Determination of Dinitramine Residues in Soil and Plant Tissue

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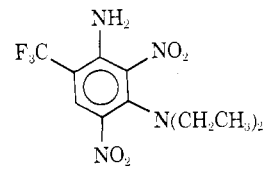
Dinitramine (N^3,N^3-diethyl-2,4-dinitro-6-trifluoromethyl-*m*-phenylenediamine; tested under the names diethamine and USB 3584; trade name Cobex) is an effective new selective herbicide used for control of a wide variety of weeds in soybeans and cotton. An analytical method for determining residual quantities of this material has been developed which involves methanol extraction, methylene chloride partitioning, Florisil column chromatography, and measurement by electron capture gas chromatography. The method is applicable to soil, forage, and crop samples, and it gives good recoveries down to 0.01 ppm without subtraction of blanks. An alternative method utilizes the same extraction and clean-up procedure, but with alkali flame ionization detection. The latter method also is quantitative at 0.01 ppm. Application of these procedures to samples from field test plots demonstrated that dinitramine is readily degraded by environmental conditions over the growing season. Soil residues fell below 10% of application levels in about 100 days, while mature plant tissue and crop samples contained less than 0.01 ppm of dinitramine.

Dinitramine (N^3,N^3-diethyl-2,4-dinitro-6-trifluoromethyl-*m*-phenylenediamine, Cobex) is a new selective herbicide which is effective in the control of annual grasses and broadleaf weeds in cotton and soybeans.



dinitramine

Dinitramine has been field tested under the names diethamine and USB 3584. An analytical method for low levels of the compound was required in order to study its persistence in the environment. This paper describes the procedure, using electron-capture gas chromatography, for assay of dinitramine residues in soils and in plant tissues.

METHOD

Apparatus. A gas chromatograph, such as the Varian Aerograph Model 1740 equipped with a tritium foil electron capture detector, is required. The column is 5 ft × 1/8 in. S.S. packed with 3% QF-1 on 100/120 Varaport-30. Prepurified nitrogen flow is 25–35 ml/min, and column and inlet temperatures are 200 and 225°, respectively. Detector temperature is 200–210°.

Reagents. Dinitramine standard solution, 0.1 ppm in Nanograde benzene, is prepared fresh every week and protected from light. Methylene chloride and *n*-hexane are redistilled. Methanol, ethyl ether, and acetonitrile are A.R. grade.

Florisil Standardization. Batches of 500 g of 60–100 mesh Florisil are heated to 130° for 48 hr, and then moistened by thoroughly mixing with 15 g of water and kept in a sealed jar.

The Florisil clean-up column is prepared as follows. To a 22-mm i.d. tube fitted with a Teflon stopcock and a glass wool pledget, add Florisil with light tapping until a 7.5-cm column is formed. Add 2.5 cm of anhydrous sodium sulfate on top of the Florisil. Wash the column with 100 ml of *n*-hexane, keeping a layer of hexane on the column. Add 200 μg of dinitramine (200 μl of a 1000-ppm benzene solution) to a flask containing 5 ml of 95/5 hexane–ethyl ether. Transfer to the column and start to drain, collecting the eluate. Rinse the flask with four successive 5-ml portions of hexane–ether, allowing each to go into the column before the next addition. Add additional hexane–ether to the column and follow the visible movement of the dinitramine. Note the volume of solvent mixture required to move the dinitramine as a yellow band to the bottom of the Florisil column. This volume of forerun is to be discarded in the sample clean-up procedure following. Continue to elute and record the volume of hexane–ether required to remove the dinitramine from the column. This volume plus an additional 50 ml will be collected in the clean-up procedure to ensure complete recovery of the dinitramine. Each fresh batch of Florisil should be standardized in this manner.

Sample Extraction. For plant tissue, grind a representative sample of frozen tissue in a Hobart food cutter in the presence of Dry Ice. When thawed and free of Dry Ice, weigh 100 g into a quart Mason jar. Extract with 400 ml of methanol for 10 min using an Omni-Mixer. In the case of soil samples, extract 100 g of thawed, air-dried soil with 400 ml of methanol in a Mason jar for 30 min on a platform shaker. In either case, after extraction, filter through Whatman No. 1 paper into a glass bottle.

Clean-Up Procedure A. This procedure is suitable for soil samples as well as soybean and some other plant tissues. Transfer 100 ml of the extract (representing a 25-g sample) to a 1-l. separatory funnel. Add 500 ml of 5% sodium chloride solution to the funnel and mix. Add 50 ml of methylene chloride to the funnel and shake vigorously for 1 min. Allow the layers to separate. Drain the CH_2Cl_2 layer through anhydrous sodium sulfate into a 300-ml round-bottomed flask. Repeat the extraction with two more 50-ml portions of CH_2Cl_2, collecting the extracts as before. Wash the sodium sulfate with 25 ml of CH_2Cl_2. Evaporate the combined CH_2Cl_2 extracts using a flash evaporator and a 40–50° water bath. Remove the flask as soon as the solvent is completely evaporated to prevent loss of dinitramine. Add 5 ml of 95/5 hexane–ether mixture to the flask, swirl to dissolve contents, and transfer to the Florisil column. Start collecting the eluate. Rinse the flask with four 5-ml portions of hexane–ether, allowing each portion to go into the column before the next addition. Elute with hexane–ether. Retain the fraction of the eluate as determined in the standardization of the Florisil. Normally the first 50–55 ml is discarded and the next 100 ml is saved in a 200-ml round-bottomed flask. Evapo-

U. S. Borax Research Corporation, Anaheim, California 92801.

Table I. Recovery of Dinitramine Added to Untreated Soils

Soil type	Added, ppm	% recovery, avg
Sandy loam	0.012	87
Sandy loam	0.04	97
Clay loam	0.2	85
Silty clay loam	0.04	120

Table II. Recovery of Dinitramine Added to Untreated Soybean and Cotton Plants and Seeds

Crop	Added, ppm	% recovery, avg
Soybean plants	0.01	88
Soybean seed	0.01	98
Cotton plants	0.005	80
Cotton plants	0.01	102
Cotton seed	0.01	97

rate to dryness as before. Dissolve the residue in 2 ml of Nanograde benzene and transfer to a small vial. Stopper and protect the solution from light with aluminum foil.

Inject a 2-μl aliquot into a gas chromatograph operated under the previously described conditions. Measure the area under the peak at the retention time of dinitramine (height $\times$ width at $^1/_2$ height is adequate).

Clean-Up Procedure B. A variation of the Florisil column elution is required for cotton plant tissue to remove an interference at the gas chromatographic retention time of dinitramine as follows. After the methylene chloride extraction and evaporation, dissolve the residue in 5 ml of a 1:1 mixture of hexane–benzene (instead of hexane–ether) and transfer to the Florisil column. Rinse the flask with four more 5-ml portions and elute the column with the same solvent mixture. NOTE: The Florisil must be previously standardized with 1:1 hexane–benzene to determine which fraction of eluate to retain. Normally, the first 50–55 ml are discarded and the next 100 ml are saved in a 200-ml round-bottomed flask. Evaporate to dryness as before and take up the residue in 2 ml of Nanograde benzene for injection into the gas chromatograph.

Modification. The addition of an oil removal step to Procedures A and B is required for plant tissues that have a high oil content; *e.g.*, soybeans and cottonseed. The residue from the methylene chloride evaporation is dissolved in 25 ml of *n*-hexane and partitioned with two 25-ml volumes of acetonitrile. (If problems with phase separation occur, the addition of one drop of concentrated hydrochloric acid helps alleviate the difficulty.) The acetonitrile extract is evaporated to dryness and the residue is dissolved in 5 ml of solvent and transferred to the Florisil column according to Procedure A for soybean seeds and Procedure B for cottonseed.

Standard Curve. Determine the gas chromatographic linear response range for dinitramine using standard solutions in benzene by plotting peak area (mm^2; height $\times$ width at $^1/_2$ height) *vs.* dinitramine injected on log–log coordinates. A linear range of 200–2000 pg is typical. If a sample extract contains dinitramine, the observed peak area must be within this linear range to obtain reliable data. Depending on dinitramine concentration in the sample, some adjustment in volume of the final concentrate or of the aliquot injected may be necessary. Some variations in detector sensitivity are observed with time. For best results, two points on the line (*e.g.*, 200 and 1000 pg) should be established every few hours. A reasonable approximation is obtained by determining one point and applying the same slope as previously established (for the same detector and conditions).

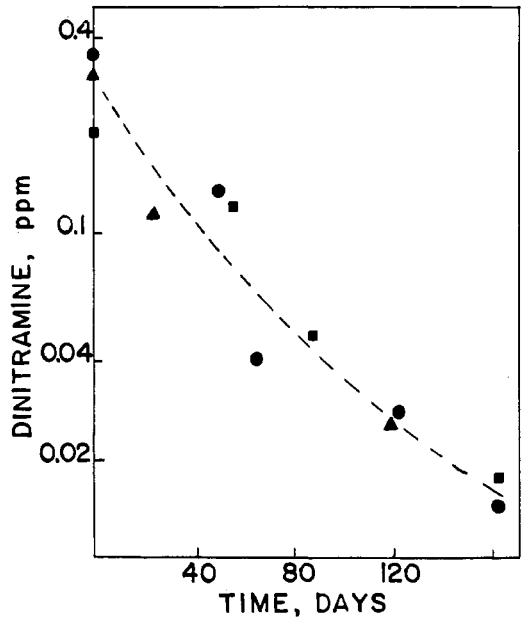


Figure 1. Dissipation of dinitramine from treated soil. Cotton plots at 1 lb/acre. ●, Georgia; ■, Mississippi; ▲, Mississippi

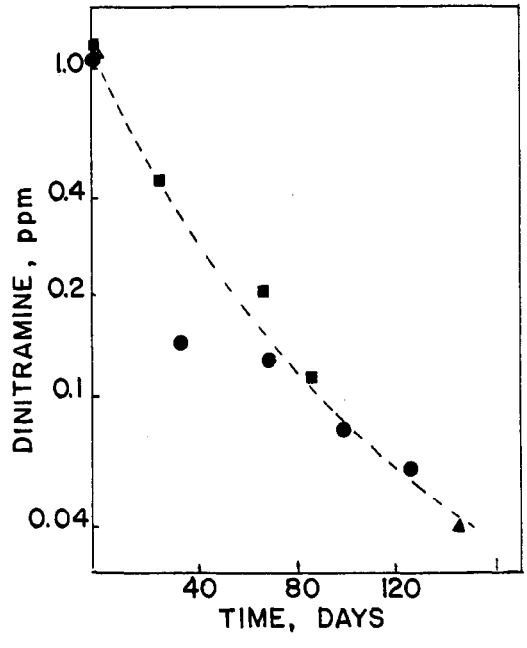


Figure 2. Dissipation of dinitramine from treated soil. Soybean plots at 1 lb/acre. ●, Iowa; ■, Mississippi; ▲, Indiana

Calculations. Measure the area of the dinitramine peak obtained from the sample and compare with the standard curve. The residue in the original sample is calculated as follows.

$$\text{dinitramine (ppm)} = \frac{(D)(\text{vol})\,10^{-6}}{(\text{wt})(\text{inj})}$$

where D = dinitramine corresponding to observed peak area, in picograms, vol = volume of final concentrate, in microliters, wt = weight of analyzed sample (25 g), and inj = volume injected into chromatograph, in microliters.

Confirmatory Method. Values obtained by the above procedure can be verified by the use of an alkali flame ionization detector. All extraction and clean-up procedures are carried out as previously described, giving a final benzene con-

centrate for gas chromatographic analysis. The flow rates of nitrogen, air, and hydrogen in the chromatograph are adjusted to maximize the signal-to-noise ratio, typically about 30, 250, and 35 cm^3/min, respectively. The sample peak area produced at the dinitramine retention time is compared with a log–log standard curve determined separately for this detector. Due to variation in detector sensitivity, a standard should be injected every few hours for calibration.

DISCUSSION

The method is quantitative to 0.01 ppm. Some sample types (*e.g.*, low organic soils) present so little gas chromatographic background that levels of dinitramine much lower than 0.01 ppm are readily determined.

Tables I and II list the recoveries, ranging from 85 to 120%, which were obtained from spiked samples of untreated soil and plant tissue.

In field studies, analysis of soil samples from treated plots showed a steady dissipation of dinitramine content. This is illustrated in Figures 1 and 2, showing reduction to 10% of the original value over a period of about 100 days. This dissipation is due to degradation rather than leaching or volatilization, and will be discussed in detail in a subsequent publication.

Plant tissue from treated field plots contained very little dinitramine. Values of 0.01–0.05 ppm have been obtained from some cotton and soybean roots, but plant tops contained detectable dinitramine only where plots were treated at exaggerated rates, and in no case was dinitramine found in cotton or soybean seeds.

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Adsorption–Desorption of Parathion as Affected by Soil Organic Matter

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Influence of soil organic matter on the adsorption–desorption of parathion was studied by using tagged insecticide. It was found that the parathion adsorption by soils is dependent on the type of association between the organic and mineral colloids. In aqueous solutions the parathion has a greater affinity for organic than for mineral adsorptive surfaces. Parathion bonding is stronger on organic than on mineral surfaces.

The adsorption–desorption of pesticides by the active soil surfaces is one of the main processes controlling soil–pesticide interactions. Although the organic colloid fraction has been shown to be the most active soil component in affecting pesticide fixation in soils (Wolcott, 1970), it was also noted that it cannot always be used as a single factor to predict the adsorptive capacity of soils (Meggitt, 1970). The character and the interaction between the organic and mineral colloids of the soil are finally defining the nature of the available adsorptive surfaces.

Soil adsorption of the organophosphorus insecticide parathion was found to be related to the organic matter content and to the soil mineralogy (Saltzman and Yaron, 1971). Swoboda and Thomas (1968), studying the adsorption mechanism of parathion by leaching experiments, found that parathion was retained in soils, mainly as a water-insoluble organic constituent of the soil, by partitioning between soil organic matter and the liquid phase. In a recent study, Leenheer and Ahlrichs (1971) stated that parathion affinity for organic surfaces depends on the magnitude of the hydrophobic nature of these surfaces, rather than on the type of organic matter. They assumed parathion adsorption on organic surfaces to be a physical adsorption with formation of weak bonds between the hydrophobic portion of the adsorbent and adsorbed molecule.

Little information on the influence of organic matter on the desorption of pesticides is found in the literature. In the review of Wolcott (1970) concerning the retention of pesticides by organic materials in soils, it is mentioned that there is some evidence that similar treatments may result in complete release of pesticides from clays, but only in a partial desorption from high organic soils.

The aim of this work was to investigate the relative importance of mineral and organic surfaces in the parathion adsorption–desorption process in some semi-arid soils, characterized by low organic matter content and different mineralogy.

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EXPERIMENTAL SECTION

Materials. Three mineral soils from various locations in Israel, having a relatively high organic matter content and different mineralogy, and a peat with 95% organic matter content, were selected for this experiment. The analytical characterization of the 20-cm upper layer is given in Table I.

The pesticide studied was the organophosphorus insecticide parathion (*O,O*-diethyl *O*-*p*-nitrophenyl phosphorothioate). Pure parathion (produced by Analabs, Inc.) and ^{14}C-labeled parathion (produced by Amersham Radiochemical Center) with a specific activity of 52 μCi/mg were used. The labeling was done in the alkyl chain.

Apparatus. For counting ^{14}C activity, a Packard 3003 Tricarb liquid scintillation spectrometer was used. The scintillation liquid consisted of 50 g of naphthalene, 7 g of PPO (2,5-diphenyloxazole), and 0.05 g of POPOP [2,2-*p*-phenylenebis-(5-phenyloxazole)], brought to 1 l. with dioxane. The purity of the material was checked periodically by gas chromatog-