# 4-(p-Nitrobenzyl)Pyridine as a Spray Reagent for Organophosphorus Pesticides and Some of Their Breakdown Products on Thin-Layer Chromatograms[1]

by MOHAMED TAWFIK H. RAGAB[2]
*Department of Agricultural Chemistry and Bureau of Conservation and Environmental Science*
*Rutgers–The State University, New Brunswick, N. J.*

The increasing use of organophosphorus pesticides has created interest in the development of a universal thin-layer chromogenic reagent for use in the detection and identification of these pesticides and as many as possible of their breakdown products. Barney II (1) has recently reported two spray tests for many organophosphorus pesticides. Benzidine which may cause injury to blood (12) and reported as a cause of bladder tumours

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1/ Presented at the ACS Winter Meeting, Phoenix, Arizona, January, 1966. After its submission a similar paper by Watts was presented at the 79th Meeting of the A.O.A.C., Washington, D.C., October, 1965. Watts (14), however, used the base tetraethylenepentamine but NaOH was used in the present study.

2/ Research Station, Canada Department of Agriculture, Kentville, Nova Scotia, Canada.

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(2, 12), was used in these sprays.

The present study deals with the use of 4-(p-nitrobenzyl) pyridine (NBP) as a universal spray reagent for organophosphorus pesticides and their postulated breakdown products on thin-layer chromatograms of alumina, cellulose, and Eastman silica gel chromatogram. Recovery studies from fortified water, fish fillet and crops have been made on alumina plates.

The NBP reaction or its modification has been previously reported for several alkylating compounds (3, 4, 10) including organophosphorus pesticides (5, 7, 10), and the herbicide dimethyl tetrachlorophthalate (11). Its use on thin-layer chromatograms for chloro-s-triazine herbicides has recently been reported (9).

## Method

### Reagents

All solvents were of ACS grade or equivalent and redistilled before use.

(a) Acetone.--ACA, refluxed with potassium permanganate (1 g/L) for one hour then redistilled (5).

(b) Chromogenic spray reagent.--4-(p-Nitrobenzyl) pyridine (K&K Laboratories, Inc., Plainview, N.Y.), 2% in acetone (a). Prepared fresh weekly.

(c) Ammonium carbonate solution.--ACS, 20%, aqueous, diluted 1:1 (v/v) with acetone before use.

(d) Sodium hydroxide solution.--ACS, 10% in 50% (v/v) ethanol.

(e) Aluminum oxide G.--(Research Specialties Co.).

(f) Cellulose, plain.--#8078-1 (Research Specialties Co.).

(g) Eastman chromatogram sheets.--Silica gel 100μ, type K301R2, 8 x 8", without fluorescent indicator.

(h) Solvent system for aluminum oxide and cellulose, immobile.--DMF 20% in acetone (a). Mobile.--Methyl cyclohexane (6).

(i) Solvent system for thin-layer sheets.--Ethyl acetate: n-hexane, 1:3 (v/v).

(j) Sodium chloride and anhydrous sodium sulfate.--ACS.

(k) Nuchar carbon 190-N.

(l) Pesticides standard solutions*.--Stock solutions to contain 10 μg/μl ethyl acetate in 10 ml volumetric flasks. Lower concentrations were obtained by serial dilutions with ethyl acetate.

Thin-Layer Chromatography

(a) Aluminum Oxide G or cellulose, plain plates.--Coat thoroughly cleaned 8 x 8" glass plates with 200μ thickness of each adsorbent in the conventional manner from a slurry of $Al_2O_3$:distilled water (1:2) and cellulose:distilled water (1:5). Leave the plates to

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* For simplicity 0.1 ml of liquid pesticide was assumed to weigh 0.1g.

dry at room temperature for 20 minutes after which slide them in the drying rack and activate in the oven for 40 minutes at 105°C. When cool, use immediately or store in a desiccator until needed. Scrape 1/4" from both sides and edge of the plate first coated by the applicator. Spot pesticide standards with 1 µl disposable micropipettes at 1/2" intervals on an imaginary line which passes between two pencil marks one on each side of the plate and 3/4" from its bottom edge. Horizontally score a line 10 cm from the imaginary starting line. Dip the spotted plates in the immobile solvent and develop them in the mobile solvent as described by Kovacs (6).

(b) Eastman chromatogram sheets.--After spotting standards on the thin-layer sheet but without scraping layer from edge or sides, mount the sheet between the two plates to form the thin-layer sandwich, clip plates, and develop in the solvent system n-hexene:ethyl acetate (3:1 v/v).

When the solvent reaches the marked front, remove the developed plates or sheets and air dry them for at least ten minutes. Spray plates or sheets heavily and homogenously with NBP solution followed lightly by ammonium carbonate solution. Leave plates or sheets for about five minutes for acetone to evaporate then heat them in the oven for ten minutes at 110-120°C. When cool, spray plates or sheets with sodium hydroxide reagent and mark positions of the colored spots with a pencil. Calculate the $R_{Co-Ral}$ of each compound and visually determine its minimum detectable amount in case of each thin-layer absorbent used.

### Application of Thin-layer Chromatography on Alumina to Residue Analysis

(a) Recovery and detection of malathion from fortified water and fish fillet.

Distilled water and chopped Carp fish fillet were fortified separately at 0.1, 0.5, and 1.0 ppm levels with malathion. The malathion was extracted with hexane and the extracts from fish were cleaned up (8). The concentrated extracts were spotted on $Al_2O_3$ thin-layer plate along with malathion standard and chromatographed according to the above procedure.

(b) Recovery and detection of some organophosphorus pesticides from fortified crops.

Chopped carrots were fortified with malathion at 0.1, 0.5 and 1.0 ppm levels and chopped asparagus with 0.1 and 1.0 ppm levels of Zinophos. Chopped carrots and lettuce were separately fortified with a mixture of malathion, parathion, and trithion at 0.1 and 1.0 ppm levels. Pesticides were extracted twice in the Lourdes homogenizer with ethyl acetate using three parts solvent to one part crop. The extracts were combined in a separatory funnel, about 1 g sodium chloride was added and contents were shaken vigorously. When layers were separated, water was drained out and the extract was filtered through a funnel plugged with a small piece of glass wool topped by about 10 gm of a mixture of 1:1 anhydrous sodium sulfate and Nuchar carbon 190-N. The extract in the funnel was rinsed three times with ethyl acetate and rinsings were filtered through the funnel. The combined filtrates were made up to a known volume with ethyl acetate and representative volumes containing the ppm level of pesticide or pesticide mixture were concentrated in a small pear-shaped ground joint flask and spotted on $Al_2O_3$ thin-layer plates along with pesticide standards and chromatographed as before.

## Results and Discussions

Table 1 presents the results obtained from the application of NBP spray reagent to the organophosphorus pesticides and some of their degradation products. The employment of more than one thin-layer adsorbent is of value in the qualitative identification of the pesticides tested. The approximate minimum detectable amount of each pesticide or pesticide derivative on the three TL adsorbents were recorded.

All the 36 compounds tested gave distinct blue spots with the exception of diazinon which gave a red spot

TABLE 1

Response of Organophosphorus Pesticides and Breakdown Products to NBP Spray Reagent

| Pesticide[1] | Aluminum Oxide G[2] | | Cellulose, plain[2] | | Eastman Silica Sheet[3] | |
|---|---|---|---|---|---|---|
| | $R_{\text{Co-Ral}}$ | Sensitivity (ug) | $R_{\text{Co-Ral}}$ | Sensitivity (ug) | $R_{\text{Co-Ral}}$ | Sensitivity (ug) |
| Malathion | 1.68 | 0.30 | 0.98 | 0.25 | 1.16 | 0.50 |
| Malaoxon | 0.00 | 0.25 | 0.30 | 0.20 | 0.30 | 0.50 |
| Parathion | 1.77 | 0.25 | 0.57 (1.17)[4b] | 0.50 | 1.31 | 0.20 |
| Paraoxon | 0.01 | 0.15 | 0.08 | 0.25 | 0.43 | 0.20 |
| Methyl parathion | 0.98 | 0.25 | 0.62 | 0.15 | 1.17 | 0.20 |
| Diazinon | 3.67 | 0.50 | 1.47 | 0.50 | 1.25 | 1.00 |
| Diazinoxon | 3.65 | 0.50 | 1.42 | 0.50 | 1.26 | 1.00 |
| Thimet | 3.38 | 0.50 | 1.19 | 2.00 | 1.46 | 2.00 |
| Thimet sulfone | 0.00 | 5.00 | 0.66 | 0.50 | 0.12 | 0.25 |
| Thimet sulfoxide | 0.84 | 0.25 | 0.27 (0.31)[b] | 0.50 | 0.20 | 0.10 |
| Trithion | 2.84 | 0.50 | 1.13 | 1.00 | 1.44 | 1.00 |
| Methyl trithion | 1.89 | 0.15 | 0.98 | 0.15 | 1.40 | 0.50 |
| Imidan | 0.55 | 0.30 | 0.25 | 0.25 | 0.79 | 1.00 |
| Dibrom | 0.09[4a] | 0.30 | 0.35 (0.23)[b] | 0.50 | 0.71 | 5.00 |
| DDVP | 0.07 | 4.00 | 0.43 | 2.00 | 0.62 | 5.00 |
| Phosdrin | 0.28 | 4.00 | 0.64 | 0.50 | 0.66 | 4.00 |
| Guthion | 0.67 | 0.20 | 0.38 | 0.25 | 0.69 | 0.50 |
| Systox | 3.94 | 1.00 | 1.09 (0.66)[b] | 2.00 | 1.45 | 5.00 |
| Di-Syston | 4.06 | 4.00 | 1.23 (0.23)[b] | 2.00 | 1.43 | 5.00 |
| Meta-Systox | 0.05 | 0.25 | 0.47 | 0.50 | 0.00 | 0.50 |
| Dylox | 0.06 | 0.25 | 0.75 | 0.50 | 0.04 | 0.50 |

## TABLE 1 (continued)

Response of Organophosphorus Pesticides and Breakdown Products to NBP Spray Reagent

| Pesticide[1] | $R_{Co\text{-}Ral}$ | Sensitivity (ug) | Cellulose, plain[2] |  | Eastman Silica Sheet[3] |  |
|---|---|---|---|---|---|---|
|  |  |  | $R_{Co\text{-}Ral}$ | Sensitivity (ug) | $R_{Co\text{-}Ral}$ | Sensitivity (ug) |
| Co-Ral | 1.00 | 1.50 | 1.00 | 4.00 | 1.00 | 5.00 |
| Bayer 29493 | 1.81 | 0.15 | 1.38 | 0.50 | 1.20 | 0.50 |
| Ronnel | 3.25 | 1.00 | 1.62 | 0.25 | 1.39 | 0.20 |
| Ethion | 3.00 | 5.00 | 1.11 | 2.00 | 1.47 | 5.00 |
| Dimethoate | 0.10 | 0.20 | 0.11 | 1.00 | 0.07 | 0.15 |
| Zinophos | 1.65 | 4.00 | 1.11 | 0.50 | 1.02 | 0.20 |
| Phosphamidon | 0.50 | 0.20 | 0.19 (0.26)[b] | 0.50 | 0.06 | 0.20 |
| Desethyl-phosphamidon | 0.15 | 0.25 | 0.09 | 0.50 | 0.05 | 0.20 |
| Bidrin | 0.32 | 0.20 | 0.09 | 0.50 | 0.00 | 0.20 |
| Ciodrin | 0.43[a] | 0.30 | 0.58 (0.07)[b] | 0.35 | 0.31 | 0.20 |
| Delnav | 1.55 | 1.00 | 1.66 | 0.50 | 1.25 | 5.00 |
| Abate | 1.31 | 1.00 | 1.47 | 0.50 | 1.12 | 1.00 |
| Dursban | 3.50 | 0.25 | 0.98 | 0.50 | 1.40 | 0.10 |
| Dimethoxon | 0.00 | 0.25 | 0.06 | 0.50 | 0.00 | 0.20 |

1. Obtained as analytical standards through the courtesy of the manufacturing companies.
2. Solvent system.--immobile : 20% dimethyl formamide in acetone, mobile - methyl cyclohexane. $Al_2O_3$ plates were washed as described by Kovacs (6).
3. Solvent system.--n-hexane : ethylacetate (3:1).

4a. Indicates streak. 4b. Indicates additional spots or streak.

and parathion, paraxon, and methyl parathion which gave persistent brilliant yellow spots. This yellow color of spots shows up with much lesser intensity soon after spotting and before the application of any of the spray reagents.

The blue color produced by NaOH is less stable than that produced by tetraethylenepentamine (14) but it can be regenerated when required with NaOH reagent. After fading, another chromogenic reagent may be applied to the same chromatogram. The use of a NaOH spray probably presents less risk than tetraethylenepentamine which is regulated by the Hazardous Substances Labeling Act.

No interference was noted when extracts of crop and fish were analyzed by this procedure although some purple coloration was observed in case of fish fillet but this did not interfere with the position of malathion on the thin-layer plates. Excellent recoveries (over 90%) of malathion from fortified water, fish, and carrots were obtained. Similar recoveries were also observed in case of asparagus fortified with zinophos and carrots and lettuce spiked separately with a mixture of malathion, parathion, and trithion.

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