

Determination of *m*-Dinitrophenyl Pesticides

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A simple and rapid method for spectrophotometric determination of *m*-dinitrophenyl pesticides is based on their reaction with potassium cyanide. The reaction is sensitive to 2 γ of active material and specific for the *m*-dinitro structure. The developed color is stable and obeys Beer's law over a wide range. The use of this reaction in determining DDT, methoxychlor, or BHC is suggested, and a color reaction was demonstrated. Its use in the determination of other *m*-dinitro compounds is also suggested.

CHEMICAL CONTROL of agricultural and forest insect pests necessarily involves introduction of toxic materials into wildlife habitats, and thus exposes wild animals, birds, and fish to possible contact with freshly applied or residual toxicants, or with contaminated food supplies. In some instances, heavy avian or fish mortality has followed such pesticidal applications (4, 8-11, 15-17). The effects of DDT upon wildlife have been extensively studied, but relatively little is known about possible effects of other pesticides. The biochemical laboratory of the Patuxent Research Refuge, Laurel, Md., is conducting studies with penned quail and pheasants in efforts to obtain such information.

2,4-Dinitro-6-methylphenol	6.77×10^3
2,4-Dinitro-6- <i>sec</i> -butylphenol	1.96×10^3
2,4-Dinitro-6-cyclohexylphenol	2.10×10^3
2,4-Dinitro-6-(1-methylheptyl)phenyl crotonate	1.87×10^3

Analytical methods for some of these compounds, including materials with *m*-dinitrophenolic groups, were found to be deficient in sensitivity, specificity, or both. Four *m*-dinitrophenolic pesticides have been used to control mites and aphids, and as fungicides; but suitable methods for their quantitative de-

termination were lacking. Nonphenolic dinitro compounds could be determined through their reaction with ketones in the presence of strong alkali, according to the methods of Fischer (7) and of Schechter and Hornstein (14). *m*-Dinitrophenols may be determined by titration of iodine liberated from $\text{KBr-KBrO}_3\text{-KI}$ (2) or by titration with standard titanous chloride in an inert atmosphere (6).

In 1865, Pfaundler and Oppenheimer (12) showed that polynitro compounds reacted with potassium cyanide to give a colored product. Subsequently, Borsche and Bocker (5) extended the reaction; and Anger (7) showed it to be specific for *m*-dinitro compounds. This reaction was applied to the spectrophotometric determination of *m*-dinitrophenolic pesticides in the current studies.

Table II. Interference by Other Pesticides

Common Name	Chemical Name	Micrograms	Absorbance
Aldrin	1,2,3,4,10,10-Hexachloro-1,4,4a,5,8,8a-hexahydro-1,4- <i>endo</i> , <i>exo</i> -5,8-dimethanonaphthalene	405	0.020
Dieldrin	1,2,3,4,10,10-Hexachloro- <i>exo</i> -6,7-epoxy-1,4,4a,5,6,7,8,8a-octahydro-1,4- <i>endo</i> , <i>exo</i> -5,8-dimethanonaphthalene	401	0.085
Chlorobenzilate	Ethyl 4,4'-dichlorobenzilate	409	0.025
DDT	1,1,1-Trichloro-2,2-bis(<i>p</i> -chlorophenyl)ethane	402	0.013
Isobornyl thiocynoacetate	Mixture of terpene thiocynoacetates	401	0.070
Isolan	Dimethyl-5-(1-isopropyl-3-methyl-pyrazolyl)carbamate	418	0.017
Kelthane	1,1-Bis(<i>p</i> -chlorophenyl)2,2,2-trichloroethanol	401	0.035
Methoxychlor	1,1,1-Trichloro-2,2-bis(<i>p</i> -methoxyphenyl)ethane	407	0.035
MGK 264	<i>N</i> -(2-Ethylhexyl)bicyclo[2.2.1]hept-5-ene-2,3-dicarboximide	402	0.025
Prolan	1,1-Bis(<i>p</i> -chlorophenyl)-2-nitropropane	503	0.015
Pyrolan	Dimethyl-5-(3-methyl-1-phenylpyrazolyl)carbamate	402	0.015
Bulan	1,1-Bis(<i>p</i> -chlorophenyl)-2-nitrobutane	403	0.085
<i>n</i> -Propyl isome	Dipropyl-5,6,7,8-tetrahydro-7-methylnaphtho[2,3]-1,3-dioxole-5,6-dicarboxylate	410	0.040
Piperonyl butoxide	α -[2-(2-Butoxyethoxy)-ethoxy]-4,5-methylene-dioxy-2-propyltoluene	507	0.030
Chlordan	1,2,4,5,6,7,8,8-Octachloro-2,3,3a,4,7,7a-tetrahydro-4,7-methanoindene	402	0.025
Heptachlor	3a,4,5,6,7,8,8-Heptachloro-3a,4,7,7a-tetrahydro-4,7-methanoindene	404	0.010
Ovex or Ovotran	<i>p</i> -Chlorophenyl- <i>p</i> -chlorobenzenesulfonate	505	0.010
Isodrin	1,2,3,4,10,10-Hexachloro-1,4,4a,5,8,8a-hexahydro-1,4- <i>endo</i> , <i>endo</i> -5,8-dimethanonaphthalene	405	0.015
Endrin	1,2,3,4,10,10-Hexachloro- <i>exo</i> -6,7-epoxy-1,4,4a,5,6,7,8,8a-octahydro-1,4- <i>endo</i> , <i>endo</i> -5,8-dimethanonaphthalene	503	0.015
DMC	1,1-Bis(<i>p</i> -chlorophenyl)ethanol	405	0.025

Procedure

Preparation of Standard Curve. Dissolve 0.1000 gram of the dinitrophenolic pesticide in ethyl alcohol and adjust volume to 1000 ml. Use aliquots, and prepare ethanolic solutions containing 5, 10, 25, and 50 p.p.m. of the pesticide. To 1 ml. of each solution, add 2 drops of 10% aqueous potassium cyanide and 0.5 ml. of 1-propanol. Heat in boiling water for 10 minutes, cool, and adjust volume to 1 ml. with 95% ethyl alcohol. Using a microcell in a Beckman DU spectrophotometer, determine absorbance at 350 μ and prepare curve by plotting absorbance against concentration.

Preparation of Sample. Extract plant materials according to the method of the Association of Official Agricultural Chemists (3) by macerating with dilute alkali, acidifying with hydrochloric acid, and extracting with chloroform. Evaporate extracts nearly to dryness, dissolve residues in 95% ethyl alcohol, and adjust to volume. Prepare suitable aliquots in ethyl alcohol, and determine *m*-dinitrophenolic compounds as outlined above.

Results and Discussion

The reaction between potassium cyanide and *m*-dinitrophenolic pesticides produced deeply colored solutions with absorption maxima at 350 m μ (Figure 1). As little as 2 γ of the phenolic compound could be determined. The colored solutions obeyed Beer's law over wide ranges of concentrations (Figure 2). Molar extinction coefficients were determined for four common pesticides (Table I), and were expressed in terms of the phenolic reactant.

As stated by Anger (7), the reaction is specific for *m*-dinitro compounds; other common insecticides do not interfere with development of color nor with spectrophotometric determination (Table II). For qualitative purposes, ethyl alcohol would probably suffice as a solvent. However, it allows evaporation to dryness with diminution of color development and loss of color sensitivity. For quantitative analysis, this is undesirable. The higher alcohols decrease sensitivity by their increased absorption. With 1-propanol, these obstacles are overcome. The boiling point (98° C.) is high enough and the absorption low enough so that no serious difficulties are encountered under the conditions of the reaction.

Little is known of the reaction itself other than its specificity for *m*-dinitro compounds. However, on the basis of the reaction of 2,4-dinitrophenol to give *m*-purpuric acid, the reaction products are phenylhydroxyamines:

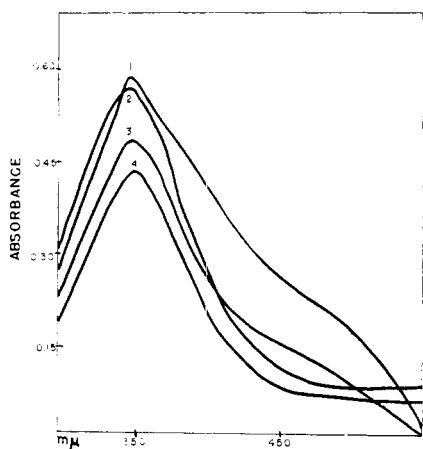
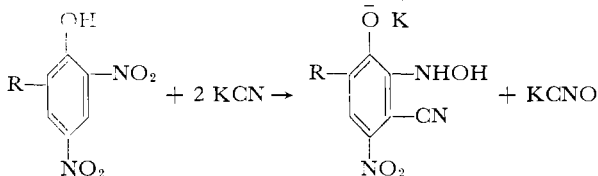


Figure 1. Absorption spectra for potassium cyanide plus *m*-dinitrophenolic pesticides

1. 2,4-Dinitro-6-methylphenol
2. 2,4-Dinitro-6-cyclohexylphenol
3. 2,4-Dinitro-6-sec-butylphenol
4. 2,4-Dinitro-6-(1-methylheptyl)phenyl crotonate

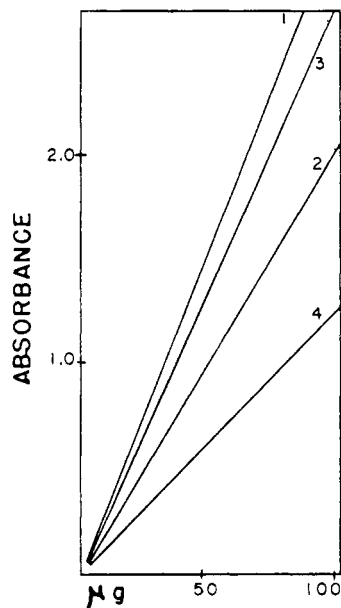
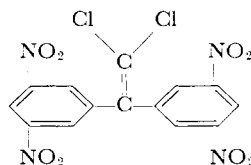


Figure 2. Absorbance vs. concentration

1. 2,4-Dinitro-6-methylphenol
2. 2,4-Dinitro-6-cyclohexylphenol
3. 2,4-Dinitro-6-sec-butylphenol
4. 2,4-Dinitro-6-(1-methylheptyl)phenyl crotonate

The reaction is general to the extent that any *m*-dinitro compound will give a color reaction with potassium cyanide. Thus it seemed probable that this reaction might be applied to the determination of DDT. According to Schechter and Haller, the main nitration product

of DDT is the tetranitro derivative (13). The presence of the



m-dinitro groups should and does lend itself to reaction with potassium cyanide. In this case, the absorption maximum shows a marked shift to lower wave length numbers (Figure 3) and from a red color to amber. The reaction of the nitrated material with potassium cyanide is almost instantaneous to give a color that is stable for at least 4 hours. Similar results were obtained with methoxychlor.

In the Schechter-Hornstein method for analysis of lindane, the material is dechlorinated to benzene and then nitrated. Again the *m*-dinitro compound

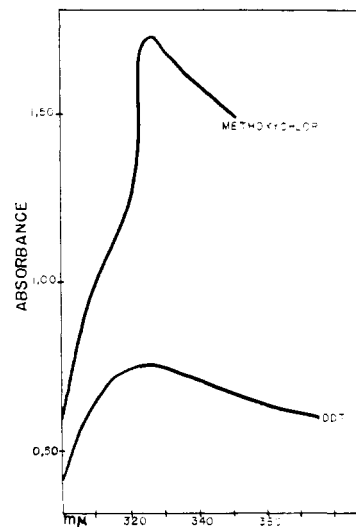


Figure 3. Absorption spectra for potassium cyanide plus nitration products from the Schechter-Hornstein procedures for methoxychlor and DDT

predominates and is the basis for their color reaction with methoxylate and methyl ethyl ketone. This *m*-dinitrobenzene also reacts with potassium cyanide to give a stable color.

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