COMMUNICATIONS

Dual Simultaneous AutoAnalyzer for Screening Some Insecticide Residues

A Total Phosphorus System and a New Anticholinesterase System

A new automated system for the determination of certain anticholinesterase insecticides is coupled with an automated total phosphorus system for organophosphorus insecticides to produce a dual detection system with simultaneous recording on a two-pen recorder of two distinct parameters from each sample. In a screening program for organophosphorus insecticide residues, one system complements the other; a sample which gives a positive response with both pens can be stated with more certainty to be an organophosphorus insecticide than if only one system was used.

A new system for the automatic determination of certain anticholinesterase (antiChE) insecticides is based on the method for the microdetermination of ChE (Levine *et al.*, 1966), which is similar to an independently developed ChE system (Humiston and Wright, 1967). Another automated antiChE system based upon the same chemistry was concurrently and independently developed (Voss, 1966, modified by Voss and Geissbühler, 1967). When we employ acetylthiocholine as substrate for the enzyme and a sulfhydryl group indicator, 5,5′-dithiobis-(2-nitrobenzoic acid) (DTNB), for measurement of hydrolyzed substrate, these new methods are more specific than the original described by Winter (1960) for the analysis of insecticides of this type and as modified and applied to certain pesticide residue determinations by Ott and Gunther (1966). The Winter (1960) method was based solely on the measurement of a nonspecific pH change due to the enzymatic liberation of acetic acid from acetylcholine iodide as indicated by phenol red.

The present new antiChE AutoAnalyzer system has been coupled with a total phosphorus AutoAnalyzer (slightly modified from that of Ott and Gunther, 1968) for organophosphorus insecticides to produce a dual detection system with simultaneous recording on a two-pen recorder of two distinct parameters from each sample.

APPARATUS

AutoAnalyzer (Technicon Corp., Ardsley, N.Y.) modules are arranged as shown in Figures 1 and 2. Detailed descriptions of the individual modules are available from Technicon.

MATERIALS AND METHODS

AntiChE AutoAnalyzer Reagents. ATChI Solution. Dissolve 0.5 gram of acetylthiocholine iodide (Nutritional Biochemicals Corp., Cleveland, Ohio) in 100 ml. of water. Keep the solution refrigerated when not in use, and add 0.5 ml. of Brij-35 (Technicon) per liter prior to first use.

Brij-35 Solution. Mix 0.5 ml. of Brij-35 per liter of water.

Buffer. Mix 6.6 grams of sodium chloride, 250 ml. of 0.2*M* Tris solution [tris(hydroxymethyl)aminomethane], and 400 ml. of 0.1*N* HCl. Bring to 1-liter volume with water and add 0.5 ml. of Brij-35 before use.

Buffer-DTNB. Dissolve 50 mg. of DTNB [5,5′-dithiobis-(2-nitrobenzoic acid), Aldrich Chemical Co., Inc., Milwaukee, Wis.] in 1 liter of Tris buffer. Prepare the solution at least one day in advance, because DTNB solubilizes slowly; refrigerate when not in use.

Diluted Plasma. Mix 25 ml. of outdated human plasma (local blood bank) with 75 ml. of 9.0% sodium chloride solution; prepare fresh daily.

Total Phosphorus AutoAnalyzer Reagents. Digestant. Prepare a solution of 42% perchloric acid in 40% sulfuric acid (to each 100 ml. of 70% perchloric acid add 100 ml. of concentrated sulfuric acid).

ANSA. Mix 150 grams of sodium bisulfite and 5 grams of sodium sulfite in about 800 ml. of water, heat to about 50° C., and add 2.5 grams of 1-amino-2-naphthol-4-sulfonic acid. Stir until dissolved, dilute to 1250 ml., and refrigerate in an amber bottle. Dilute 200 ml. of this stock solution to 1 liter in another amber bottle for a working solution.

Levor IV (Technicon). Just before use, add 0.5 ml. of this detergent per liter to each reagent pumped into the total phosphorus AutoAnalyzer, except for the digestant and the 5% NaOH solution.

AntiChE Procedure. AutoAnalyzer. The flow diagram for the new antiChE system (Figure 1) is essentially the same as the Levine *et al.* (1966) flow diagram for ChE except that diluted outdated human plasma is the source of ChE, used as a reagent rather than as a sample, and is first automatically incubated at 37° C. with the insecticide

Figure 1. AutoAnalyzer system for determination of antiChE insecticides

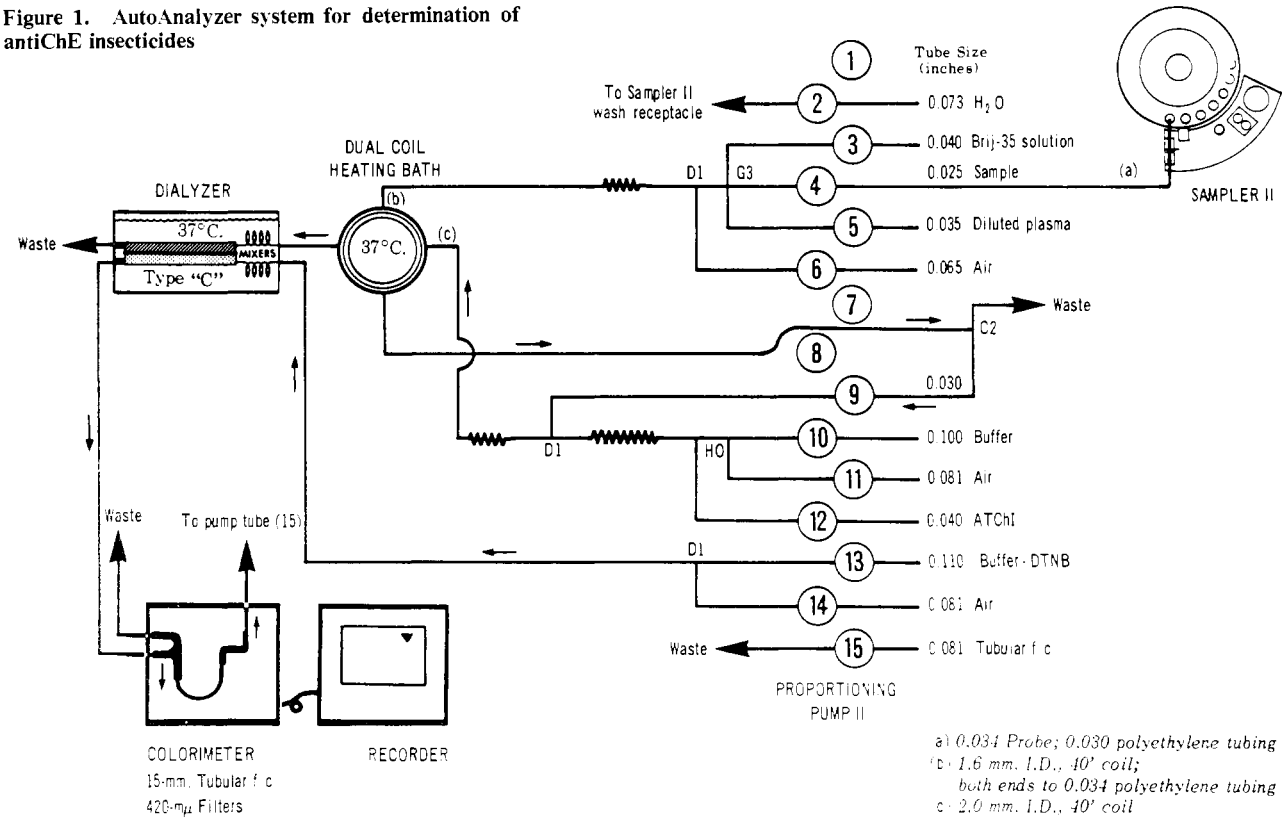
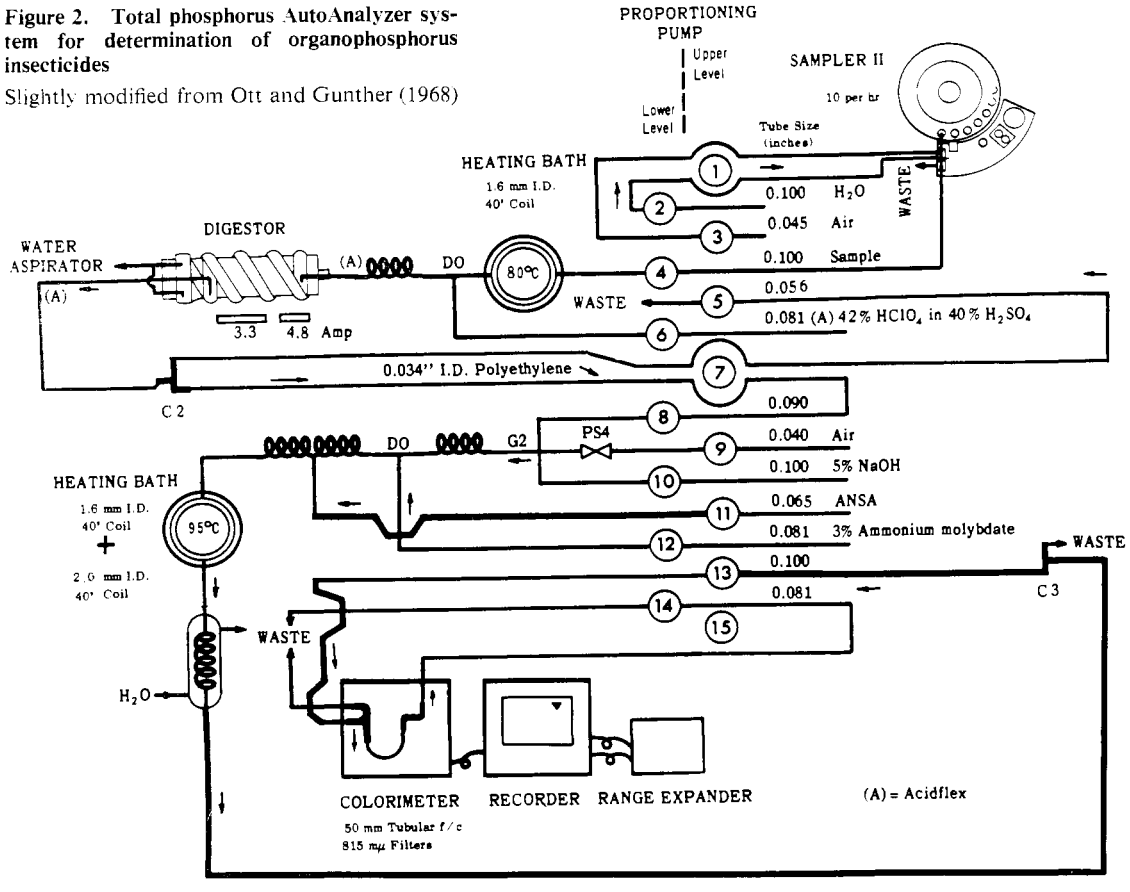


Figure 2. Total phosphorus AutoAnalyzer system for determination of organophosphorus insecticides

Slightly modified from Ott and Gunther (1968)



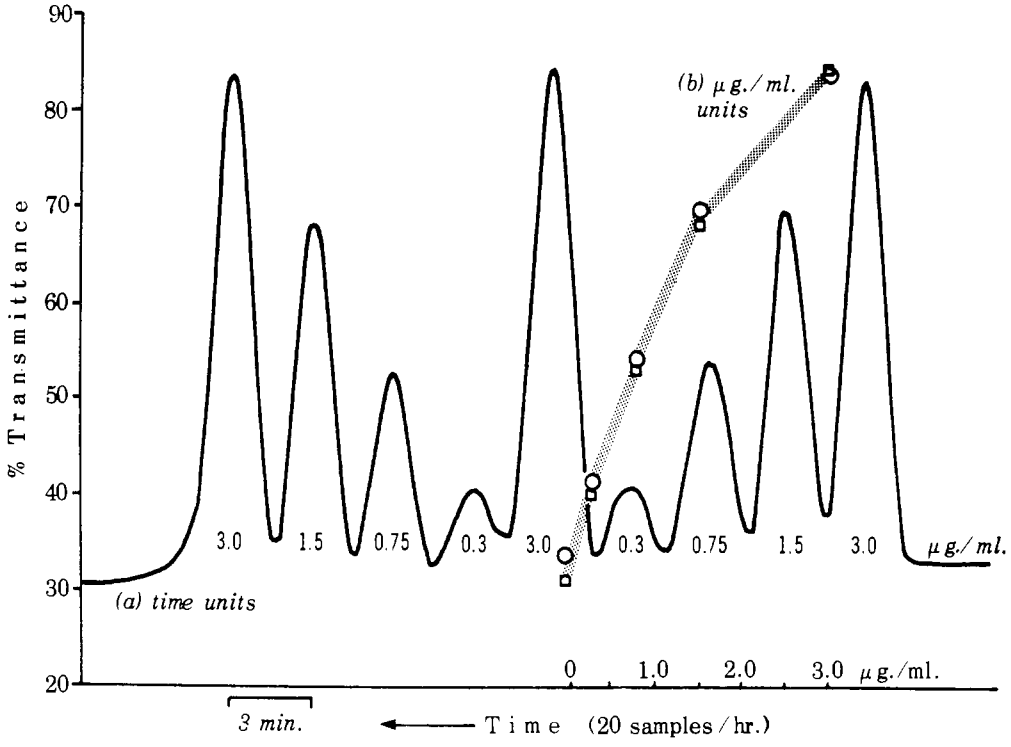


Figure 3. Typical antiChE AutoAnalyzer chart recording and superimposed chart reader standard curve obtained from aqueous standard solutions of Phosdrin

○ Plotted from peak maxima from series on right □ Plotted from series on left

sample. The excess ChE is mixed with buffer and substrate and incubated again at 37° C. in a second coil of the heating bath, where thiocholine is released. The thiocholine enters the 37° C. dialyzer and passes through the membrane to pick up the color reagent (DTNB), and the absorbance of the resulting colored anion is continuously recorded at 420 mμ in a 15-mm. tubular flow cell. Maximum (base line) color is the result of a constant amount of ChE between inhibitor samples, while peaks for the inhibitor samples are seen due to a reduction in color formation.

Recorder tracing of a sample takes place about 20 minutes after pickup by the sampler II module which is operated in dual simultaneous analyses at 10 samples per hour with a special 1 to 3 cam providing 1.5 minutes of sampling time, followed by 4.5 minutes of wash time before the next sample. More rapid antiChE analysis and much lower minimum detectability are permitted if this system is operated alone. To increase response, a larger sample aliquot is pumped and/or a scale expander (Technicon) is used. Figure 3 illustrates the use of the system for the analysis of Phosdrin; rapid quantitation of unknowns is made possible with a standard curve drawn on a Technicon chart reader as shown.

Oxidation of Samples. If samples are to be oxidized (see Discussion and also Ott and Gunther, 1966) for conversion of some organophosphorus insecticides to more potent ChE inhibitors before AutoAnalysis with the anti-ChE system, any suitable method may be used, from the many reported for this purpose. Probably the most universal and efficient oxidative procedure available is exposure

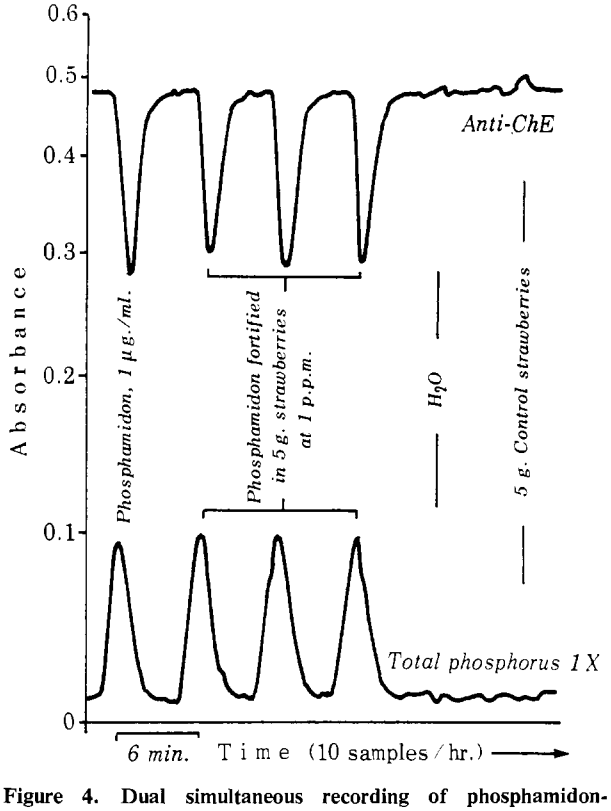


Figure 4. Dual simultaneous recording of phosphamidon-fortified strawberries

Including comparison with equivalent amounts of control strawberries and of phosphamidon standard

of samples, after development on thin-layer or paper chromatograms, to bromine vapors; Thimet (phorate) is converted to a strong inhibitor(s) by exposure in the bottom of a test tube to bromine vapors for about 5 minutes. Attempts to use dilute bromine water for oxidation of Thimet have been unsuccessful here. After a bromine vapor oxidative procedure, excess bromine is allowed to dissipate. then the oxidized sample is solubilized in ethyl alcohol and diluted with water to a 10% ethyl alcohol solution as the sample for the AutoAnalyzer.

Total Phosphorus Procedure. The basics of the total phosphorus system has been briefly discussed by Gunther and Ott (1966) and thoroughly by Ott and Gunther (1968), but the preparation of the special reagents required is redescribed above. The flow diagram is shown in Figure 2, including a time delay coil in the first heating bath for matching the timing with the antiChE system. This coil is not necessary for total phosphorus analysis alone. In the dual system, it can be at ambient temperature; an elevated temperature is not essential.

Dual AutoAnalyzer Procedure and Results. In dual detection operation the small diameter stainless steel pickup probe of the antiChE system is simply taped to the glass pickup probe of the total phosphorus system. One sampler II module operated as described above thus supplies an aliquot of each sample to the analytical systems also joined with a two-pen recorder. To match the two systems so that the time elapsed from sample pickup to recorder readout is the same, the flow rate of air used for segmentation can be changed in one or both systems, without upsetting relative reagent concentrations, while fine adjustment may be made by shortening a transmission tubing length in one system and lengthening in the other if necessary. Typical results from simultaneous dual analysis of simulated insecticide residues in fortified strawberry stripping solutions are shown in Figures 4 and 5. Strawberries are tumbled for 1 hour in CH_2Cl_2, the solvent is evaporated off, and 5.0 ml. of water plus a boiling chip are added to each 5.0-gram aliquot, followed by oven incubation for 10 minutes at 110 ° C. prior to AutoAnalysis.

Results from phosphamidon solubilized in 5% ethyl alcohol are shown in Figure 6. Note the peak maxima reproducibility within a given system between replicated solution concentrations even when separated by solutions of higher concentrations. Results from technical grade parathion in 1% ethyl alcohol solutions are shown in Figure 7.

DISCUSSION

Recordings from relatively high concentration solutions of technical grade parathion by the antiChE system do not offer consistent peak maxima reproducibility (see Figure 7) but quantitatively are precise if peak height measurements are made in absorbance units rather than using peak

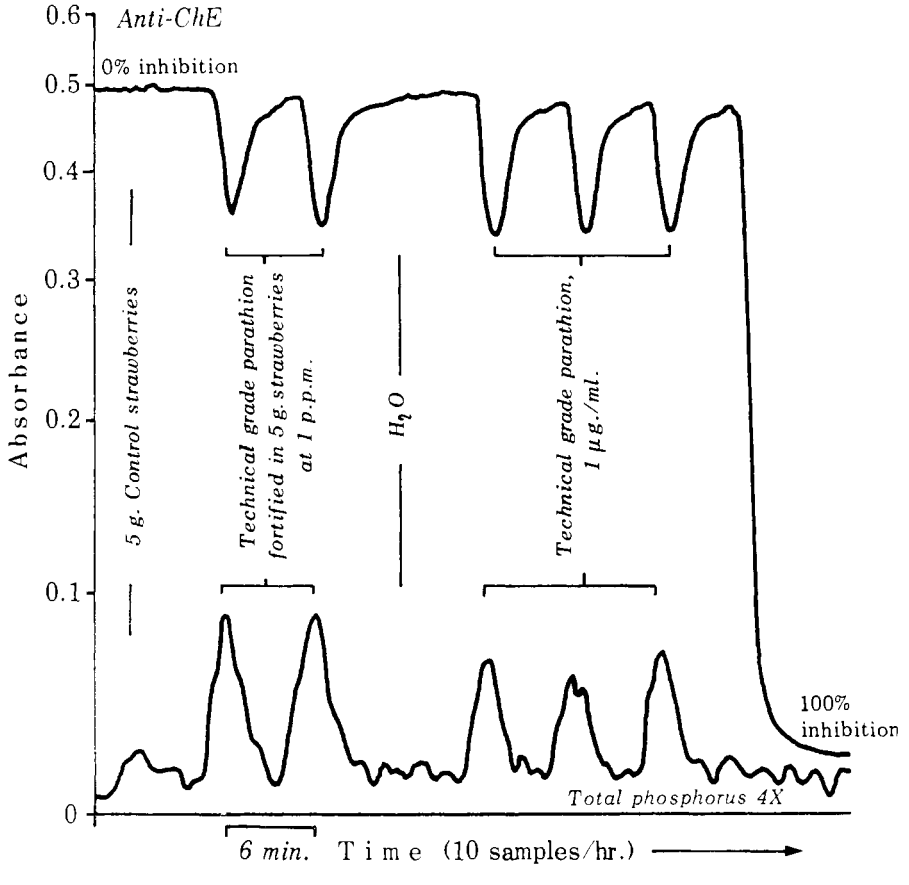


Figure 5. Dual simultaneous recording of technical grade parathion-fortified strawberries

Including comparison with equivalent amounts of control strawberries and of technical grade parathion standards. Signal from colorimeter of total phosphorus AutoAnalyzer expanded 4× with a scale expander

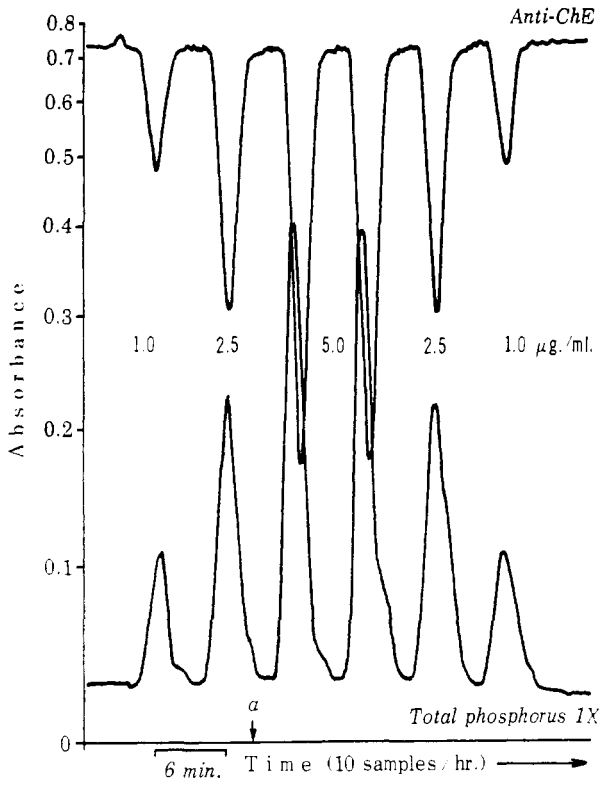


Figure 6. Dual simultaneous recording of phosphamidon standard solutions

a. Sampling origin of last sample

maxima measurements alone. For screening purposes peak maxima observation is generally adequate; screening is defined by Ott and Gunther (1968). The tailing and poor return to base line with technical grade parathion as compared with the good return to base line with phosphamidon are related to the differences in adsorption of the compounds to the sample Tygon pump tubing. Actually this variation alone provides some small degree of specificity to the antiChE system—e.g., a nontailing peak indicates that the compound is not the inhibitor(s) in technical grade parathion, nor paraoxon; a tailing peak indicates that the inhibitor is not Phosdrin, phosphamidon, nor oxidized Thimet. Use of Technicon Acidflex tubing for pumping the inhibitor samples and polyethylene tubing for the first incubation coil as suggested by Voss (1966) might reduce the tailing effect for some insecticides. However, it has been the experience in these laboratories that Acidflex tubing is better for pumping aqueous solutions of paraoxon, while Tygon tubing is better for parathion solutions. Desicote (Beckman Instruments, Inc., Fullerton, Calif.) treated tubings decrease adsorption. Best results have been obtained with Esco silicone rubber tubing (Technicon) of 2.0-mm. bore and 1.0-mm. wall thickness. However, this is apparently not available in a wide range of bore sizes. Compensation for tailing peaks to improve return to base line when operating the antiChE system independently can be obtained by use of a special 10 per hour 1 to 6 cam in the sampler II module.

If it is desirable or necessary to operate the systems independently, an extra sampler II module may be needed —for example, some organophosphorus insecticides re-

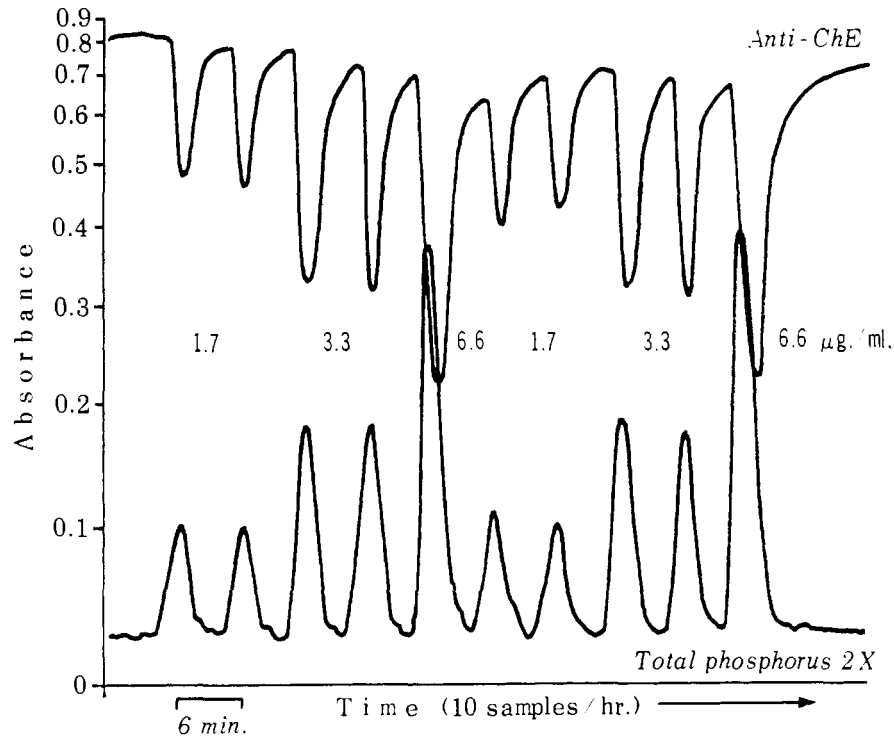


Figure 7. Dual simultaneous recording of technical grade parathion in standard solutions

quire oxidation to more potent ChE inhibitors before a response is detectable with the antiChE system and some compounds require alkaline hydrolysis for adequate detectability in the total phosphorus system (Ott and Gunther, 1968). Thus, it may be desirable to analyze all samples in the dual simultaneous mode and then independently with aliquots of the same samples which have first been oxidized and hydrolyzed, respectively, use appropriate manual methods such as bromine vapor treatment for oxidation and incubation 15 minutes at 110° C. in 0.25*N* NaOH solution for hydrolysis (covered with aluminum foil and a boiling chip present).

In screening programs for organophosphorus insecticide residues, the dual system approach offers greater validity than either single system can offer, with relatively no more time or effort. Generally more rigorous care in cleanup and sample preparation is required for total phosphorus than for antiChE assay; therefore, almost any sample preparation method adequate for the former is adequate for dual operation. These requirements are discussed by Ott and Gunther (1968) and in manual methods for the total phosphorus assay of organophosphorus insecticide residues. Given cleanup suitable for total phosphorus assay and efficient oxidation of relatively poor ChE inhibitors to strong inhibitors, the dual system can be used for screening samples of food and feed for many organophosphorus insecticide residues. A few crops and organophosphorus insecticides need no cleanup or oxidation. Considerable qualitative and quantitative information can be obtained if the dual system is used following a segregative technique such as thin-layer chromatography (Abbott *et al.*, 1967). Preseparation of metabolites of insecticides will help circumvent the lack of correlation between antiChE and total phosphorus analyses of such insecticides as Trithion, Di-Syston, and Thimet with their many phosphorus-containing metabolites of differing antiChE activities. This problem can otherwise be minimized if unseparated metabolites are all oxidatively converted to one oxygen analog (Manuel, 1968).

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Daniel E. Ott

Department of Entomology
University of California
Riverside, Calif. 92502

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