THE DEVELOPMENT OF ENZYME-LINKED IMMUNOSORBENT ASSAYS FOR THE DETECTION OF PESTICIDES IN MILK

A Thesis submitted to the University of Salford in candidature

for the degree of

DOCTOR OF PHILOSOPHY

By

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DECLARATION

I declare that this Thesis has not previously been submitted, and is not currently being submitted for any degree other than that of the degree of Doctor of Philosophy of the University of Salford.

The work embodied in this Thesis was carried out by myself in the Multidisciplinary Research and Innovation Centre, The North East Wales Institute, Deeside, Clwyd, under the joint supervision of Dr. C. J. Smith and Dr. R. Bisby.

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5. SUMMARY AND CONCLUSIONS

The basic concept of the ELISA technique, i.e. the use of a combination of solid phase and enzyme-linked immuno reagents to monitor immune reactions without interference from other molecules is relatively simple. In this Thesis an attemp was made to evaluate the usefulness of the ELISA technology for the detection of pesticides.

The object of this Thesis was the development of an ELISA for the detection of aldrin/dieldrin pesticides based on polyclonal antibody and an ELISA for the detection of ethyl parathion based on monoclonal antibody.

The Thesis described theoretical concepts and practical requirements which enable any of the assays for each antigen to be developed. Such development involves no only examination of various ELISA methodologies but also the production, characterisation and isolation of polyclonal and monoclonal antibodies, and critical assessment of the assays and assessment of the results using the methods developed.

5.1 The development of ELISA for the detection of aldrin/dieldrin based on polyclonal antibody

Polyclonal antibodies were raised against aldrin/dieldrin using appropriate hapten-protein conjugate as immunogen. Polyclonal antisera, harvested from immunised animals was not sensitive enough to be employed as the basis for ELISA. The sensitivity was improved by removing anti-bovine serum albumin antibodies by means of affinity chromatography. The purified serum was used as the basis for ELISA systems and assay format optimised for maximum efficiency.

The possibility of using ELISA assays for the detection of aldrin/dieldrin in milk was examined using non-competitive. It has been shown that the fat content dramatically affects the antigen antibody interaction

Cross reactivity studies with compounds structurally related to aldrin/dieldrin indicates that the antibody recognises the shape of the molecule (for interaction) rather than any attraction forces.

Another part of the study involved the application of the ELISA to determine if it was capable of detecting aldrin/dieldrin in milk from different species, buffalo, cow, sheep, goat and donkey milk were collected from different cities in Egypt. The standard curves for each type of milk were prepared using competitive ELISA and the samples were tested for aldrin/dieldrin. The level of pesticide was obtained from the standard curve for each type of milk. The assay also was applied in a high protein system, viz .eggs, and proved to be capable of detecting aldrin/dieldrin at very low levels.

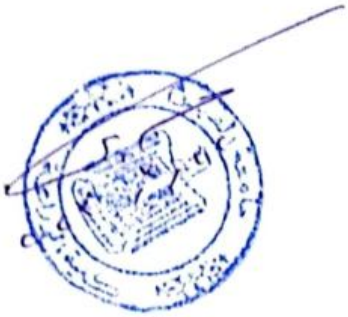
5.2 The development of ELISA for the detection of ethyl parathion based on monoclonal antibod

Antibodies were raised in vivo against ethyl parathion using appropriate hapten-protein conjugate .Polyclonal antisera harvested from immunised animal was not sensitive enough to be employed as the basis for ELISAs for the detection of ethyl parathion. Specific fusion technique, employed to isolate specific antibody producing cells in vitro, were successful in isolating one hybrid clones which produced sensitive and specific monoclonal antibodies. The antibody was used to develop an ELISA systems which was optimised for maximum efficiency. The ELISA was capable of detecting ethyl parathion at picomole concentrations.

A series of cross-reactivity assays were carried out using compounds of similar chemical structure to ethyl parathion. The apparently that antibody interact with the phosphorus aromatic side of the molecule rather than the oxyalkyl part which is common in all the related pesticides.

The possibility of using this assay to detect levels of parathion in milk was examined using an indirect competitive assay. The results indicate that the assay can be used equally well in milk as in water.

Ii is hoped that the current assays are successful enough to permit their production on a commercial scale. It could be thus employed as a rapid and simple method for the detection of these two pesticides.



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