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A Fruit Fly Bioassay with Phosphotriesterase for Detection of Certain Organophosphorus Insecticide Residues

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There has been an increasing demand for analyses to be performed on samples suspected of containing pesticides or other toxic chemicals. Bioassays, such as the fruit fly test, are routinely used in veterinary diagnostic laboratories to rapidly screen for the presence of toxic residues in feed and tissues. However, these bioassays are nonspecific and are used only to indicate if other sophisticated analytical methods should be applied for futher investigation. Usually the analytical methods for positive identification are different for different classes of pesticides; therefore, it would be advantageous if the initial bioassay could also provide information as to what classes of pesticides were present. This would be particularly helpful in the case of poisonings involving the acetylcholinesterase-inhibiting organophosphorus and carbamate pesticides, which differ in antidote, although their mode of action is identical. In this report we have presented evidence to show that certain organophosphorus pesticides present at residue levels can be quickly and easily detected and distinguished from other toxic compounds.

The bioassay described involves the use of fruit flies and a bacterial enzyme, phosphotriesterase (PTE), which can specifically and efficiently degrade certain organophosphorus pesticides. PTE was first discovered in Flavobacterium sp. isolated from paddy water by an enrichment culture technique (Sethunathan and Yoshida 1973). These soil bacteria were initially isolated for their ability to degrade parathion and diazinon, and later it was found that they could degrade a wide range of organophosphorus pesticides (Munnecke 1980; Barik and Munnecke 1982). Some of the biochemical properties of PTE, such as pH range, effect of metal ions, and inhibition by detergents and organic solvents, have been characterized (Brown 1980). The enzyme cleaves the phosphotriester linkage of the organophosphorus molecule and greatly reduces its toxicity (Serdar et al. 1982).

This bioassay is based on the principle that PTE can only degrade or detoxify certain organophosphorus pesticides but not other pesticides such as organochlorines and carbamates. This provides a discrimination for those pesticides that are substrates of the enyzme from other toxic substances by using the standard fly test. Results presented in this report suggest that this method can provide a simple and quick alternative to confirm the presence of certain organophophorus pesticides.

MATERIALS AND METHODS

Wild type Drosophila melanogaster were propagated by standard methods (Scores and Lichtenstein 1967) by using a medium containing per liter: 100 g glucose, 10 g agar, 100 g yeast and 3 g p-hydroxybenzoate as a fungistat. Adult flies of approximately the same age were used for each assay to avoid any fluctuation

Table 1. % Degradation of Organophosphorus Pesticides by PTE*.

Group I	
Bromophos-Ethyl	53.2
Coumaphos	100.0
Diazinon	72.1
Fensulfothion	100.0
Leptophos O_2 Analog	100.0
Parathion	98.4
Paraoxon	100.0
Pirimiphos Ethyl	94.3
Pyrazophos	89.1
Quinalphos	99.3
Group II	
Chlorfenvinphos	7.4
Chlorothiophos	11.4
Chlorpyrifos	37.2
Crufomate	0.9
Dichlofenthion	32.6
EPN	29.3
Fenithrothion	7.9
Malathion	16.4
Methyl Parathion	31.3
Pirimiphos Methyl	17.1
Trichloronate	19.8
Group III	
Chlorpyrifos-Methyl	0
Ethoprop	0
Etrimifos	0
Famphur	0
Fenthion	0
Isofenphos	0
Jodfenphos	0
Ronnel	0
Sulfopros	0
Sulfotepp	0

*Individual pesticide was mixed with PTE in 10 mM Tris-HCl buffer (pH=8.5) and incubated at 37°C for 30 min. The remaining pesticide was then extracted by hexane and the % degradation was determined by gas chromatography.

in pesticide susceptibility due to age differences. Flies were anesthetized with ether before being transferred and were checked for their full recovery before exposure to the pesticide in testing vials.

Flavobacterium sp. (ATCC #27551) were grown in nutrient broth containing per liter: 10 g tryptone, 5 g yeast extract, and 10 g sodium chloride. Overnight culture was harvested by centrifugation, and the cell pellet was washed and dried three times with acetone. The hard pellet was then pulverized with mortar and pestle. The resulting dried cell powder can be stored at 4°C for several months. For the bioassay, a fresh PTE enzyme solution was made by dissolving 0.05 g of dry cell powder in 5 ml of Tris-HCL buffer (10 mM, pH=8.5).

Small double filter papers (Whatman 984H, 2.4 cm size) were impregnated with exact amount of pesticide solution by means of a microsyringe. The solvent (2,2,4-trimethylpentane, hexane, acetone, or ethyl acetate) was allowed to evaporate for 30 min. Control filters impregnated with the same volume of solvent showed no acute effect and indicated that 30 min are sufficient to allow evaporation of all the solvent.

A fixed amount of either PTE enzyme solution or buffer was also impregnated on the filters and incubated at 37°C for 30 min to allow pesticide degradation. At the end of the incubation, exactly 10 flies along with a few drops of sugar or honey water were added into each testing vial containing the filters. The vials were allowed to stand at room temperature overnight (between 16 to 20 h) and the percent fly survival in each vial was determined by visual inspection.

RESULTS AND DISCUSSION

By using a gas chromatograph equipped with a nitrogen-phosphorus detector McDaniel et al. (manuscript in preparation) have shown that organophosphorus pesticides can generally be classified into 3 groups according to their susceptibility to PTE degradation (Table 1). Group I compounds, including such widely used pesticides as parathion, diazinon, coumaphos and paraoxon, are almost completely degraded by PTE. Group II compounds are not readily degraded by PTE, but still undergo extensive degradation with PTE treatment. Group III compounds are not substrates of PTE and are undegraded (See Table I).

Since parathion is one of the more commonly used pesticides and is also a good substrate for PTE, it was chosen to be used for the initial development of this bioassay. Results in Table 2 show that all the flies were killed by parathion in the test vials where PTE was not added. Even at the lowest amount of parathion applied (1.2μg) no fly survived overnight. In contrast, all the test flies survived if the pesticide-impregnated filters were treated with PTE (0.87 mg) prior to the fly test.

Table 2. PTE Effect on Group I Organophosphorus Pesticides.

Pesticide	(μg)	PTE (mg)	% Survival
Parathion	1.2	0	0
	1.2	0.87	100
	12.4	0	0

Table 2. PTE Effect on Group I Organophosphorus Pesticides. Cont.

Pesticide	(μg)	PTE (mg)	% Survival
	12.4	0.87	100
	41.1	0	0
	41.1	0.87	100
	82.6	0	0
	82.6	0.87	50
	82.6	1.74	100
	124	0.87	0
Diazinon	11.0	0	0
	11.0	0.87	100
	22.0	0	0
	22.0	0.87	100

When the amount of parathion was increased to 82.6μg, only 50% of the flies survived in PTE-treated test vials, and suggested that more enzyme may be needed for detoxifying all the parathion. This was found to be true when the amount of PTE was doubled, as also shown in Table 2. These results indicate that to get a conclusive result, it is important not to have an excessive amount of pesticide so that the amount of PTE becomes the limiting factor. This can be achieved by performing multiple tests using different dilutions of the same sample. Essentially the same results were obtained with diazinon (Table 2).

Since group II organophosphorus compounds are not as suitable as substrates for PTE as group I compounds, one would expect that the detoxification be less efficient under the same condition. This is shown in Table 3 for malathion, chlorpyrifos, and methyl parathion. However, at between 1 to 10μg levels, conclusive results can still be obtained for this group. To ensure that the pesticide level falls in between this range, again multiple tests using different dilutions of the same sample should be performed. Alternatively, by increasing the amount of PTE, one can overcome this overloading problem, as demonstrated in Table 2 for parathion.

Table 3. PTE Effect on Group II Organophosphorus Pesticides.

Pesticide	(μg)	PTE (mg)	% Survival
Malathion	6.8	0	0
	6.8	0.87	100
	13.5	0	30
	13.5	0.87	70
	23.7	0	0
	23.7	0.87	0
	47.4	0	0
	47.4	0.87	0
Chlorpyrifos	4.8	0	0

Table 3. PTE Effect on Group II Organophosphorus Pesticides. Con t.

Pesticide	(µg)	PTE (mg)	% Survival
	4.8	0.87	100
	9.5	0	0
	9.5	0.87	100
	16.4	0	0
	16.4	0.87	0
	19.0	0	0
	19.0	0.87	0
Methyl Parathion	1.3	0	0
	1.3	0.87	100
	13	0	0
	13	0.87	0

Since group III organophosphorus pesticides, and other insecticides such as carbamates are not degraded by PTE, one would predict that the survival in the bioassay should not be affected by the enyzme treatment. Results shown in Table 4 and Table 5 for group III organophosphorus and carbamates pesticides respectively, indicate that for a wide range of pesticide concentrations, there was no difference in survival between enzyme-treated and untreated samples. This demonstrates the basis of this modified fly test which is to rely on the specificity of PTE toward detoxification of certain pesticides. Table I provides the reference for the detoxification capability of this enzyme.

Table 4. PTE Effect on Group III Organophosphorus Pesticides.

Pesticide	(µg)	PTE (mg)	% Survival
Fenthion	1.5	0	0
	1.5	0.87	0
	7.7	0	0
	7.7	0.87	0
	46.0	0	0
	46.0	0.87	0

Table 5. PTE Effect on Carbamate Pesticides.

Pesticide	(µg)	PTE (mg)	% Survival
Carbofuran	1.0	0	100
	1.0	0.87	100
	7.6	0	20
	7.6	0.87	10
	11.5	0	0

Table 5. PTE Effect on Carbamate Pesticides. Cont.

Pesticide	(μg)	PTE (mg)	% Survival
	11.5	0.87	0
	22.9	0	0
	22.9	0.87	0
Aldicarb	9.5	0	0
	9.5	0.87	0

This test is based on the experimental fact that phosphotriesterase, isolated from soil bacteria, can only degrade and thus detoxify certain organophosphorus insecticides but not other toxic substances such as carbamate and organochlorine compounds. By coupling the standard fruit fly test with the enzyme treatment, the presence of some of the more commonly used pesticides, such as parathion, diazinon, and malathion can be detected and confirmed.

Bioassay results presented in this paper demonstrate that residue levels of certain organophosphorus insecticides can be distinguished from other toxic residues. However, analysts should be cautious in drawing the conclusions from these bioassay results. For example, while positive results (detoxification) would represent conclusive evidence for the presence of residues, negative results do not preclude the presence of organophosphorus insecticides since group III compounds in Table I are not hydrolyzed by PTE. By varying the amount of sample (pesticide) and/or the amount of enzyme on the filter papers, one could eliminate the possibility of saturating the enyzme and of not detecting insecticides present at levels below the assay's sensitivity. The bioassay sensitivity (between 1 μg to 10 μg) is approximately equivalent to a range of 0.1 to 1 ppm in samples.

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