting streams, ponds etc., sediment of those water bodies, and soil. Each presents different problems in acquiring representative samples for analysis. We are currently assessing the utility of numerous sampling procedures.

Soil

One compound of interest to us is applied at rates of < 100 g/ha and is tightly bound to surface soil. As a result, shallow samples (< 2 cm) are required to obtain pesticide concentrations high enough for determination, and this is impracticable with a conventional auger. Where sample numbers can be kept small, it is possible to take deeper cores (e.g. 0-10 cm) and carefully section these to obtain shallow samples, but to examine a large area in the search for pesticide traces deposited by run-off, this process becomes too labour-intensive. We have, therefore, examined other approaches.

To detect sediment (run-off) movement down slopes, trays (aluminium, 25 cm square and 0.5 cm deep) have been employed in an attempt to retain the soil particles moving across the ground surface, which might be expected to have relatively high pesticide concentrations. Boxes, similar but 5 cm deep, are also being examined to determine whether their greater depth will act as a more efficient trap for solids in run-off water by slowing the flow.

We have used 50 cm square "quadrats" of stainless steel, pressed into the earth prior to carefully removing the surface soil within using a spade. It is possible to obtain reliable samples of ca. 2.5 cm depth using this procedure. Natural surface irregularities can render shallower sampling by this technique impractical. The same limitation is expected to apply to the "shallow depth soil sampler" which is described in the EPA "FARM" manual (Smith et al., 1985) and which we are evaluating.

Water

We have employed cylindrical tube samplers to collect "integrated depth" water samples (i.e. where water is sampled equally from all depths in the water body within the reach of the sampler, including the surface) and closeable bottle sub-surface water samplers have been used where water is to be collected from a single depth in the water column, excluding the surface film. The surface film can be sampled separately using a stainless steel mesh disc sampler and is often important in studies of drift or direct overspray. Automatic samplers linked to triggers such as flow meters or rain gauges can be valuable for mid-stream sampling of flowing water, since they reduce or remove the need for a rapid manual response to rainfall "events".

Sediment

We currently use long-handled augers with detachable tips for the collection of sediment core samples. The collection of deep cores (to 15 cm) may dilute the residues of interest with older unexposed sediment, but the collection of shallow samples from the surface of sediment in water bodies has so far proved impractical.

As an alternative, where water depths allow, traps can be positioned to collect sediment (including soil particles in run-off) falling through the water column. Sophisticated designs based on funnel shaped traps are available for use in deep waters. In ponds of ca. 1 m depth we have employed simple glass beakers, covered in netting to exclude most detritivores and attached to canes for recovery. Table 1 contains an attempted mass balance of a soil adsorbed pesticide using "beaker traps" for 14 days in a semi-artificial pond after its application as a slurry on soil (simulated run-off).

TABLE 1. Mass balance over the area of a pond using "beaker traps".

Sampling time	Pesticide residue (mg)* Sediment traps	Sediment traps (Cumulative)	Susp. solids	Water	Total found	Total as % nominal
1 h	1487	1487	520	184	2191	88
6 h	165	1652	208	131	1991	80
1 d	119	1771	107	100	1978	79
3 d	136	1907	35	61	2003	80
7 d	65	1972	6	39	2016	81
14 d	25	1998	6	23	2026	81

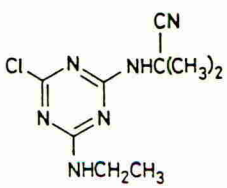
* 2500 mg were added, absorbed on a soil slurry, at time 0 hours.

THE POTENTIAL OF IMMUNOASSAY (ELISA) FOR SCREENING SOIL AND WATER FOR PESTICIDE CONTENT

A considerable amount of development work is being undertaken by many groups to apply immunoassay techniques, in particular enzyme linked immunosorbant assay (ELISA) to the determination of pesticide residues in environmental samples, especially water and soil (Vanderlaan et el., 1990). The ELISA format is typically based on a 96 well micro-titre plate which has the advantage of allowing some 20 - 30 duplicate samples to be screened simultaneously in addition to standards and QC checks. Owing to the selectivity of the antibody response, sample preparation can often be minimal. The sensitivities of many assays are such that a limit of determination of 0.01 mg/kg can be achieved with directly incubated soil, and residues in water in the range 0.01 to 0.1 μg/l can be determined following pre-concentration by solid phase extraction (often C_{18}) from a volume of about 150-250 ml. These features allow ELISA to be used for screening larger numbers of samples simultaneously than with instrumental techniques such as GC and HPLC. The simplicity of the technique allows the construction of field test kits, for some compounds.

We have been developing ELISA screening procedures for both soil and water for flufenoxuron (I) and cyanazine (II).

(I)



(II)

As part of the evaluation, the ELISA was compared with the existing instrumental method. Comparative results for determination of cyanazine in water by ELISA and GC-MS and flufenoxuron in soil by ELISA and HPLC show the good agreement achieved between methods (Tables 2 and 3). With the analysis of cyanazine in water, these results are supported by comparative analysis of field samples by the two methods. The results indicate that ELISA can confidently be used to screen samples prior to selection for chemical analysis and, for these examples, gives a worthwhile reduction of overall analysis time.

TABLE 2. Cyanazine added to water (μg/l); comparative analysis by ELISA and GC-MS.

Method	ELISA				GC-MS			
Sample	A	B	C	D	A	B	C	D
Set 1	0.055	<0.01	0.41	0.21	0.071	<0.01	0.53	0.28
Set 2	0.055	<0.01	0.71	0.31	0.075	<0.01	0.56	0.30
Set 3	0.065	<0.01	0.47	0.29	0.075	<0.01	0.57	0.27
Set 4	0.060	<0.01	0.51	0.32	0.075	<0.01	0.58	0.26
Set 5	0.060	<0.01	0.71	0.32	0.075	<0.01	0.58	0.25
Mean	0.059	<0.01	0.56	0.29	0.074	<0.01	0.56	0.27
Added	0.075	-	0.60	0.25	0.075	-	0.60	0.25

Note: Results are means of duplicates (GC-MS) or triplicates (ELISA).

TABLE 3. Flufenoxuron in soil samples (mg/kg); comparative analysis by ELISA and HPLC.

Method	ELISA					HPLC
Sample	1	2	3	mean	SD	
A	0.10	0.19	0.16	0.15	0.04	0.20
B	0.26	0.18	0.06	0.16	0.10	0.23
C	0.36	0.28	0.19	0.27	0.08	0.23
D	0.26	0.18	0.11	0.18	0.07	0.20
E	0.13	0.09	0.12	0.11	0.02	0.09
F	0.07	0.10	0.10	0.09	0.02	0.13
G	0.08	0.11	0.08	0.09	0.02	0.07
H	0.20	0.22	0.07	0.16	0.08	0.07

Two current disadvantages of ELISA are the considerable investment of time and facilities needed for assay development and the slow acceptance of immunoassay procedures for generating pesticide data for regulatory use. To address these concerns, the availability of suitable ELISA reagents (antibodies) for pesticides from diagnostics suppliers is increasing and

more comparative data showing the good comparability of ELISA and instrumental methods are being obtained.

USE OF DEUTERATED STANDARDS IN CONJUNCTION WITH GC-MS AS A QUALITY CONTROL TO MONITOR THE INTEGRITY OF LOW PESTICIDE CONCENTRATIONS IN FIELD WATER SAMPLES DURING TRANSPORT, STORAGE AND ANALYSIS

Where field water samples are taken for pesticide analysis at low concentrations (close to the EEC maximum limit of 0.1 μg/l for individual pesticides in drinking water) it is very important to be sure that no loss of residues occurs before analysis. The principle of adding an internal standard to samples at the time of analysis to assist quantification of residues and check method performance is well established. It works well, provided that the behaviour of the standard parallels that of the analyte throughout the procedure. To use the same approach successfully for field samples requires that the standard and analyte match as exactly as possible over a wide range of chemical characteristics including rate of breakdown (hydrolysis), volatility etc. These criteria can be met by the use of isotopic substitution.

In work with cyanazine (I) we have used penta-deuterated (d_5) cyanazine (-$NHCD_2CD_3$ form) as internal standard. At the end of each sampling day, d_5-cyanazine in methanol was added to field water samples by syringe to give a concentration of 1 μg/l. Cyanazine and d_5-cyanazine were quantified in the same extracts from the water by positive ion chemical ionisation (CI) GC-MS using the characteristic M+1 ions at 241/243 (cyanazine, ^{35}Cl and ^{37}Cl isotopes) and 246/248 (246 only used for quantification) for the d_5 analogue. This procedure enabled the integrity of samples during transportation and storage to be confirmed by checking the d_5 cyanazine recovery obtained. Good recoveries have been achieved on several sampling occasions for water samples stored at 4°C for periods of up to about 4 weeks between sampling and analysis (Table 4). Under these conditions, cyanazine water concentrations as low as 0.01 μg/l have been determined. The procedure has the added advantage that the d_5-recovery also includes the analytical method recovery for each sample.

TABLE 4. Recovery of d_5-cyanazine added to water samples in the field.

Year	Number of results	d_5-Cyanazine recovered* Range (%)	Mean (%)
1	21	52 - 79	74
	121	80 - 120	98
	7	121 - 154	132
2	6	68 - 79	75
	93	80 - 120	98
	4	121 - 128	123
	3	160 - 270	-

* From 1.0 μg/l added in the field.

THE UTILITY OF FIELD AND TRIP BLANKS IN ENVIRONMENTAL MONITORING STUDIES

Another requirement of monitoring for pesticides at low concentrations in water is to ensure that none of the equipment or procedures introduces contamination which may interfere with the determination of the pesticide residue and give rise to invalid false positive results. To ensure such contamination is avoided in our studies, all water sampling equipment used is made of inert materials (glass and PTFE) and is thoroughly washed before use, rinsed with solvent (e.g. acetone) and the solvent wash analysed to the required limit of determination.

Some 15% of the sample bottles taken to the field are filled with laboratory distilled water, checked to be interference free by analysis. About one third of these are never opened and act as trip blanks. Water in each of the remainder is transferred to empty sample bottles at selected field sampling locations to simulate field sampling. These samples act as field blanks.

During a recent field study where several hundred samples have been taken over a 3 year period, 56 field blanks and 21 trip blanks taken in this way were found to be clean to a limit of determination equivalent to 0.02 $\mu g/l$, thus giving confidence in any positive values found above this concentration in field water samples. In addition to these precautions, all the sampling equipment was checked before use to ensure that it caused no loss of low concentrations of pesticide from water.

ACKNOWLEDGEMENTS

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REFERENCES

Carter, A.D. (1991) Methods of monitoring soil water regimes and the interpretation of data relevant to pesticide fate and behaviour. BCPC Monograph No. 47, Pesticides in Soils and Water, pp. 143-149.

Clark, L.; Gomme, J.; Carter, A.; Harris, R.C. (1990) WRc/Soil Survey Inert Suction Sampler. Brighton Crop Protection Conference, Pests and Diseases, 8C-11, 1011-1016.

Smith, C.N.; Brown, D.S.; Dean, J.D.; Parrish, R.S.; Carsel, R.F.; Donigian, A.S., Jr. (1985) Field Agricultural Runoff Monitoring (FARM) Manual, EPA/600/3-85/043, Athens, Georgia: USEPA.

Vanderlaan, M.; Stanker, L.H.; Watkins, B.E.; Roberts, D.W. (Eds.) (1990) Immunoassays for trace chemical analysis, ACS Symposium Series 451, Washington, DC: American Chemical Society.

SEED POTATOES - CONTAMINATION WITH TECNAZENE FROM THE ENVIRONMENT

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ABSTRACT

Seed potatoes, cv. Desiree, delivered from Scotland, were kept in a commercial ware potato store containing a tecnazene treated crop for a period of 8 weeks in both chitting trays and hessian sacks. Samples were taken for tecnazene analysis at 10-14 day intervals. Tecnazene levels in the peel rose to an average of 1.5 ppm in the bagged potatoes and 3.6 ppm in the trayed potatoes. This difference could have been due to the deposition of tecnazene contaminated dust on potatoes in the trays.

INTRODUCTION

The sprout suppressant effects of tecnazene have been known since the mid-1940's (Brown, 1947; Brown and Reavill, 1954) and it has now been used for nearly 40 years on stored ware potatoes for this purpose (Anon., 1989).

There are many causes of poor and/or delayed emergence of seed potatoes in the field. These include diseases such as gangrene or dry rot, physiological disorders such as little potato or coiled sprout, and occasionally contamination of the seed by chemicals such as tecnazene, chlorpropham, glyphosate or glufosinate. Analysis of the recovered seed sometimes detects the presence of tecnazene but subsequent investigation usually fails to find any deliberate contamination. This trial sets out to show to what extent, if any, contamination could occur through adsorption following delivery onto the premises of the ware grower in the presence of a tecnazene treated crop.

MATERIALS AND METHODS

Location and layout

The trial was located in a commercial ware potato store (c.1500t capacity) in Shropshire. The seed in the trial was placed on top of the main air duct which separated the two halves of the tecnazene treated ware crop. The front section of the building was used for grading this crop and noticeable amounts of dust were generated during the grading operation, which occurred intermittently throughout the period of the trial.

Four 50kg bags of Scottish Desiree seed potatoes (grade SE1) were used in the trial. Three were placed flat along the length of the duct and the fourth was tipped into 3 standard wooden chitting trays, stacked one on top of the other.

Sampling

Single samples of 4 tubers each were removed from 2 of the bags on 14 March 1988 for tecnazene analysis by DAFS (Department of Agriculture and Fisheries for Scotland) before being officially sealed and despatched to England. Further single samples of 8 tubers each were taken from all 4 bags on arrival at ADAS Wolverhampton on 18 March. The trial was then set up at the farm and single samples, again of 8 tubers each, were collected from each tray and each bag at 10-14 day intervals from 28 March to 18 May. The ware crop in the store was sampled from the face and top of the heap at the time the trial was set up to establish the amount of tecnazene in the crop contaminating the seed.

Residue analysis

All samples taken in England were immediately placed in polythene bags and sealed to prevent loss of tecnazene by volatilisation during transit. They were sent by 24 hour carrier to the ADAS Pesticide Residues Unit, Cambridge for analysis.

Packaging and transport of samples does present something of a dilema. Sealed polythene bags can result in tuber rotting if transport is delayed, especially in warm weather, whereas ventilated polythene bags, although reducing the risk of rotting in transit, do increase the risk of tecnazene loss by volatilisation. Because these samples were transported in sealed polythene bags, as far as possible delays in delivery over a weekend were avoided by collecting and despatching on the same day early in the week.

Even though samples were placed in sealed polythene bags, to minimise the risk of cross contamination, samples from the trays and bags were packaged separately.

At Cambridge, samples received the minimum washing necessary consistent with removing adhering soil. A 2mm thick peel was taken for analysis using a standard hand-held kitchen potato peeler. The analysis itself was carried out by gas chromatography utilising electron capture detection, following extraction by ethyl acetate.

Tecnazene residues in potatoes can be measured on a whole tuber basis or in the peel only. The former is the more relevant method in terms of the setting of maximum residue levels (MRL's) to restrict consumption of tecnazene in food. The latter is the more relevant in terms of measuring residues which might inhibit sprouting, since most of the residue is concentrated in the surface layers of the potatoes, where the eyes are located (Dalziel and Duncan, 1980). (As a rule of thumb, levels in the peel can be converted to levels in the whole tuber by dividing by 10. For example, 49mg/kg tecnazene in the peel would equate to 4.9mg/kg in the whole tuber).

RESULTS AND DISCUSSION

Dormancy in the seed potatoes was breaking at the time of delivery. Tecnazene only works as a sprout suppressant before dormancy break (Dent, 1985) and therefore it is not surprising that the different levels of

contamination observed did not seem to visibly affect sprout growth. This point may be important in determining whether slow emergence observed in the field can be correctly ascribed to tecnazene residues.

The results of the tecnazene analyses are presented below.

1. DAFS analysis before despatch on 14 March:-

Sample 1 <limit of determination (0.002 mg/kg)

Sample 2 <limit of determination

2. ADAS analysis on receipt of seed on 18 March:-

Bag No	Tecnazene mg/kg in peel
1	0.2
2	0.1
3	0.1
4	0.1

3. Analysis of the ware crop sampled on 18 March - 49 mg/kg tecnazene in the peel.

4. Analysis of samples from bags and trays during the trial:-

TABLE 1 Tecnazene mg/kg in peel

	Bag 1	Bag 2	Bag 3	Mean	Tray 1 (top)	Tray 2 (middle)	Tray 3 (bottom)	Mean
28 March	0.2	0.2	0.1	0.2	3.0	2.3	2.1	2.5
7 April	3.6	3.8	1.2	2.9	5.0	4.0	3.4	4.1
20 April	1.0	1.6	1.2	1.3	4.4	3.6	4.5	4.2
4 May	1.6	1.8	1.7	1.7	3.0	3.5	4.0	3.5
18 May	1.7	1.9	1.2	1.6	3.6	3.3	3.8	3.6
Mean	1.6	1.9	1.1	1.5*	3.8	3.3	3.6	3.6*

* Difference statistically significant at p = 0.002

It is difficult to explain the tecnazene levels in bags 1 and 2 on 7 April. These measurements certainly do not fit in with the general pattern of results in this trial.

In the bags, average tecnazene levels rose to about 1.6-1.7mg/kg over the period of the trial. In the trays however, tecnazene levels rose quickly to an average of 4 mg/kg and subsequently appeared to level off at around 3.5mg/kg. At the first 2 sampling dates there appeared to be a gradient in tecnazene levels between the top and bottom trays. This gradient subsequently disappeared.

The levelling off of tecnazene residues in both the bags and trays towards the end of the trial, and the disappearance of the gradient in the trays, could be due to a balance being reached between adsorption + deposition, and volatilisation in the case of the trays. Certainly dust visibly accumulated on the seed in the trays (and on the structures of the building) due to grading of potatoes while the trial was in progress and given the relatively high level of tecnazene in the ware crop it is not unreasonable to assume that dust did contaminate the seed. This would account for the higher average tecnazene levels in the trays where the seed was directly exposed to dust contamination, rather that in the bags, where the seed was not nearly so exposed. As far as possible, dust contamination of seed in the bags was avoided by sampling from the centre of the bags.

CONCLUSIONS

1. Tecnazene can be adsorbed by seed potatoes stored in a contaminated ware store.

2. Seed stored in trays carried more tecnazene at all sampling dates than that stored in bags. Deposition of tecnazene contaminated dust created during grading probably accounted for this difference.

3. There are indications that tecnazene residues may level off after a period of time.

4. No visible difference in sprout suppression was seen between the seed in the trays and bags, despite significant differences in recorded tecnazene levels. This may not have been the case had the seed been dormant at the time of delivery.

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REFERENCES

Anon. (1989) Fusarex for long-term storage of quality potatoes. ICI Agrochemicals Bulletin.

Brown, W. (1947) Experiment on the effect of chlorinated nitrobenzenes on the sprouting of potato tubers. Ann. appl. Biol., **34**, 422-429.

Brown, W.; Reavill, M.J. (1954) Effect of tetrachloronitrobenzene on the sprouting and cropping of potato tubers. Ann. appl. Biol., **41**, 435-447.

Dalziel, J.; Duncan, H.J. (1980) Studies on potato sprout suppressants. 4. The distribution of tecnazine in potato tubers and the effect of processing on residue levels. Potato Res., **23**, 405-411

Dent, T. J. (1985) Review of current usage of pesticide chemicals for the control of post-harvest losses in stored potatoes. Chemistry and Industry, 4 Feb. 1985.