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RAPID ASSAYS FOR ENVIRONMENTAL AND BIOLOGICAL MONITORING

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ABSTRACT

Rapid, inexpensive, sensitive, and selective enzyme-linked immunosorbent assays (ELISAs) now are utilized in environmental science. In this laboratory, many ELISAs have been developed for pesticides and other toxic substances and also for their metabolites. Compounds for which ELISAs have recently been devised include insecticides (organophosphates, carbaryl, pyrethroids, and fenoxycarb), herbicides (s-triazines, arylureas, triclopyr, and bromacil), fungicides (myclobutanil), TCDD, and metabolites of naphthalene and toluene. New rapid assays have been developed for mercury.

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INTRODUCTION

Advantages of ELISAs include high sensitivity and selectivity, minimal sample preparation, high sample throughput, and low cost. ELISAs are well suited for monitoring large numbers of environmental and biological samples. The ELISA technique has some shortcomings, e.g., cross-reactivity with compounds structurally related to the analyte, matrix interferences, and limited availability of polyclonal antibodies. Immunoassay usually compares favorably with instrumental analysis if the target compound is hydrophilic, nonvolatile, and of high molecular weight.

MATERIALS AND METHODS

Various aspects of ELISA development have been extensively reviewed (Jung et al., 1989; Hammock, et al., 1990; Szurdoki et al., 1995c). The steps below describe briefly the development of our ELISAs.

A. Hapten Design and Synthesis

Ideal haptens are structurally very similar to the analyte molecule and possess a spacer arm terminated by a functional group for coupling to proteins. Avoiding masking characteristic groups on the target molecule (e.g., attaching the spacer group at a carbon atom of the analyte far from polar moieties) is often necessary for the production of high affinity antibodies. Hapten design is also based on the need for either a compound-specific or a class-selective assay. In addition, ease of chemical synthesis, stability and solubility of the hapten are important criteria.

B. Conjugation of Haptens to Macromolecular Carriers

To elicit strong immune response, the immunizing hapten is conjugated to an immunogenic carrier protein. This hapten and others are also conjugated to enzymes and

other proteins for use in ELISAs. Mild reaction conditions are used to preserve enzyme activity. For the protein conjugates, stability under conditions of the coupling reaction, immunization, and ELISA are important. The conjugation method used depends on the hapten's functional group. The conjugation reactions are confirmed and hapten densities estimated.

C. Immunization

In our lab, polyclonal antibodies are obtained primarily from rabbits. Immunogen protein conjugates emulsified with an adjuvant are injected intradermally. Following the initial immunization, boost injections are given every 4 weeks, and blood samples are drawn 10 days after boosts to monitor antibody production. When the resulting antibodies display sufficiently high titers, animals are sacrificed. The serum is isolated by centrifugation, preserved with sodium azide and stored at -20 °C.

D. Antiserum Characterization

Antibody titers are determined using various plate coating antigens, obtained from several haptens and proteins, to identify useful antibody-coating antigen combinations for the assay development. Inhibition experiments are then conducted with the target analyte and chemicals with similar structure to reveal antibody selectivity.

E. Assay Development, Optimization, and Validation

Several common variants of two principal ELISA formats, using either immobilized antigen or antibody, are employed. The effect of a number of coating antigens and enzyme tracers, produced from several haptens and proteins or enzymes, on the assay performance is usually investigated. Assay conditions including incubation times, temperature, pH, and salt concentration are optimized. Solvent and matrix interferences are then studied. Sample preparation/cleanup is devised if the assay performance would be significantly compromised by the matrix of interest. ELISAs are validated with spike-recovery studies using water, soil, urine, blood, and other environmental and biological sample matrices.

RESULTS AND DISCUSSION

Rapid assays (Table 1) have recently been developed in our laboratory for the following compounds:

A. Insecticides

ELISAs have been developed for the detection of the 4-nitrophenol metabolites of some common organophosphorus insecticides (e.g., methyl and ethyl parathion, fenitrothion) in water, soil, urine, and food samples (Li et al., 1991; Harris et al., 1995a). Carbaryl, a major carbamate insecticide, is applied to many fruit, vegetable, and field crops. An ELISA has been used for the analysis of carbaryl in soil, water, foods, and urine (Marco et al., 1993a). Pyrethroids are widely used in agriculture, forestry and for household purposes. Assays have been devised for fenvalerate, fenpropathrin, and their metabolites (Wengatz et al., 1995). An ELISA was developed for the insect growth regulator (IGR) fenoxycarb (Székács et al, 1995).

B. Herbicides

s-Triazine herbicides are used worldwide. From a library of haptens and polyclonal and monoclonal antibodies, ELISAs for the detection of several parent *s*-triazines and their metabolites in natural waters, soil, urine, and foods have been developed (Goodrow et al., 1990; Schneider and Hammock, 1992; Lucas et al., 1993; Kido et al., 1995; Jaeger et al., 1995). ELISAs were devised for several arylurea herbicides (diuron, monuron, and linuron) (Schneider et al., 1994; Karu et al., 1994). An ELISA for triclopyr has been developed (Harris et al., 1995b). This herbicide is used for post-emergence control of a number of broadleaf and woody plants in forests. Bromacil is used to protect citrus and pineapple and is also applied as a general weed killer on noncrop land. Immunoassays in various formats were devised for the analysis of this herbicide (Szurdoki et al., 1992, 1995c; Bekheit et al., 1993; Wengatz et al., 1995).

TABLE 1
Selected Rapid Assays.

Compound (Description)	Limit of Detection [pg/mL] Assay Format (Antibody*)	Reference
4-nitrophenol (insecticide metabolite)	200 ELISA (P)	Li et al., 1991; Harris et al. 1995a
carbaryl (insecticide)	50 ELISA (P)	Marco et al., 1993a
fenoxycarb (insecticide [IGR])	4,000 ELISA (P)	Székács et al., 1995
atrazine (*s*-triazine herbicide)	30 ELISA (M)	Schneider and Hammock, 1992
diuron (arylurea herbicide)	40 ELISA (P) 600 ELISA (M)	Schneider et al., 1994 Karu et al., 1994
triclopyr (herbicide)	1,000 ELISA (P)	Harris et al., 1995b
bromacil (herbicide)	10 ELISA (P) 50 near-IR immunoassay (P)	Szurdoki et al., 1992 Wengatz et al., 1995
mercapturic acid conjugate of naphthalene (urinary metabolite)	5 ELISA (P)	Marco et al., 1993b
Hg^{2+} (toxic metal ion)	75 sandwich chelate assay	Szurdoki et al., 1995

* P: polyclonal; M: monoclonal.

C. Fungicide

An ELISA was developed for the systemic fungicide myclobutanil (Székács and Hammock, 1995). This compound is widely used to prevent fungal infection in both agriculture and industry.

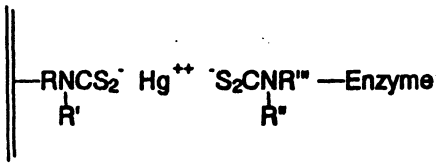

FIGURE 1

Structures of the reagents used in the sandwich chelate assay. R, R', R", and R'" are alkyl groups.

D. Other Toxic Chemicals, Metabolites

As part of a project to develop noninvasive methods for monitoring human exposure to toxic compounds (Harris et al., 1995a), ELISAs have been developed to detect mercapturic acid metabolites of naphthalene (Marco et al., 1993b; Zheng et al., 1995), toluene, and benzyl bromide (Jaeger et al., 1995). Assays have been devised for generalized detection of mercapturic acid conjugates as urinary biomarkers of exposure to electrophiles. ELISAs are being developed for 2,3,7,8-tetrachlorodibenzodioxin (TCDD). New rapid assays for mercury, based on the formation of sulfur containing chelates have been devised (Szurdoki et al., 1995a, 1995b). One of these assays involves a sandwich chelate formed by a chelator supported on the well of the ELISA plate, the mercuric ion, and a chelator linked to the reporter enzyme (Figure 1).

E. New Applications of Immunoassays and Antibodies

Simultaneous quantification of several structurally related triazine herbicides has been achieved by mathematical evaluation of data obtained by a set of antibodies with different cross-reactivities for each congener (Wortberg et al., 1995). Techniques of molecular biology have been used to develop recombinant antibodies (Ward et al., 1993). Our

reagents are currently being applied for various novel immunoassay formats (e.g., near-IR immunoassay, immunosensor) (Wengatz et al., 1995).

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