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THE ANALYSIS OF MICRO AMOUNTS OF BINAPACRYL, EPN, METHYL PARATHION,
AND PARATHION

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The Analysis of Micro Amounts of Binapacryl, EPN, Methyl Parathion, and Parathion

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Aromatic nitrated insecticides can be determined by a microchemical method based on the Averell-Norris procedure. The method is more sensitive than other known methods, and interference from plant extracts is low.

Aromatic nitrated insecticides have been analyzed by various methods. The method of Averell and Norris (1) for the determination of parathion is one of the most widely used. Kolbezen and Barkley (2) determined Chlorthion (*O*-(3-chloro-4-nitrophenyl) *O,O*-dimethyl phosphorothioate) in a manner analogous to the Averell-Norris parathion method. Young (3) increased the sensitivity of this method for determining 1-chloro-2-nitrobenzene in pineapple. Rosenthal, *et al.* (4) determined dinitrooctylphenol by a distillation process in the analysis of residues of Karathane, a mixture of dinitro(1-methylheptyl)phenyl crotonate (78%) and dinitro(1-methylheptyl)phenol and related compounds (22%). EPN, methyl parathion, *p*-nitrophenol, and paraoxon residues have been determined by use of the Averell-Norris procedure. Binapa-

cryl (2-*sec*-butyl-4,6-dinitrophenyl-3-methyl-2-butenate) is determined by the method of Niagara Chemical Division of Food Machinery Corp. (5).

The microcolorimetric method presented utilizes the reactions of the Averell-Norris procedure and has definite advantages over the methods cited above. The method has a sensitivity of approximately ten-fold over other methods and there is a low degree of interference from plant extracts. The plant material is extracted with *n*-hexane:isopropyl alcohol, which does not extract a large amount of interfering substances from the sample, and during the subsequent reduction and filtration steps the plant interferences are largely removed.

The method consists of reducing the nitro grouping, diazotizing, and then coupling with *N*-1-naphthylethylenediamine dihydrochloride, and concentrating the resultant color by extracting into chloroform. This method has also been applied to determination of Guthion (*O,O*-dimethyl-*S*-(4-oxo-1,2,3-benzotriazin-3(4*H*))-ylmethyl) phosphorodithioate), a nitrogen ring compound (6), by first hydrolyzing to break the ring, then

reducing, diazotizing, coupling, and extracting into chloroform.

Analysis of Guthion standard solutions by this method gave absorbance readings of nearly twice those obtained by the method of Meagher, *et al.* (7).

METHOD

Reagents

(a) *Binapacryl standard solution*.—(Niagara Chemical Division, Food Machinery Corp.). One ml contains 10 μ g in redistilled *n*-hexane.

(b) *Parathion and methyl parathion standard solutions*.—(American Cyanamid Co.). One ml contains 10 μ g of redistilled *n*-hexane.

(c) *EPN standard solution*.—(E. I. duPont de Nemours & Co.). One ml contains 10 μ g in redistilled *n*-hexane.

(d) *Cotton*.—Acetone-extracted and oven-dried.

(e) *n-Hexane*.—Redistilled (over metallic sodium).

(f) *Skellysolve B*.—Any *n*-hexane with a low reagent blank can be used.

(g) *Acid-alcohol solution*.—Combine 2 parts 95% ethyl alcohol and 2 parts concentrated HCl, mix, and cool. Make fresh daily.

(h) *Lanolin solution*.—1% in chloroform.

(i) *Zinc powder*.—Reagent grade, 80–200 mesh.

(j) *Sodium nitrite*.—0.25% in distilled water. Make fresh weekly and keep refrigerated.

(k) *Ammonium sulfamate*.—2.5% in distilled water. Make fresh weekly and keep refrigerated.

(l) *Dye solution*.—1% *N*-1-naphthylethylenediamine dihydrochloride in distilled water. Add 0.5 g decolorizing carbon, mix, and filter through two fluted papers. Store in dark bottle under refrigeration and make fresh every other day.

Determination

Pipet aliquots of the standard insecticide solution containing 0–50 μ g into 125 ml Erlenmeyer flasks. Add 0.5 ml lanolin solution, and

evaporate the samples to dryness in a 40°C water bath, using a small stream of filtered air to speed evaporation. Add 5 ml of the alcohol-acid solution plus 0.8 g zinc, insert a small funnel into the opening of the flask, and reflux gently on a hot plate 10 minutes. Remove, filter through a small cotton plug into glass-stoppered test tubes, and wash with 20 ml 50% ethyl alcohol. Add 1 ml 6*N* HCl, mix, and let stand until samples come to room temperature. Add 1 ml sodium nitrite solution, mix well, and let stand 10 minutes in the dark. Add 1 ml ammonium sulfamate solution, mix well, and let stand 10 minutes in the dark. Add 2 ml dye solution, mix, and let stand 20 minutes in dark, mixing at intervals. Add 4 ml chloroform, shake, pipet off chloroform, and filter it through a small plug of cotton. Determine absorbance at 565 $m\mu$, using chloroform as a reference. The color is stable for at least 1 hour.

Experimental and Results

The standard curves obtained by this method for 0–50 μ g of the nitrated pesticides follow Beer's Law. The ranges of absorbance readings of the standards and the maximum wavelengths, determined with a Beckman Model DB recording spectrophotometer, are reported in Table 1.

The extraction and cleanup procedures applicable to the nitrated insecticides can be used for this method. Van Middelem, *et al.* (8) have reported on the extraction and cleanup of parathion residues on leafy vegetables. The suggested cleanup procedure given in the *Official Methods of Analysis* of the AOAC (9) is also useful.

Known amounts of insecticides were added to apple and pear fruits and to apple and pear leaf tissue prior to extraction. The apple and pear fruits were extracted by tumbling, for a 5 minute period, with a 2:1 mixture of distilled *n*-hexane:isopropyl alco-

Table 1. Characteristics of the color produced from some nitrated aromatic insecticides

Insecticide	Range in μ g	K Factor of Color Produced	Range in Absorbances ^a	Maximum Absorbance ^a , $m\mu$
Binapacryl	0–100	0.0142	0.010–1.425	565
EPN	0–50	0.0318	0.012–1.600	565
Methyl parathion	0–50	0.0173	0.008–0.871	560
Parathion	0–50	0.0263	0.008–1.325	560

^a Determined by Beckman Model DB recording spectrophotometer.

Table 2. Average recovery of EPN, binapacryl, parathion, and methyl parathion added to apple and pear fruits, tomatoes, leaf lettuce, and apple and pear leaves

Sample	Insecticide	Added, ppm	Recovery, %
Apple fruits ^a	EPN	0	0
		0.09	85.4
		0.50	106.0
	Binapacryl	0	0
		0.10	68.7
		0.50	90.3
		0 ^b	0
		0.47 ^b	97.6
	Parathion	0	0
		0.09	113.0
		0.50	58.3
	Methyl parathion	0	0
		0.09	120.0
		0.50	73.3
Pear fruits ^c	Binapacryl	0	0
		0.45	92.0
Tomatoes	EPN	0	0
		0.05	72.0
		0.25	80.0
	Binapacryl	0	0
		0.05	106.0
		0.25	80.0
	Parathion	0	0
		0.05	109.0
		0.25	88.0
	Methyl parathion	0	0
		0.10	86.5
		0.25	72.0
Leaf lettuce	EPN	0	0.02
		0.05	80.0
		0.25	84.0
	Binapacryl	0	0
		0.05	113.0
		0.25	100.0
	Parathion	0	0
		0.05	96.0
		0.25	104.0
	Methyl parathion	0	0
		0.10	58.5
		0.25	96.0
Apple leaves	Binapacryl	0	0
		0.05 ^d	99.0
		0.50 ^d	87.5
		5.00 ^d	90.5
Pear leaves	Binapacryl	0	0
		0.05 ^d	96.0
		0.50 ^d	94.0
		5.00 ^d	95.0

^a Transparent apple.^b Golden Delicious apple.^c d'Anjou pear.^d μ g added per sq. cm.

hol. Three ml of the mixed solvent was added per gram of sample. The apple and pear leaf tissue samples were extracted by shaking with distilled *n*-hexane, in a small flask, for 1 minute.

Tomato and leaf lettuce samples were ground to a fine state of subdivision, frozen, and allowed to thaw slightly. Known amounts of insecticide were added to different samples and the fortified samples tumbled for 30 minutes with a 2:1 mixture of distilled *n*-hexane:isopropyl alcohol. Three ml of mixed solvent was added per gram of sample.

When a mixed solvent was used, the isopropyl alcohol was removed from the extract by repeated washing with distilled water. All extracts were finally dried by filtration through anhydrous sodium sulfate.

The extracts of apple and pear fruits and of apple and pear leaf tissues did not require any cleanup procedure before analysis.

The extracts of tomato and leaf lettuce did require this cleanup, however. An aliquot of the tomato and leaf lettuce extract was evaporated to approximately 25 ml and shaken for 3 minutes with 1.5 g of a 2:1 mixture of Attaclay:Nuchar 190-N. The sample was filtered through a plug of acetone-washed cotton and overlaid with sodium sulfate, and the flask and filter were washed 4 times with 15 ml portions of distilled benzene.

The extracts, both cleaned up and not cleaned up, were carefully evaporated to dryness, and the insecticide content was determined in the same manner as the standard curve.

The recovery of EPN, binapacryl, parathion, and methyl parathion added to apple and pear fruits, tomatoes, leaf lettuce,

and apple and pear leaf tissue are presented in Table 2.

Discussion

To check possible interferences, a number of insecticides were analyzed by this method. When 100 μ g of Guthion was analyzed without the hydrolysis step, a slight color was obtained while Karathane and Chlorthion gave absorbance readings of 0.650 and 0.250, respectively. The following insecticides in 100 μ g quantities gave no interference: aldrin, Aramite, captan, carbophenothion, chlordane, Co-Ral, DDE, DDT, Delnav, demeton, diazinon, dimethoate, Dipterex, Disyston, endosulfan, endrin, Eradex, ethion, Genite, heptachlor, Kelthane, Kepone, lindane, malathion, menazon, methoxychlor, mirex, naled, phorate, Phosdrin, phosphamidon, Phostex, schradan, Sevin, sulfotepp, Sulphenone, TDE, tetradifon, toxaphene, V-C 13 Nemacide, and Zectran.

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