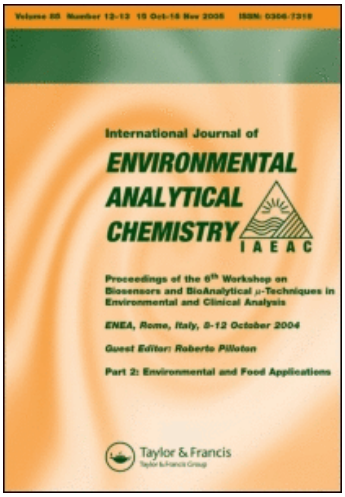

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THE USE OF ELISA FOR THE DETERMINATION OF PESTICIDE RESIDUES IN FOOD

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Several commercially available enzyme-linked immunosorbent assay (ELISA) kits have been evaluated for the determination of pesticide residues in foods. Whilst designed for analysing residues in water, experience has shown that, with certain modifications to the way in which ELISAs are applied to food extracts, they can be used as semi-quantitative screening procedures for pesticide residues.

KEY WORDS: ELISA, pesticides, residues, food.

INTRODUCTION

Enzyme-linked immunosorbent assay (ELISA) kits are commercially available for the determination of a variety of pesticide residues in water and potentially offer time and cost savings over conventional instrumental techniques.

However, there are obvious difficulties in the application of these kits to the analysis of pesticide residues in food. Food is a far more complex matrix than water and hence the assay may suffer from interferences. In addition, with all foods except liquids, there is a need to extract the residues with an organic solvent prior to analysis by ELISA.

However, ELISA antibodies are generally intolerant of solvent concentrations greater than approximately 10%, thus organic extracts cannot be used without dilution.

This paper summarises our experiences in this field and highlights some of the important practical aspects which must be considered in order to achieve repeatable and reliable ressults.

MATERIALS

The work drawn upon in this paper was using the following Millipore EnviroGard kits:

- benomyl, including carbendazim (MBC) and thiabendazole (TBZ)
- 2,4-dichlorophenoxyacetic acid (2,4-D)
- pentachlorophenol (PC)
- triazines

All were of the microplate format except the PCP kit which was in tube format.

In all cases, absorbance measurement was performed using a Titertek Multiskan Microplate reader. For the PCP kit, the final solutions were transferred by pipette from the tubes to a microplate.

DISCUSSION

Analytical expertise

The successful use of ELISA kits requires experienced, trained analysts. Manufacturers often imply that a lower level of manipulative skills is required than for chromatographic techniques. Even the importance of basic laboratory skills, such as the correct use of micropipettes, should not be underestimated.

Extraction

As stated earlier, ELISA kits are generally intolerant of organic solvent concentrations greater than approximately 10%. However, because they are highly selective and sensitive to a particular analyte, it is possible to extract the sample with methanol and subsequently dilute the extract with water in the ratio 1:10 or greater. MBC, TBZ and 2,4-D have all been analysed in fruits and vegetables in this manner.

For the determination of PCP and triazines in milk, no extraction was necessary; milk samples could be applied directly to the plate.

Matrix effects

Standards *must* be prepared in matrix matched extract that is pesticide free. The importance of matrix matching cannot be over-emphasised. Table 1 illustrates the variations in response caused by different matrix concentrations. In the case of PCP and triazines analysis, fresh whole milk and previously frozen whole milk were found to respond differently. Absorbance differences could also be observed between whole milk and semi-skimmed milk.

Cross reactivity

Interference from structurally similar compounds can cause false positives and also affect quantification. The analyst must be aware of possible cross reactivity from such

Table 1 Matrix effect of orange extracts on 2,4-D ELISA response.

	Absorbance
Blank matrix	0.415
Blank matrix spiked at 1 µg/l	0.310
Blank matrix at 10 x dilution	0.601
Blank matrix at 10 x dilution spiked at 1 µg/l	0.386

compounds, e.g trichlorophenols with the PCP kit. In certain cases, this cross reactivity can be beneficial. For example, 2,4-D may be applied to crops as aqueous solutions of salts, or as oil emulsions of esters. For determination by gas chromatography, the various forms must all be derivatised to a single compound, usually the methyl ester. However, because the ELISA kit responds to all forms of 2,4-D, it can effectively be used to screen samples without the need for prior conversion to a single compound. This is especially important in situations where the treatment history of the sample is unknown. Because of the potential for cross reactivity to cause false positive results, it is essential to confirm positive data generated using ELISAs by a second technique, usually chromatography.

Quantification

To date ELISAs should be regarded, at best, as a semi-quantitative screening technique for food extracts. The reliability of quantification appears to vary greatly between kits. For MBC and TBZ, in fruits and vegetables, quantitative agreement between ELISA and HPLC results was good (Table 2). For 2,4-D in oranges, quantitative agreement with GC-MS was poor (Table 3).

However, in all cases, ELISA was able to detect residues at low levels, e.g. 2,4–D residues in oranges at 0.2 mg/kg. Hence, it was possible to screen for positive samples. False negatives have never been observed.

Table 2 Comparison of HPLC and ELISA techniques for fruit and vegetable samples (Reproduced from Mountfort *et al*, 1).

	Level found by HPLC (mg/kg)		*Level found by ELISA (mg/kg)*
Commodity	*Carbendazim*	*Thiabendazole*	*(expressed as carbendazim)*
Pea	0.62	[b]	0.8
Aubergine	[b]	[b]	[b]
Aubergine	0.02	0.02	0.02
Pepper	[b]	0.04	[a]
Pepper	[a]	[b]	
Pepper	[b]	[a]	[a]
Pepper	0.03	[b]	[a]
Melon	[a]	0.04	[a]
Melon	[b]	[b]	[a]
Melon	[a]	[b]	[b]
Melon	[a]	[b]	[b]
Melon	[a]	[b]	[a]
Melon	0.06	[b]	0.02
Melon	[b]	[b]	0.08
Mangetout	0.27	0.99	0.23
Mangetout	0.11	[b]	0.05
Mangetout	4.20	[b]	3.10
Mangetout	3.00	[b]	3.60
Mangetout	0.16	[b]	0.44
Mangetout	0.11	[b]	0.05
Mangetout	0.23	[b]	0.11
Mangetout	0.03	[b]	0.07
Mangetout	0.13	[b]	0.04

[a]Levels were detectable, but less than 0.02 mg/kg
[b]Levels were less than 0.01 mg/kg
Additionally, 50 samples were found to contain no residues by both techniques.

Table 3 Quantitative comparison of ELISA and GC-MS for the analysis of 2,4-D in oranges.

	mg/kg			
2,4-D by ELISA	12	11	5	4
2,4-D by GC-MS	0.28	0.42	0.30	0.32

Cost implications

ELISA procedures typically involve approximately half the staff effort of instrumentally based chromatographic procedures. Using ELISA as a screening tool can significantly reduce the number of samples requiring analysis using these more expensive chromatographic procedures.

Achievements to date

An ELISA screening procedure for MBC and TBZ residues in several different fruit and vegetables, has been accredited by the United Kingdom Accreditation Service (UKAS) to the European standard, EN 45001. ELISA procedures for 2,4-D residues in oranges; for PCP in milk, and for triazines in milk have all been successfully validated and have been used to screen samples in annual surveillance programmes.

CONCLUSION

Some commercial ELISA kits designed to detect pesticide residues in water can, with care and experience, be successfully applied as a screening technique for residues in food extracts.

Acknowledgements

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References

1. K. A. Mountfort, S. L. Reynolds, S. A. Thorpe and S. N. White, *Food and Agricultural Immunology*, **6**, 17–22 (1994).