Fiber Optic Biosensor for Cyclodiene Insecticides

Kathleen E. Brummel,[†] Jeremy Wright,*[,†] and Mohyee E. Eldefrawi[‡]

Department of Biomedicinal Chemistry, School of Pharmacy, University of Maryland at Baltimore, 20 North Pine Street, Room 500, Baltimore, Maryland 21201, and Department of Pharmacology and Experimental Therapeutics, School of Medicine, University of Maryland at Baltimore, 655 West Baltimore Street, BRB 4-029, Baltimore, Maryland 21201

Chlorendic caproic acid (CCA) was used to synthesize hexachlorocyclopentadienefluorescein (FL) and bovine serum albumin (BSA) conjugates. Anti-CCA antibodies (CCA−Abs), which were raised against BSA−CCA and immobilized on quartz fibers, bound FL−CCA selectively and reversibly. Fluorescence generated by evanescent excitation of the bound FL−CCA was used to monitor the binding event. The affinity of CCA−Abs for FL−CCA (K_D = 1.9 nM) was calculated from the time courses of association and dissociation of FL−CCA. The cyclodiene insecticides chlordane, heptachlor, dieldrin, endrin, aldrin, and endosulfan competed with FL−CCA for binding to CCA−Abs and reduced fluorescence in a concentration-dependent manner with the following rank order: chlordane > heptachlor > dieldrin > aldrin > endosulfan. This fiber optic fluoroimmunosensor detects cyclodiene insecticides at the ppb level, has low cross-reactivity with γ-hexachlorocyclohexane, and does not detect (*p*,*p*'-dichlorodiphenyl)trichloroethane (DDT).

Keywords: *Cyclodiene insecticides; fiber optic biosensor; fluoroimmunoassay; detection*

INTRODUCTION

The cyclodiene insecticides dieldrin, aldrin, heptachlor, chlordane, endrin, and endosulfan are polychlorinated hydrocarbon compounds. A characteristic polychlorinated bicyclic ring system is found in each member of this series. They were first synthesized via a Diels−Alder reaction in the 1940s as an insecticide alternative to DDT (Ungnade and McBee, 1958; ATSDR, 1989). Cyclodiene insecticides were heavily used in agriculture and were popular because of their broad spectrum action, high insecticidal activity, and persistence at the site of exposure. In addition, they were highly effective against certain pests such as fire ants and termites (EPA, 1974).

The high lipid solubility of polychlorinated hydrocarbon insecticides caused them to be readily taken up and bioconcentrated in animal fat depots, such as adipose tissues, nerves, and brain (Geyer *et al.*, 1993). Heptachlor was reported to bioaccumulate 200−37000 times from water into hydrobiota (WHO, 1984). Aldrin, heptachlor, and chlordane were shown to be highly resistant to degradation in soil and persist for years. Endosulfan was shown to be biodegradable and is still used today. Heptachlor epoxide and dieldrin persisted in both aerobic and anaerobic environments (Tu and Miles, 1976; Pfaffenberger *et al.*, 1994). The biomagnification throughout the food chain and persistence in the environment (Geyer *et al.*, 1993; Metcalf *et al.*, 1973; Cole *et al.*, 1976) rendered cyclodiene insecticides ecologically harmful and environmentally hazardous (Wurster, 1971; Rimkus and Wolf, 1987; Van Wijnen and Stijkel, 1988; Bloomquist, 1992; Kilburn and Thornton, 1995). This led to a ban on the use of cyclodiene insecticides in the 1970s by the Environmental Protection Agency (EPA). However, residues can still be found in soil from applications made 20 years ago (ATSDR, 1989; Kilburn and Thornton, 1995; Rimkus and Wolf, 1987; Van Wijnen and Stijkel, 1988; Murphy, 1995).

A continuing problem is that leaching into the environment occurs from hazardous waste sites which contain unknown amounts of cyclodiene insecticide residues that are unevenly distributed. Due to the over abundance of chemical waste containing mixtures of many pesticides, a quick and highly specific detection method is warranted in order to locate and properly dispose of these toxic compounds. A recent special report discusses the current status and future applications for monitoring pesticide residues in the environment (Krämer, 1996). Several analytical methods are available for analysis of cyclodiene insecticides. Extraction techniques along with gas chromatography/mass spectrometry (GC/MS) have been employed on these compounds (ATSDR, 1989; Rimkus and Wolf, 1987; Sovocool *et al.*, 1977; Van Wijnen and Stijkel, 1988). Because γ-aminobutyric acid (GABA) receptor is the molecular target for cyclodienes, a ligand binding assay to this receptor was used to measure the amount of α-endosulfan in blood samples (Saleh *et al.*, 1993). Enzyme-linked immunosorbant assay (ELISA) has been developed for the detection of organochlorine pesticide residues; the limit of detection was 0.01−0.03 ppm (Wigfield and Grant, 1992). A radioimmunoassay for dieldrin and aldrin was developed, using their carboxyl-substituted analogues bound to horse serum albumin (HSA) to produce polyclonal antibodies (Abs) (Langone and Van Vunakis, 1975). The Ab specificity was directed toward the distal part of the hapten, i.e. the hexachlorobicyclic ring system. Dieldrin was detected at 150 pg, aldrin at 700 pg, and heptachlor and chlordane at higher concentrations. Little cross-reactivity was seen with other chlorinated hydrocarbons such as lindane. A cyclodiene-sensitive monoclonal Ab (mAb), derived from an aldrin analogue, was also produced and used in pharmacological studies on GABA receptor (Esser *et al.*, 1991).

Fiber optic immunoassays have been shown to be

* Author to whom correspondence should be addressed [telephone (410) 706-2114; fax (410) 706-0346; e-mail wright@pharmacy.ab.umd.edu].

[†] School of Pharmacy.

[‡] School of Medicine.

S0021-8561(97)00119-2 CCC: $14.00

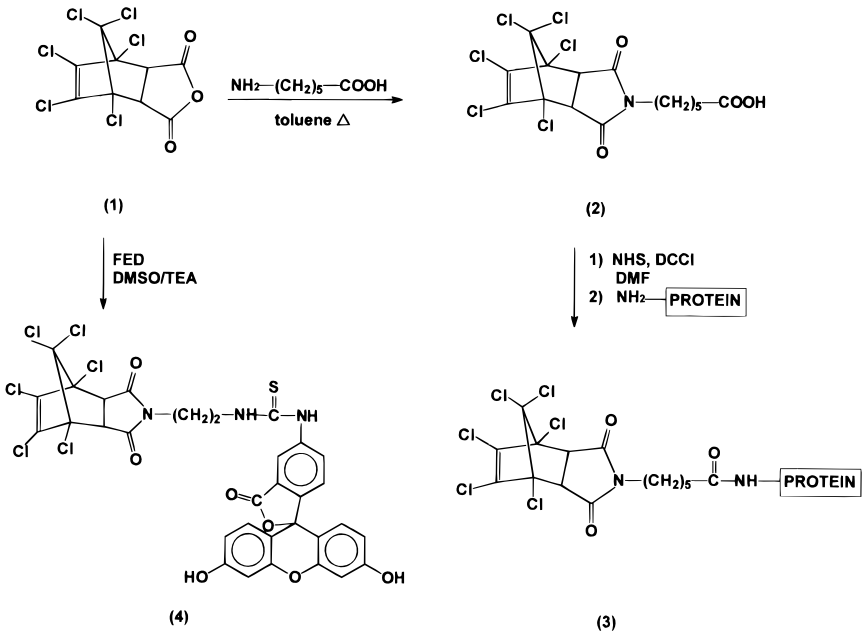

Figure 1. Synthetic scheme of CCA−hapten (**2**) and CCA−BSA antigen (**3**). Fluorescent probe, FL−CCA (**4**), is synthesized via chlorendic anhydride (**1**) with FED.

highly specific and sensitive toward target analyte(s) and are promising for future field use (Rogers *et al.*, 1991; Anis and Eldefrawi, 1993; Eldefrawi *et al.*, 1993). The fiber optic biosensor consists of Abs immobilized on the surface of a quartz fiber and is used to detect fluorescent analytes by means of the evanescent wave, which causes excitation of the bound fluorescent analyte. Measurement of the target analyte(s) is achieved by competitive displacement of the fluorescent analyte by selective transduction of a parameter of the biomolecule−analyte reaction into a quantifiable signal (Byfield and Abuknesha, 1994).

In this study, a fiber optic evanescent fluorosensor was developed using, as biological sensing elements, polyclonal Abs isolated from rabbit immune serum and immobilized covalently onto the quartz fibers. A fluorescent conjugate of the target compound (designated as the probe), which fluoresces when bound to the Ab, was used as the optical signal generator. The presence of a cyclodiene insecticide in the flow buffer competitively displaced the fluorescent probe, thereby reducing the fluorescence. This biologically optimized molecular recognition (Ab−Ag interaction) was used to detect and quantitate cyclodiene and other chlorinated hydrocarbons.

MATERIALS AND METHODS

Chemicals. Chlordane, dieldrin, aldrin, heptachlor, endrin, lindane, DDT, endosulfan, and chlordecone (99.3−100% purities) were obtained from ChemService (West Chester, PA). Chlorendic anhydride (97% purity), 6-aminocaproic acid, and ethylenediamine dihydrochloride as well as other chemicals were from Aldrich Chemical (Milwaukee, WI). Fluorescein isothiocyanate (90% purity), bovine serum albumin (96−98% purity), casein, goat anti-rabbit IgG (whole molecule), alkaline phosphatase conjugate, *p*-nitrophenyl phosphate, and other chemicals used in the ELISA were purchased from Sigma and Sigma Immuno Chemical Co. (St. Louis, MO). Commercial anti-heptachlor neat serum (from BioDesign International, Kennebunk, ME) was purified with a Protein A affinity column from Pierce (Rockford, IL).

Apparatus. Experiments were performed on the ORD portable fluorometer (ORD, Inc., North Salem, NH), as described by Rogers *et al*. (1989). The instrument was used to measure the fluorescence generated by the fluorescent probe (FL-CCA), when bound to the Abs on the surface of the quartz fiber within the evanescent zone, which extends 1000 Å from the surface. The quartz fiber (1 × 60 mm) (Wale Apparatus, Hellertown, PA) was placed inside a flow cell of 46 μL capacity, which was exchanged by selected solutions every 14 s. The instrument used a 485 nm excitation filter with a 20 nm band pass and a 530 nm emission filter with a 30 nm band pass. A photon source was generated with an InGaN/AlGaN/GaN blue-light-emitting diode operated at 20 mA (Nichia Chemical Industries LTD, Japan) and was detected with a Hamamatsu S-1087 silicon detector.

Chemical Structure Elucidation. Nuclear magnetic resonance (NMR) spectra were obtained by a General Electric QE-300 Fourier transform spectrometer. Thin layer chromatography (TLC) was run with analytical silica gel plates (Fisher Scientific, Pittsburgh, PA). Elemental analysis (C, H, N, Cl) was performed on the synthesized compounds at Atlantic Microlab, Inc., Norcross, GA.

Synthesis of 6-(1,4,5,6,7,7-Hexachlorobicyclo[2.2.1]-heptane-2,3b-succinimido)caproic Acid [Chlorendic Caproic Acid (CCA, 2), Figure 1).] 6-Aminocaproic acid (0.7 g, 5.3 mmol) was added to a solution of chlorendic anhydride (**1**) (2 g, 5.4 mmol) and equimolar triethylamine (TEA) in 15 mL of toluene in a round bottom flask fitted with a Dean-Stark water separator. The solution was refluxed for 8 h, toluene was removed under vacuum, and the residue was triturated with 20 mL of 1% HCl. The remaining tan solid was collected by vacuum filtration and crystallized from a hexane/methylene chloride solution (5:1). Yield was 2.3 g (88%) of white crystals: mp 150−151 °C; TLC (4/1 ethyl acetate/methanol/1% acetic acid) R_f =0.7; ^{1}H NMR (DMSO-d_6): δ 1.2 (m, 2H), 1.3 (m, 2H), 1.4 (m, 2H), 2.1 (t, 2H), 3.28 (t, 2H), 4.06 (s, 2H), 12.02 (s, 1H). Anal. C, H, N, Cl.

Synthesis of CCA−BSA (3) and Fluorescein (4) Conjugates (Figure 1). Fluoresceinthiocarbamoylethylenediamine (FED) was synthesized according to Pourfarzaneh *et al.* (1980). Briefly, ethylenediamine dihydrochloride was reacted with fluorescein isothiocyanate (5:1 molar ratio) in methanol with a small amount of TEA. An orange precipitate (**4**) was collected, washed with methanol (70% yield), and reacted directly with an equimolar amount of chlorendic anhydride (**1**) in dimethylformamide (DMF) and TEA. The reaction mixture was acidified with 0.5 M HCl, and the orange precipitate was isolated by filtration. TLC (5/1 ethyl acetate/methanol/1% acetic acid) showed an R_f = 0.8. NMR showed all characteristic peaks of hexachlorocyclopentadiene fluorescein (FL-CCA, compound **4**). Carboxylic hapten (**2**) (0.96 g,

0.2 mmol), *N*-hydroxysuccinimide (NHS) (0.2 mmol), and dicyclohexylcarbodiimide (DCC) (0.45 g, 2.2×10^{-4} mol) were dissolved in 1.0 mL of anhydrous DMF. The solution was stirred for 3.5 h at 15 °C, and the precipitated dicyclohexylurea was removed by centrifugation. The DMF supernatant was added to a solution of 200 mg of BSA, 20 mL of water, and 4.2 mL of DMF. The white reaction mixture was stirred gently at 4 °C for 24 h to complete the conjugation. Extensive dialysis against four changes of 4 L of 10 mM phosphate-buffered solution (PBS) over 4 days was used to purify the conjugate. The product was lyophilized, and the resulting white solid was analyzed. C, H, N, Cl analysis showed approximately 37 molecules of CCA coupled to 1 molecule of BSA.

Indirect ELISA Analysis. Microtiter plates (96-well) were coated with 50 μL of 5 μg/mL CCA−BSA in a 0.1 M carbonate buffer and stored at 4 °C overnight (18 h). The plates were then washed twice with Tris-buffered saline (TBS) and 0.5% Tween 20 (wash buffer), and then all the wells were blocked with 300 μL/well of 10% skim milk with 2% sodium azide (blocking buffer) for 2 h. Subsequently, the plates were washed four times with wash buffer and incubated with 50 μL/well of a 1:1000 dilution of commercial anti-heptachlor Ab in blocking buffer for 1 h. After 1 h the plates were again washed four times with wash buffer and treated with 50 μL/well of goat anti-rabbit IgG (whole molecule) alkaline phosphatase conjugate in blocking buffer for 1 h. The plate was rinsed six times with wash buffer and developed with *p*-nitrophenyl phosphate in a 50 mM carbonate buffer and 5 mM $MgCl_2$ solution at 50 μL/well. A color change was detected immediately, and the OD values at 405 nm and 630 nm were determined on an EL312e Biokinetics plate reader. CCA−BSA (**3**) was detected by commercial anti-heptachlor at dilutions of 1:8000, an indication of successful formation of a CCA−BSA conjugate. The same procedure was used to synthesize CCA−casein for use in ELISA assays.

Preparation of Polyclonal Ab to CCA. A New Zealand White rabbit (Hazelton, PA) was injected intramuscularly at three or four sites with 1 mL containing 1:1 Freund's complete adjuvant and 1 mg/mL CCA−BSA conjugate (**3**). The rabbit was boostered four times intramuscularly with 1 mg/mL CCA−BSA and Freund's incomplete adjuvant in a 1:1 ratio (0.8−1 mL per booster) over a period of 3 months. Immune serum (20 mL) was collected through cardiac puncture, and Ab titers were determined by ELISA. A CCA−casein conjugate and BSA (all at 5 μg/mL) were coated on 96-well microtiter plates and tested with immunized rabbit serum and control rabbit serum. Titers were detected with goat anti-rabbit IgG (whole molecule) alkaline phosphatase conjugate and *p*-nitrophenyl phosphate as a substrate. Strong responses were obtained at 1:64000 for the CCA−casein-coated wells and less response to the BSA. No response was seen for the unimmunized serum with CCA−casein or BSA.

Purification of Anti-CCA Abs. The rabbit antiserum was purified using an ImmunoPure Plus IgG purification kit following the manufacturer protocol. Briefly, the column was first equilibrated with 5 mL of ImmunoPure binding buffer, and then 4 mL of serum, diluted 1:1 with binding buffer, was added. The column was immediately washed with 15 mL of binding buffer in order to remove any non-IgG proteins. The bound IgG was then eluted with 5 mL of elution buffer, and 5 × 1 mL fractions were collected. Absorbance was monitored at 280 nm in a Beckman DU-50 spectrophotometer. Two concentrated IgG fractions were collected. The purified IgG was dialyzed against 3 × 4 L of 10 mM PBS over a period of 2 days. Subsequent analysis for protein concentration (Lowry *et al.*, 1951) indicated 12.48 mg/mL.

Covalent Immobilization of Abs on the Quartz Fibers. This was accomplished by a modified protocol of Bhatia *et al.* (1989). The fibers were washed successively in HNO_3, H_2SO_4, and HCl−EtOH, rinsed in double-distilled water, and dried at 70 °C. The clean fibers were then silanized with (3-mercaptopropyl)methoxysilane to introduce an active SH group, and then reacted with the heterobifunctional cross-linking reagent *N*-[(γ-maleimidobutyryl)oxy]succinimide. The fibers were then individually incubated in 40 μL of Ab solution (50 μg/mL) placed inside a microcapillary tube (Kimax 51) at 4 °C for 14 h. The fibers were then washed with 50 mM PBS and stored until use in PBS containing 0.05% of NaN_3.

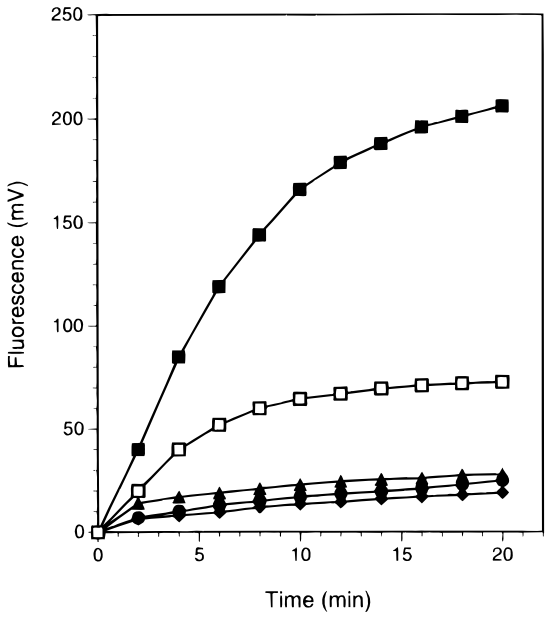

Figure 2. Specificity of the fluorescent signal transmitted by quartz fibers coated with anti-CCA Abs when perfused with flow buffer containing 10 nM FL−CCA (■, □), 10 nM FL−α-BGT (●), 10 nM FL−benzoylecgonine (▲), or 10 nM FL−atrazine (◆). The quartz fibers coated with anti-heptachlor Abs fluoresce when perfused with buffer containing 10 nM FL−CCA ($_a$).

Fluorescence Measurements. The Ab-coated fibers were mounted in the flow cell of the ORD fluorometer and perfused with PBS containing 0.1% casein (100 μg/mL) for a period of 5 min to establish baseline fluorescence. FL−CCA (10 nM) was added to the buffer, and the fluorescence generated in the evanescent zone, as a result of binding of CCA−FL to the Ab on the surface of the fiber, was transmitted through the fiber to the silicon detector, translated into voltage, and recorded.

RESULTS

Specificity of the Fluoroimmunosensor. The binding of FL−CCA to anti-CCA Ab-coated fibers resulted in a fluorescent signal, the amplitude of which increased with higher FL−CCA concentrations. At 10 nM FL−CCA the fluorescence increased with higher anti-CCA Ab density on the fiber. When the anti-CCA Ab-coated fibers were perfused with flow buffers, each containing 10 nM of FL−CCA, FL−atrazine, FL−α-bungarotoxin (α-BGT) or FL−benzoylecgonine, strong fluorescence was generated only with FL−CCA (Figure 2). Very low fluorescence (<10%) was generated by the other FL reagents, most likely due to nonspecific binding. Fibers coated with the commercially available anti-heptachlor Abs had lower binding capacity of FL−CCA (Figure 2) and slightly higher nonspecific binding of the other three fluorescent reagents (data not shown). Nonspecific binding to the anti-heptachlor Ab-coated fiber amounted to 28% of FL−CCA binding compared to only 10% for the anti-CCA Ab-coated fiber. These values were used to correct for specific binding in subsequent experiments. Fibers coated with control rabbit IgG or BSA failed to bind FL−CCA and transmitted no fluorescence above the base line level. Perfusion with 10 nM FL−CCA rapidly increased the fluorescence transmitted by anti-CCA Ab-coated fibers and reached a steady state in 30−40 min, while removal of FL−CCA from the flow buffer decreased fluorescence in a time-dependent manner (Figure 3). Since fluores-

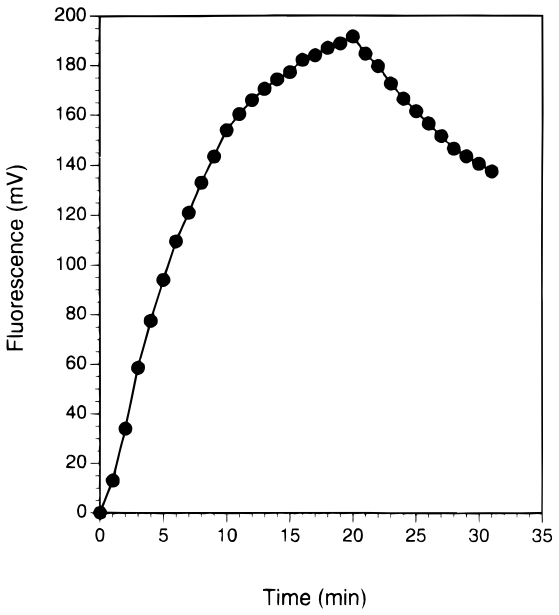

Figure 3. Time course of fluorescent increase as a result of association of FL−CCA (10 nM) with anti-CCA-coated quartz fibers (50 μg/mL). After 20 min, FL−CCA was withdrawn from the flow buffer and the dissociation time course monitored for 10 min.

cence originates in the evanescent zone, it is safe to assume that the increase and decrease in fluorescence represent binding to, and dissociation of FL−CCA from, the fiber. All studies were performed with single-use fibers.

Kinetics of Association and Dissociation of FL−CCA with Fibers Coated with Anti-CCA Abs. The apparent association rate constant (k_{app}) was calculated from the slope of the initial 15 min of the rising portion of the linear plot of $\ln(Fl_{ss}/Fl_{ss} - FL_t)$ *vs* time. Fl_{ss} is the steady state value measured after 60 min; t is the time interval at which fluorescence was measured. The dissociation rate constant (k_{-1}) was calculated by determining the halftime ($T_{1/2}$) of dissociation following removal of FL−CCA from the flow buffer after reaching the steady state. The semilogarithmic plot of fluorescence versus time was then used to obtain $T_{1/2}$ followed by k_{-1}, calculated from the formula $k_{-1} = 0.693/T_{1/2}$. The association rate constant k_1 was calculated from the formula $k_1 = (k_{app} - k_{-1})/[FL-CCA]$. The affinity constant K_D was calculated using the formula $K_D = k_{-1}/k_1$. The K_D value for FL−CCA binding to anti-CCA Abs was determined to be 1.9 nM. For the commercial anti-heptachlor Abs we saw two dissociation components. The major component was the slow dissociating one with $T_{1/2} = 13$ min and $K_D = 2.8$ nM.

Competitive Displacement of FL−CCA by Cyclodiene Insecticides. The affinity of the immunosensor for cyclodiene insecticides was determined from a plot of fluorescence *vs* time. Fluorescence resulting from binding of FL−CCA to anti−CCA Ab-coated fibers was reduced in the presence of chlordane (0.1−1000 nM) in a concentration dependent manner (Figure 4A). Linear transformation of the percent inhibition of binding at steady state allowed accurate determination of the concentration required to obtain 50% inhibition (i.e. IC_{50}) (Figure 4B). The following was the rank order of competitive inhibition of FL−CCA binding by the insecticides: CCA > chlordane > heptachlor > dieldrin > aldrin (Figure 5). The IC_{50} values for the insecticides was calculated from the linear transformation of the competitive inhibition data (Table 1), using the Cheng−Prusoff (1973) equation $[K_i = IC_{50}/(1 + [L]/K_D)]$, where [L] is the concentration of the fluorescent probe. To calculate the K_i values, [L] was 10 nM and K_D was 1.9 nM for anti-CCA. Chlordane and heptachlor had K_i values of 1.38 and 1.66 nM, respectively; followed by dieldrin with a K_i of 7.59 nM and aldrin with a K_i of 18 361 nM.

Cross-Reactivity with Other Chlorinated Hydrocarbons. Because the cyclodiene insecticides mimic CCA, this compound was used to generate the antisera and the fluorescent probe. It was thought that the anti-CCA Ab would have highest affinity for CCA, but would cross-react with structurally related chlorinated hydrocarbon compounds. The concentration selected to generate cross-reactivity data was 0.1 μM of CCA, which gave complete inhibition of fluorescence, but significant partial inhibition by aldrin, the least inhibitory compound. The anti-CCA Ab immunosensor showed 60 and 64% cross-reactivity to heptachlor and chlordane, respectively, but only 20 and 50% to endrin and dieldrin, respectively. The cross-reactivity at different concentrations of chlordane levels changes the ranking of chlordane and heptachlor. This change still results in detection of heptachlor and chlordane better than dieldrin and aldrin. Its cross-reactivity to endosulfan was very low (9%). DDT, lindane, and chlordecone showed cross- reactivities ranging from 0 to 8% (Table 1). Cross-reactivities of immunosensors using the commercially available anti-heptachlor Abs for the cyclodiene insecticides were different (Table 1), with higher cross-reactivity for the cyclodiene insecticides than for CCA.

DISCUSSION

The specificity of the fiber optic biosensor coated with anti-CCA Abs is evidenced by the strength of the fluorescent signal produced when it binds FL−CCA. The unrelated chemicals FL−α-BGT, FL−benzoylecgonine, or FL−atrazine (Figure 2) produce low, if any, fluorescence at the same concentrations. The signal is time-dependent and is detectable in 1 min. Binding of the FL−CCA to the CCA−Ab-coated fiber is reversible (Figure 3), as expected of the Ag−Ab interaction. The anti-CCA Ab detects chlordane by inhibition of the fluorescence signal in a dose dependent manner (Figure 4A). As little as 0.1 nM chlordane is easily detectable and within 1 min.

It is evident that this fluoroimmunosensor can detect and quantitate cyclodiene insecticides (Figures 4 and 5 and Table 1). It utilizes the same competitive immune assay strategy described previously for detection of polychlorinated biphenyls (Zhao *et al.*, 1995) and imazethapyr herbicide (Anis and Eldefrawi, 1993). It is highly sensitive, detecting 0.3 ppb FL-CCA (equivalent to 1 nM) and chlordane and heptachlor at 1 nM (Figure 5). Dieldrin and aldrin are detected at 10 and 1000 times higher concentration; therefore the biosensor has lower sensitivity toward these compounds. The sensitivity of the biosensor is comparable to that of a radioimmunoassay, reported to detect 0.15 ppb dieldrin and 0.7 ppb aldrin (Langone and Van Vunakis, 1975). Their detection levels of heptachlor and chlordane by the radioimmunoassay were 13 and 26 times less than dieldrin. The hapten used to synthesize the Ag to raise Abs used in that radioimmunoassay was a carboxylic acid derivative of dieldrin. An ether derivative of aldrin was also used to raise mAbs that bound chlordane and endosulfan (Esser *et al.*, 1991). This mAb cross-reacted

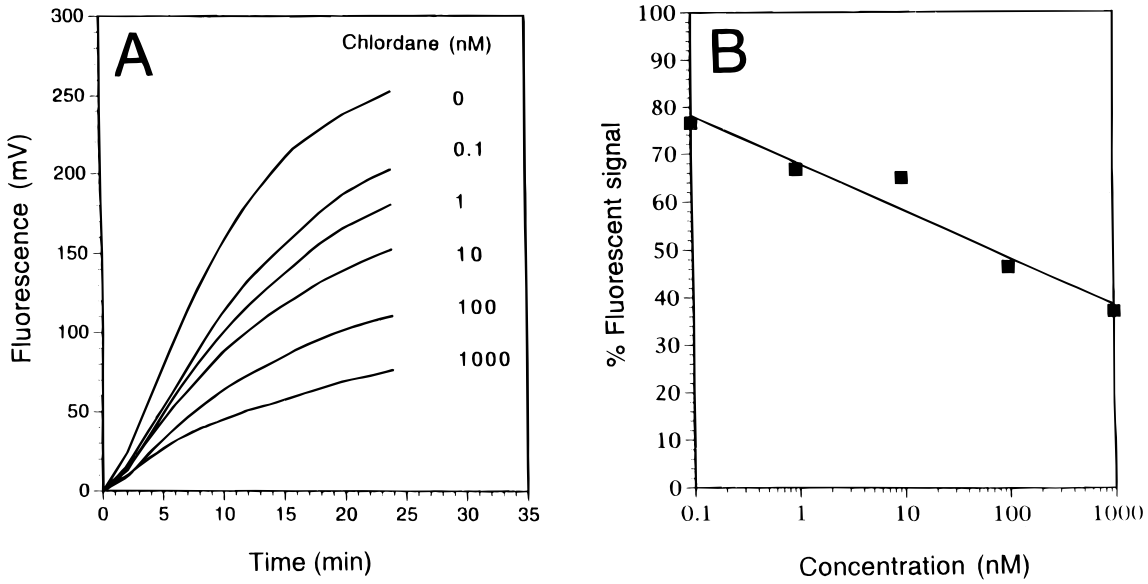

Figure 4. Concentration-dependent inhibition of FL−CCA binding to anti-CCA-coated quartz fibers by chlordane (A). The logarithmic transformation of inhibition versus percent fluorescent signal (B).

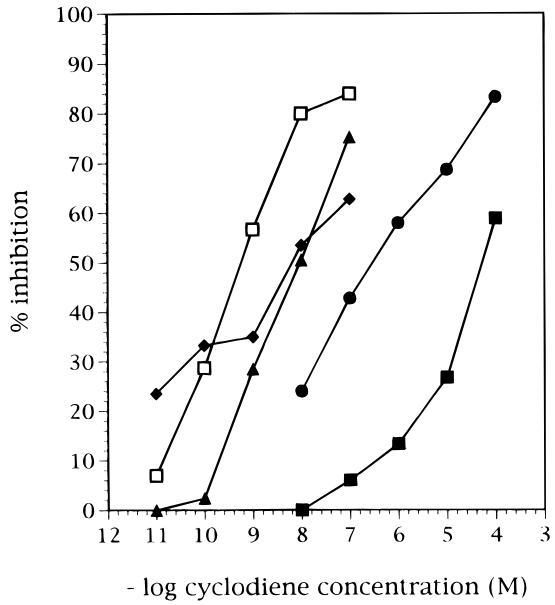

Figure 5. Dose-dependent competitive inhibition of fluorescence of anti-CCA-coated fibers (50 μg/mL) by CCA (□), aldrin (□), dieldrin (●), heptachlor (▲), and chlordane (◆). Each symbol represents the mean of duplicate or triplicate experiments with SD < 14%.

with heteroadamantanes, which are nonchlorinated and bear little structural resemblance to cyclodiene insecticides. However, they bind to the same site on the GABA receptor that binds cyclodiene insecticides. Thus the carboxylic acid-derived Abs appear to be selective.

Heptachlor and chlordane contain a hexachlorobicyclic ring system similar to that of CCA (Figure 6) and also have a five-membered ring system with one or two chlorine atoms. It is evident that heptachlor and chlordane compete with FL−CCA binding to the anti-CCA Ab-coated fiber better than the other cyclodiene insecticides tested (Table 1). However, the K_i values of chlordane (K_i = 1.38 nM) and heptachlor (K_i = 1.66 nM) are 10 times lower than that of CCA (K_i = 0.16 nM). Aldrin, dieldrin, and endrin, with more complex stereochemical structure and less likelihood of being recognized by the anti-CCA Ab, have even lower K_i values (Table 1).

Table 1. Cross-Reactivities of Anti-CCA and Commercial Anti-Heptachlor-Coated Fibers with Insecticides

		cross-reactivities[b]	
compound	K_i (nM) of anti-CCA[a] Ab	anti-CCA Abs	anti-heptachlor Abs
CCA	0.16 ± 0.013	100	100
chlordane	1.38 ± 0.011	63.7	123
heptachlor	1.66 ± 0.004	60.1	121
dieldrin	7.59 ± 0.003	51.0	114
aldrin	>1000	7.1	135
endrin	>1000	20.2	90
endosulfan	>1000	9.0	38
lindane	>1000	8.3	0
chlordecone	>1000	2.4	0
DDT	>1000	0	0

[a] Mean K_i values of duplicate or triplicate experiments ± SD, i.e. the mean value of all the sums of all measurements. [b] Cross-reactivity values of chlorinated insecticides (each obtained from triplicate experiments) are calculated as follows: the rate of fluorescence decrease caused by the addition of 100 nM of CCA to the flow buffer is considered as 100. The initial rate of fluorescence decrease in the presence of 100 nM of each compound is divided by that for CCA to obtain the cross-reactivity index. A cross-reactivity value above 100 indicates detection of the compound at lower concentrations than CCA.

The commercial anti-heptachlor Ab has lower affinity for FL−CCA (Figure 2) but higher affinity for all the cyclodiene insecticides (Table 1). The higher cross-reactivity means that the anti-heptachlor Abs, although having lower affinity for the probe, have higher sensitivity in detecting cyclodiene insecticides. Thus, while the use of CCA as a hapten to generate anti-cyclodiene Abs is successful, other antigens may generate Abs with even higher sensitivity for cyclodiene insecticides. The cross-reactivity of the biosensor with lindane and chlordecone, although low (<10%), is still detectable. Since cross-reactivity was tested at 0.1 μM concentrations (40 ppb) of the different chlorinated hydrocarbons, it is possible that this biosensor can yield false positive results if either of these insecticides is present at ppm concentrations. This problem may be overcome by increasing the threshold cut off level 10-fold (i.e. 400 ppb). There are obvious approaches to improve sensitivity and selectivity of the biosensor and reduce the possibility of false-positive results. One is to use mAbs selected for their high affinities for each cyclodiene

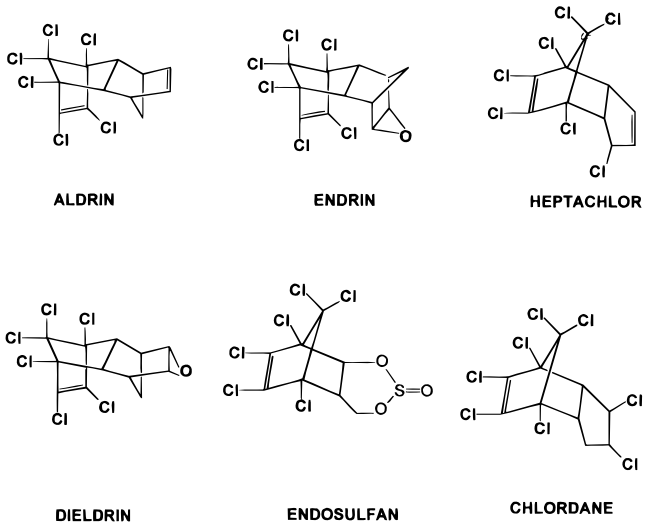

Figure 6. Structure of several cyclodiene insecticides.

insecticide. Higher affinity of the mAb for a cyclodiene insecticide than other cyclodienes, which are close in structure, requires selection of the ideal hapten. This strategy to improve sensitivity and selectivity of the biosensor are currently under study.

Advantages of this solid phase fluoroimmunosensor over other immune assays include the use of less hazardous materials compared to radioimmunoassay (Langone and Van Vunakis, 1975) and speed compared to both radioimmunoassay and ELISA (Engvall, 1980). This particular fiber optic sensor is best suited for single measurements. Regeneration of the sensor takes time and eliminates the advantages of quick screening if a large number of samples is involved. However, there are other biosensors such as the light addressable potentiometric sensor (LAPS) with high throughput that was used successfully for the detection of organophosphorous insecticides (Fernando *et al.*, 1993). As shown in Figure 4A, chlordane reduces not only the steady-state fluorescence, but also the rate of association, in a concentration-dependent manner. The reduction in the initial rate of fluorescence increase was shown to parallel the reduction in steady-state fluorescence (Anis and Eldefrawi, 1993; Devine *et al.*, 1995). Thus, rate measurements obtained in minutes could be used for quick screening purposes. Such rate measurements of cocaine alkaloids in coca leaf extracts gave values comparable to those obtained by fluorescence polarization and gas chromatography (Toppozada *et al.*, 1997). The fiber optic fluorometer is a portable system, and variants of the instrument are available commercially from Research International, Woodinville, WA (Ligler *et al.*, 1993). Such portable instruments can be used in on-site analysis for rapid screening of large numbers of samples to define the boundaries of a contaminated site. It is obvious that the immunosensor will identify cyclodiene insecticides as a group. However, biosensors using mAbs raised against different haptens would have various sensitivities for the different cyclodienes. The advantages from previous studies on other environmental pollutants demonstrated a quick screening technique. Little sample pretreatment was necessary to assay soil samples for the detection of imazethapyr (Anis and Eldefrawi, 1993; Zhao *et al.*, 1995). This was performed by extracting the soil with water, sedimenting the particles, and then filtering and buffering the extract with PBS. Dose−response curves for imazethapyr obtained from these soil extracts were superimposable upon laboratory generated curves. Detection of cyclodiene insecticides in soil is expected to yield a generic method. Until highly selective mAbs are produced, samples giving positive values by the above method would also have to be assayed by GC/MS or HPLC if identification of the individual cyclodiene insecticide is required.

ABBREVIATIONS USED

CCA, chlorendic caproic acid; FL−CCA, hexachlorocyclopentadiene fluorescein.

ACKNOWLEDGMENT

We thank Dr. Amira T. Eldefrawi and Ms. Vania I. Cortes for assistance in preparation of the manuscript.

LITERATURE CITED

Agency for Toxic Substance and Disease Registry U. S. Public Health Service (ATSDR). In *Toxicological Profile for Aldrin/Dieldrin*. Oak Ridge National Laboratory, Eds.; Dynamic Corp., 1989.

Anis, N. A.; Eldefrawi, M. E. Reusable fiber optic immunosensor for rapid detection of imazethapyr herbicide. *J. Agric. Food Chem.* **1993**, *41*, 843−848.

Bhatia, S. K.; Shriver-Lake, L. C.; Prior, K. J.; Georger, J. H.; Calvert, J. M.; Bredehorst, R.; Ligler, F. S. Use of thiol terminal silanes and heterobifunctional crosslinkers for immobilization of antibodies on silica surfaces. *Anal. Biochem.* **1989**, *178*, 408−413.

Bloomquist, J. R. Intrinsic lethality of chloride-channel-directed insecticides and convulsants in mammals. *Toxicol. Lett.* **1992**, *60*, 289−298.

Byfield, M. P.; Abuknesha, R. A. Biochemical aspects of biosensors. *Biosens. Bioelect.* **994**, *9*, 373−400.

Cheng, Y.; Prusoff, W. H. Relationship between the inhibition constant (K_i) and the concentration of an inhibitor that causes a 50% inhibition (IC_{50}) of an enzyme reaction. *Biochem. Pharmacol. J.* **1973**, *22*, 3099−4106.

Cole, L. K.; Metcalf, R.; Sanborn, J. R. Environmental fate of insecticides in terrestrial model ecosystems. *Int. J. Environ. Studies* **1976**, *10*, 7−14.

Devine, P. J.; Anis, N. A.; Wright, J.; Kim, S.; Eldefrawi, A. T.; Eldefrawi, M. E. A fiber optic cocaine biosensor. *Anal. Biochem.* **1995**, *227*, 216−224.

Eldefrawi, M. E.; Eldefrawi, A. T.; Anis, N. A.; Valdes, J. J. Reusable fiber optic biosensors for detection of drugs and toxicants. In *Uses of immobilized Biological Compounds for Detection, Medical, Food and Environmental Analysis*; Guilbault, G. G., Mascini, M., Eds.; NATO ASI Ser. E; Kluwer Academic: Dordrecht, The Netherlands, 1993, pp 387−395.

Engvall, E. Enzyme immunoassay ELISA and EMIT. *Methods Enzymol.* **1980**, *70*, 419−439.

Environmental Protection Agency (EPA). Pesticide products containing heptachlor or chlordane: intent to cancel registrations. *Fed. Register* **1974**, *39*, 41298−41300.

Esser, T.; Karu, A. E.; Toia, R. F.; Casida, J. E. Recognition of tetramethylenedisulfotetramine and related sulfamides by the brain GABA-gated chloride channel and a cyclodiene-sensitive monoclonal antibody. *Chem. Res. Toxicol.* **1991**, *4*, 162−167.

Fernando, J. C.; Rogers, K. R.; Anis, N. A.; Valdes, J. J.; Thompson, R. G.; Eldefrawi, A. T.; Eldefrawi, M. E. Rapid detection of anticholinesterase insecticides by a reusable light addressable potentiometric biosensor. *J. Agric. Food Chem.* **1993**, *41*, 511−516.

Geyer, H. J.; Scheunert, I.; Rapp, K.; Gebefugi, I.; Steinberg, C.; Kettrup, A. The relevance of fat content in toxicity of lipophilic chemicals to terrestrial animals with special reference to dieldrin and 2,3,7,8-tetrachlorodibenzo-p-dioxin (TCDD). *Ecotoxicol. Environ. Saf.* **1993**, *26*, 45−60.

Kilburn, K. H.; Thornton, J. C. Protracted neurotoxicity from chlordane sprayed to kill termites. *Environ. Health Perspect.* **1995**, *103*, 690−694.

Krämer, P. Biosensors for measuring pesticide residues in the environment: past, present, and future. *J. AOAC Intl.* **1996**, *79*, 1245−1254.

Langone, J. L.; Van Vunakis, H. Radioimmunoassay for dieldrin and aldrin. *Res. Commun. Chem. Pathol. Pharmacol.* **1975**, *10*, 163−171.

Ligler, F. S.; Golden, J. P.; Shriver-Lake, L. C.; Ogert, R. A.; Wijesuria, D.; Anderson, G. P. Fiber-optic biosensors for the detection of hazardous materials. *Immunomethods* **1993**, *3*, 122−127.

Lowry, O. H.; Rosebrough, N. J.; Farr, A. L.; Randall, R. J. Protein measurement with the folin phenol reagent. *J. Biol. Chem.* **1951**, *193*, 265−275.

Metcalf, R. L.; Kapoor, I. P.; Lu, P.; Schuth, C. K.; Sherman, P. Model ecosystem studies of environmental fate of six organochlorine pesticides. *Environ. Health Perspect.* **1973**, *4*, 35−44.

Murphy, D. L. Bioaccumulation of chlordane by channel catfish (*Ictalurus punctatus)* and toxicity to a human liver epithelial cell line. Ph.D. Dissertation, University of Maryland, 1995.

Pfaffenberger, B.; Hardt, I.; Huhnerfuss, H.; Konig, W. A.; Rimkus, G.; Glaush, A.; Schurig, V.; Hahn, J. Enantioselective degradation of alpha-hexachlorocyclohexane and cyclodiene insecticides in roe-deer liver samples from different regions of Germany. *Chemosphere* **1994**, *29*, 1543−1554.

Pourfarzaneh, M.; White, G. W.; Landon, J.; Smith, D. S. Cortisol directly determined in serum by fluoroimmunoassay with magnetizable solid phase. *Clin. Chem.* **1980**, *26*, 730−733.

Rimkus, G. and Wolf, M. Contamination of game in Schleswig-Holstein. 2. Residues of dieldrin, heptachlor epoxide and other cyclodiene insecticides in the liver fat of hares (Lepus europaeus L.). [German] *Z. Lebensm.-Unters.-Forsch.* **1987**, *184*, 308−312.

Rogers, K. R.; Valdes, J. J.; Eldefrawi, M. E. Acetylcholine receptor fiber-optic evanescent fluorosensor. *Anal. Biochem.* **1989**, *182*, 353−359.

Rogers, K. R.; Cao, C. J.; Valdes, J. J.; Eldefrawi, A. T.; Eldefrawi, M. E. Acetylcholinesterase fiber optic biosensor for detection of anticholinesterases. *Fundam. Appl. Toxicol.* **1991**, *16*, 810−820.

Saleh, M. A.; Zied, M. A.; El-Baroty, G.; Abdel-Reheim, E.; Abdel-Rahman, F.; Wallace, C.; El-Sebae, A. H.; Blancato, J. N. Gamma aminobutyric acid radioreceptor-assay a possible biomarker for human exposure to certain agrochemicals. *J. Environ. Sci. Health* **1993**, *B28*, 687−699.

Sovocool, G. W.; Lewis, R. G.; Harless, R. L.; Wilson, N. K.; Zehr, R. Z. Analysis of technical chlordane by gas chromatography/mass spectrometry. *Anal. Chem.* **1977**, *49*, 734−740.

Toppozada, A. R., Wright, J., Eldefrawi, A. T; Eldefrawi, M. E. Evaluation of a fiber optic immunosensor for quantitating cocaine in coca leaf extracts. *Biosens. Bioelectron.* **1997**, *12*, 113−124.

Tu, C. M.; Miles, J. R. W. Interactions between insecticides and soil microbes. *Residue Rev.* **1976**, *64*, 17−65.

Ungnade, H. E.; McBee, E. T. The chemistry of perchlorocyclopentenes and cyclopentadienes. *Chem. Rev.* **1958**, *58*, 249−320.

Van Wijnen, J. H.; Stijkel, A. Health risk assessment of residents living on harbor sludge. *Int. Arch. Occup. Environ. Health* **1988**, *61*, 77−87.

Wigfield, Y. Y.; Grant, R. Evaluation of an immunoassay kit for the detection of certain organochlorine (cyclodiene) pesticide residues in apple, tomato, and lettuce. *Bull. Environ. Contam. Toxicol.* **1992**, *49*, 342−347.

World Health Organization (WHO). In *Environ. Health Criteria* 38 Hept; World Health Organization: Genova, 1984, pp 9−11.

Wurster, C. F. Aldrin and dieldrin. *Environment* **1971**, *13*, 33−45.

Zhao, C. Q.; Anis, N. A.; Rogers, K. R.; Kline, R. H.; Wright, J.; Eldefrawi, A. T.; Eldefrawi, M. E. Fiber optic immunosensor for polychlorinated biphenyls. *J. Agric. Food Chem.* **1995**, *43*, 2308−2315.

Received for review February 10, 1997. Revised manuscript received May 9, 1997. Accepted May 9, 1997.[⊗] This study was supported in part by U.S. Environmental Protection Agency (EPA) Grant CR-820460 to M.E. and Fellowship U914418-01-1 awarded to K.E.B.

JF9701190

[⊗] Abstract published in *Advance ACS Abstracts,* July, 1, 1997.