A Carboxylesterase Inhibition Assay to Estimate Parathion, Malathion and Diazinon in Lettuce Extracts

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Previous work has demonstrated that the response curves for the inhibition of beef liver carboxylesterases by organophosphorus pesticides appear to be unique for each compound used (1, 2). Moreover, the amount of the pesticide required to inhibit these enzymes is reduced considerably if the compound is converted to its corresponding oxygen analog (2).

This paper describes an enzyme assay method for parathion, malathion and diazinon in lettuce extracts after sweep codistillation and thin layer chromatographic separation.

Materials

Organophosphorus Compounds: Parathion (O,O-diethyl O-p-nitrophenyl phosphorothioate), malathion (diethyl mercaptosuccinate S-ester with O,O-dimethyl phosphorodithioate), and diazinon [O, O-diethyl-O-(2-isopropyl-6-methyl-4-pyrimidinyl) phosphorothioate]. All compounds were analytical standard quality, and all stock solutions were made up in hexane.

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Extraction Procedure

Samples were extracted according to the method described by McLeod et al (3). Five hundred grams of fresh lettuce was homogenized in a Waring Blendor. A 50 gram sub-sample was extracted with 150 ml redistilled acetonitrile for 5 minutes in the Blendor at medium speed. The mixture was then filtered on a Buchner funnel with a coarse fritted disc and the residue was re-extracted with a second 150 ml of acetonitrile. The acetonitrile extracts were combined and concentrated to approximately 50 ml in a flash evaporator. This left an aqueous concentrate which was then extracted with three 50 ml portions of hexane. The hexane was evaporated to 25 ml so that 1 ml of hexane extract was equivalent to 2 grams of plant material. This extraction procedure has been shown to give recoveries of parathion, malathion and diazinon in the range of 97-100% (4). Fortified samples were prepared by adding 1 ml of the pesticide stock solution to a given volume (usually 5 ml) of the extract.

Cleanup Procedure

Lettuce extracts were spiked with parathion, malathion and diazinon at levels of 1.0, 8.0, and 0.75 ppm. respectively - the tolerance levels for these compounds in Canada. The equivalent of 10 grams of plant material was spiked with 10 µg of parathion or 80 µg of malathion or 7.5 µg of diazinon prior to the cleanup procedure.

The Storherr sweep co-distillation apparatus (Kontes) (5) was used for the cleanup of the lettuce extracts. This technique has been used previously for the cleanup of edible oils (6), milk (7), and plant materials (8) prior to pesticide residue analyses. The Storherr tubes (30 cm. long x 1 cm diameter) were packed with silanized glass wool (Applied Science Laboratories, State College Pa.). The cleanup was carried out at 170° ± 3°C with a nitrogen flow of 600 ml/min. The equivalent of ten grams of plant material (5 ml hexane extract) was injected into the Storherr tube at a rate of 1 ml/min. After sample application the column was flushed with 10 ml of redistilled hexane at the same rate. The resultant hexane solution was concentrated to a volume of 100 µl under a stream of dry nitrogen and used for TLC.

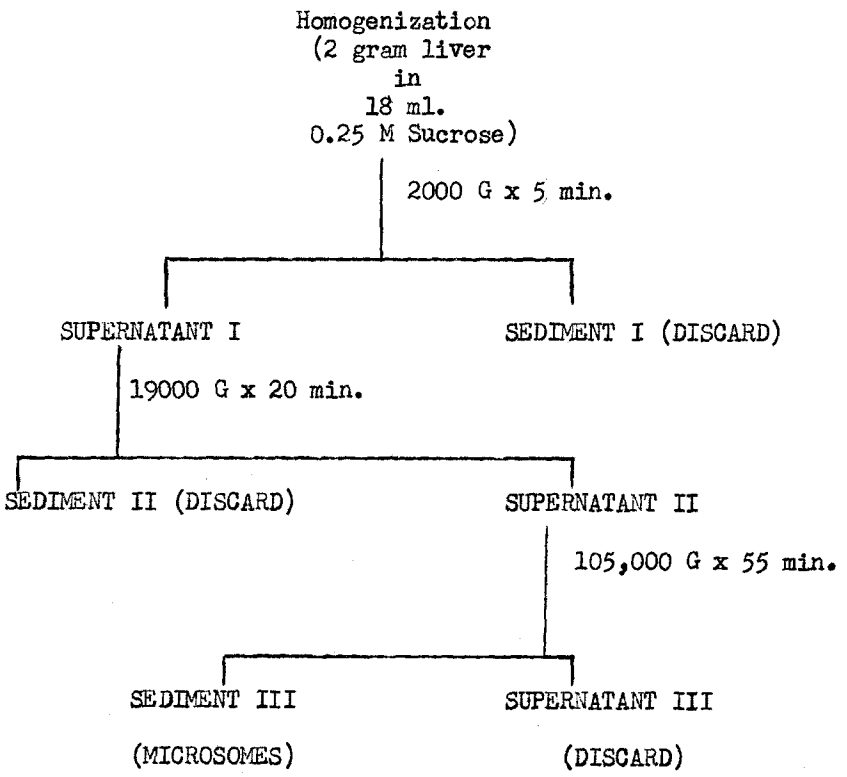
Thin Layer Chromatography

The concentrated hexane extract from the Storherr tube was spotted on a 500 µ thick Silica Gel GF_{254} ® plate. This silica gel contains a fluorescent indicator and shows up as a green color under short wave U.V. illumination (254 mµ). Pesticides present in sufficient amount (1-10 µg) will show up as dark spots on the green background, and in this way it is possible to detect the pesticides without chemical or biochemical developing reagents. The pesticide spot was circled and the plate exposed to bromine vapors for 5 minutes to convert the parent compound to its corresponding oxygen analog (9). The circled areas were scraped

from the plate into 10 ml. glass-stoppered centrifuge tubes. Ten ml. of absolute ethanol was added to each tube, the tube and contents vigorously shaken, centrifuged at 500 G x 2 min. and suitable aliquots of the ethanol mixtures were taken for the enzyme assay.

Enzyme Assay Procedure

The enzyme assay procedure was essentially the same as that described previously (10) except for a few modifications: 1) the enzyme source was a beef liver microsomal preparation obtained in the following way:



The microsomes were resuspended in 0.25M sucrose (in a volume equivalent to the original homogenate), distributed into vials and stored in the freezer. For each assay the contents of one of these vials was diluted to 50 ml with phosphate buffer (0.1 M, pH 6.3) and sub-diluted from this with the same buffer. All enzyme preparations were kept in an ice-bath during the assay.

2) The substrate was o-nithrophenyl propionate (ONPP, 1.0×10^{-3}M) which was easier to prepare and contained fewer impurities than o-nitrophenyl butyrate. The enzyme assay procedure is outlined below:

SAMPLE (in 50 or 100 µl ETOH)

+

2 ml Enzyme preparation

↓ INCUBATION (15 min. 25°C)

+

2 ml ONPP (2.0×10^{-3}M)

↓ INCUBATION (15 min. 25°C)

+

1 drop DFP (0.25M) to stop reaction

↓

ABSORBANCE measured at
370 mµ on
BECKMAN MODEL "B" spectrophotometer

$$\text{PER CENT INHIBITION} = \frac{\text{Abs (control)} - \text{Abs (pesticide)}}{\text{Abs (control)}} \times 100$$

The samples put through the Storherr tube included:

1) The pesticide standard in hexane equivalent to the Canadian tolerance level on ten grams of plant material (11).
2) The extract alone (10 gm equivalent).
3) The extract spiked with the tolerance level of the pesticide.

The samples analyzed by the enzyme assay included

A) The solvent alone (ETOH).
B) Blank silica gel extracted with solvent.
C) The area of silica gel from the lettuce extract where the pesticide would normally show, extracted with solvent.
D) The area of silica gel from the spiked lettuce extract where the pesticide was present.

Although the silica gel alone showed some inhibition as compared to the solvent (approximately 10%), there was no difference between the silica gel blank, and the silica gel lettuce blank. This indicated that there were no inhibiting compounds still in the lettuce extract, at least in the areas corresponding to the three pesticides.

The results for the three pesticides in the presence and absence of lettuce extracts is given in Table I.

TABLE I

Inhibition Results for Parathion, Malathion, and Diazinon in Lettuce Extracts

COMPOUND	QUANTITY USED FOR ASSAY	PER CENT INHIBITION[1] STANDARD	SPIKED EXTRACT
Parathion	5.0×10^{-8} g.	20.7 ± 1.37[2]	20.2 ± 1.02
Malathion	2.0×10^{-6} g.	15.4 ± 1.46	15.0 ± 1.02
Diazinon	1.9×10^{-7} g.	24.6 ± 0.26	23.9 ± 0.35

[1]Duplicate samples were carried through the cleanup procedure. Inhibition analyses were performed in triplicate and the per cent inhibition calculated as follows:

$$\% \, I = \frac{\text{Absorbance (B or C)} - \text{Absorbance (D or E)}}{\text{Absorbance (B or C)}} \times 100$$

[2]Standard error of the mean

In all cases there was good agreement between the standard and the spiked extract. A "t" test was performed on the data, and there was no significant difference at the 5% level between the standard and spiked samples. These results indicate that the carboxylesterase inhibition method is applicable to the study of some organophosphorus residues in plant materials.

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