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A Method to Determine Dinoseb Residues in Crops and Soil by Gas Chromatography

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A method is described for the determination of residues of dinoseb (2-*sec*-butyl-4,6-dinitrophenol) in alfalfa, corn, cottonseed, field beans, almonds, peanuts, peas, potatoes, soybeans, grapes, oranges, peaches, pears, barley, wheat, and soil at levels ranging from 0.05 to 100 ppm. Dinoseb is first extracted by hot hydrolysis in methanol–sulfuric acid and subsequently partitioned into diethyl ether and adsorbed onto basic alumina. After elution with sodium bicarbonate, ether partition, and diazomethane methylation, the dinoseb methyl ether is adsorbed onto acidic alumina and eluted with ether. Electron-capture gas chromatography provides a sensitive means of quantifying residues of dinoseb down to 20 pg. Average recoveries ranged from 77 to 99%.

Dinoseb (2-*sec*-butyl-4,6-dinitrophenol) is the active ingredient in several herbicides which are formulated as the alkanolamine salts of the ethanol series, as the ammonium salt, or as the free phenol. [Typical formulations include PREMERGE 3 Dinitro Amine Herbicide, DOW Selective Week Killer, and DOW General Weed Killer, which are products of The Dow Chemical Company.] These herbicides are valuable and effective in the control of many broadleaf weeds in crops and have been used extensively for many years by farmers and state and federal experiment station investigators. The lack of translocation of dinoseb in plants (Bandal and Casida, 1972) together with its short residual life on plants and in soil allow its use in many crop situations without risk of residues.

The literature is deficient in extensive and well-validated methodology for dinoseb determination in crops. Yip and Howard (1968) reported work on several dinitrophenols in some fruits and legumes. McKellar (1971) reported a method for dinoseb determination in milk and cream. Guardigli et al. (1971) developed a TLC procedure for dinoseb residues. Dekker and Selling (1975) in the Netherlands presented a method for dinoterb (2-*tert*-butyl-4,6-dinitrophenol) in soil. Edgerton and Moseman (1978) applied the methodology of McKellar to determine dinoseb in feed and rat tissues and excreta.

The method described here has been practiced for 10 years by four analysts in some 32 projects on 16 different crops plus soil, involving 37 substrates which were succulent, oily, dry fibrous, cellulosic, highly carbohydrate, or ionic (soil). Large numbers of recovery determinations validating the method in these substrates have been condensed into tables of average values.

EXPERIMENTAL SECTION

Gas Chromatograph. A Tracor Model 222 equipped with a linearized nickel-63 electron-capture detector (ECD) was used and operated at 95:5 argon/methane flow of 70 mL/min through the column plus 20 mL/min as detector purge, with temperatures of 200–220 °C (column), 350 °C (detector), and 250 °C (injector). Earlier work utilized a Barber Colman Model 5000 equipped with a strontium-90 ECD, which was operated at 90 mL/min nitrogen flow, with temperatures of 200 °C (column), 250–350 °C (detector), and 225 °C (injector). In both instruments, a 1.8 m × 3 mm i.d. glass U-column packed with 5% DC-200 on 80–100 mesh Gas-Chrom Z was used. An alternate packing would be 3% OV-101. In these instruments, 20 pg of dinoseb methyl ether produced a 5–10% FSD, with a base line noise of 0.1–0.2%. Retention time was typically 3–4 min.

Reagents. Solvents used were either distilled in glass or pesticide residue quality.

Basic and acidic alumina, Woelm type, obtained from Waters Associates as activity grade 1, were stored continually in an oven at 130 °C. Prepared columns were cooled before use.

Standards of dinoseb and dinoseb methyl ether were obtained from the Agricultural Products Department of Dow Chemical U.S.A. in 99+% purity. Solutions of dinoseb were kept in the dark, and those of dinoseb methyl ether were refrigerated except just prior to use, when they were allowed to come to room temperature.

Diazomethane methylating solution was prepared in ether from Diazald according to the directions on the bottle from Aldrich Chemical Co., Milwaukee, WI. Caution should be exercised in the preparation and use of diazomethane because it is toxic and can cause skin sensitivity and is potentially explosive under certain conditions.

Sample Preparation and Extraction. Crops should receive a preliminary chopping (Hobart Food Cutter) or grinding (Wiley Laboratory Mill), as appropriate, and be thoroughly mixed to provide a homogeneous sample. Weigh 10 g of pulverized sample (5 g of low-density samples such as straw or fodder) into a 4-oz square bottle and add 40 mL of methanol containing 2 mL of 6 N sulfuric acid. (More methanol may be required to cover straw or fodder.) Prepare a recovery sample by spiking a duplicate control sample with 1 mL of the appropriate concentration of dinoseb in methanol and letting stand 15 min. After heating the bottles for 1 h at 70 °C in a water bath or oven and cooling to the touch, blend each sample using a Lourdes MM-1 multimixer or Brinkmann Polytron PT-20ST homogenizer for 3 or 1 min, respectively. Add 5 g

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of Johns-Manville Hyflo-SuperCel filtering aid, cap the bottle with a Poly-Seal cap, and shake vigorously for 15 min. Filter the sample through a 0.5-cm pad of Hyflo-SuperCel in a Buchner fritted disc funnel, washing the bottle and filter cake with methanol to 100 mL of filtrate.

Process soils through the Wiley Mill while frozen, using dry ice to prevent thawing. Weigh 50 g of soil into a 200-mL centrifuge bottle (Corning 1261) and add the dinoseb spike in the case of recovery determinations and 100 mL of methanol solution containing 5 mL of 6 N sulfuric acid. Cap the centrifuge bottle and heat at 70 °C for 30 min, shake immediately for 15 min and then cooled the bottle to room temperature before centrifuging.

Cleanup and Derivatization. Transfer 10 mL of filtrate to an 11-dram capsule vial containing 15 mL of water and 8 g of NaCl. Partition twice with 10 mL of diethyl ether, pipetting the ether after each partition onto a 3-cm column of basic alumina in a 1-cm i.d. chromatographic tube. A layer of glass wool above the alumina surface will prevent its disturbance when adding ether. Elute the residue from the column into an 11-dram vial with 15 mL of 0.025 M sodium bicarbonate. Air pressure of 0.5 psi will keep the column flowing at 2–3 mL/min. Add 0.1 mL of concentrated phosphoric acid and 5 mL of diethyl ether and shake 2 min. Transfer the ether to a 40-mL conical centrifuge tube having a ⅋ 19 joint (Kimble 45201), add 1 mL of diazomethane solution, and let stand 15–30 min. Add 3 mL of hexane. Evaporate the excess diazomethane and ether on a steam bath to 2.5–3 mL using a Vigreaux distilling column (Kontes 286710). Clean up the residue further on a 2.5-cm column of acidic alumina contained in a disposable transfer pipet and elute the dinoseb methyl ether into the conical centrifuge tube with 5 mL of diethyl ether. Evaporate the ether on a steam bath with 3 mL of trimethylpentane present as a keeper, then dilute to 10 mL with trimethylpentane. If 5 g of straw or 50 g of soil was weighed out initially, final volume should be 5 or 50 mL, respectively, to provide a substrate concentration of 0.1 g/mL (or 0.005 μg of dinoseb methyl ether/mL at 0.05 ppm).

If after gas chromatographing the sample, interferences are found to be present in some substrates, they may be removed by partitioning the trimethylpentane solution with a few milliliters of 0.1 N NaOH and reinjecting the sample.

Gas Chromatography. Injection volume is 4 μL. The dinoseb peak height of the sample is compared to a standard curve prepared from peak heights of solutions of the dinoseb methyl ether primary standard weighed out and diluted with trimethylpentane over the range of 0.005 to 0.10 μg/mL. Treated samples out of this range were diluted.

It should be noted that dinoseb methyl ether is chromatographed but results are reported in dinoseb equivalents. This is done automatically by weighing out 1.058 times as much of dinoseb methyl ether as of dinoseb, but labeling the solutions at the concentrations of dinoseb.

Calculations. Once the sample concentration in micrograms/milliliter has been determined from the standard curve, ppm dinoseb is obtained by multiplying the sample concentration by 10 and by any dilution factor. Parts per million dinoseb in the sample should be corrected for the degree of recovery by the following formulas:

$$\%\ \text{recovery} = \frac{\text{ppm(sample)} - \text{ppm(control)}}{\text{ppm(added)}} \times 100$$

$$\text{ppm corr} = \frac{\text{ppm(sample)} - \text{ppm(control)}}{\%\ \text{recovery}} \times 100$$

Table I. Recovery of Dinoseb from Various Substrates

substrate	ppm added, range	no. of determ	% recovered range	% recovered av
alfalfa				
green forage	0.1-1.0	14	80-110	98
dry forage	0.1-1.0	17	70-100	85
almonds				
hulls	0.1-1.0	24	55-103	77
nutmeats	0.1-0.5	8	82-96	89
barley				
green forage	0.1-1.0	9	86-103	95
straw	0.1-1.0	8	79-90	84
grain	0.05-0.5	9	83-102	89
beans, field				
green forage	0.1-1.0	14	84-104	94
stover	0.1-1.0	13	80-95	87
beans	0.05-0.5	11	81-100	90
corn, field and sweet				
green forage	0.1-0.5	14	70-93	83
fodder	0.1-0.5	12	65-108	84
kernels plus cobs	0.05-0.1	9	66-90	79
kernels	0.05-0.1	12	78-108	88
cotton seed				
field trash	0.1-1.0	10	80-105	93
cotton seeds	0.1-0.5	6	85-107	98
oil	0.1-0.5	5	83-95	86
grapes	0.05-0.5	28	82-110	95
oranges	0.1-0.5	6	84-98	90
peaches	0.05-0.5	6	92-106	99
peanuts				
green forage	0.1-1.0	26	71-110	86
hay	0.1-1.0	23	72-93	86
hulls	0.1-0.5	20	63-102	89
nutmeats	0.05-0.5	42	62-110	85
pears	0.05-0.5	6	87-102	97
peas, English				
green forage	0.1-1.0	23	77-94	86
peas	0.05-0.5	19	80-95	88
peas, Southern				
vines and pods	0.1-1.0	7	80-94	85
peas	0.05-0.5	10	80-96	86
potatoes	0.05-0.5	27	79-102	93
soil	0.1-1.0	53	64-116	88
soybeans				
green forage	0.1-1.0	43	66-105	87
straw	0.1-1.0	34	72-92	82
soybeans	0.05-0.5	33	63-108	85
wheat				
green forage	0.1-1.0	7	85-93	90
straw	0.1-1.0	7	83-101	93
grain	0.05-0.5	11	81-108	90
all substrates		626		88

RESULTS AND DISCUSSION

The method described above has found extensive use in obtaining much of the analytical data needed to support petitions to EPA for establishing tolerances for dinoseb in crops. The recovery determinations validating the method in these crops are summarized in Table I. The overall average of 626 determination is 88%.

Space does not permit inclusion of chromatograms for all the substrates; however, typical chromatograms of analyses using the Barber Colman and Tracor instruments are shown in Figure 1. The shape of the solvent peak detected by the Barber Colman has always been broad and gets broader as the detector gets dirtier. This did become a problem in time, even with detector cleaning, resulting in the dinoseb peak being high upon the solvent tail. The design of the newer detectors permits rapid clearing of solvent from the cell, leaving a sharp solvent tail. Thus, it is seen that the same substrates later produced sharper looking chromatograms.

The recovery averages can be grouped according to type of substrate: succulent (fruits and potatoes), 90–97%; oily

Table II. Recovery of Dinoseb in Green Forage at Various Spiking Levels

spiking level, ppm	no. of determ	av % recov
0.1	3	91
0.5	3	94
1.0	5	89
5.0	3	94
10.0	2	95
20.0	1	98
50.0	2	98
100.0	1	96

(nutmeats and cottonseeds and oil), 85–98%; dry fibrous (dry forage, fodder, straw and hulls), 77–93% cellulosic (green forage), 85–98%; highly carbohydrate (grains, beans and southern peas), 84–90%; and soil, 88%. There is not a significant variation in degree of recovery vs. type of substrate.

Dinoseb recovery is not concentration dependent over the range of 0.1 to 100 ppm. Average recovery in soybean forage for one project is shown in Table II. In this case, the average of 20 determinations was 93%.

Stability of dinoseb residues in samples in freezer storage was examined for the case of soybean forage. Samples were spiked at two levels at the time of initial crop grinding and then were maintained frozen with the treated samples for 21 months until all the samples were analyzed. The recoveries were 74% at 0.1 ppm and 90% at 1.0 ppm. The control sample used for these storage recoveries contained 0.04 ppm apparent residue.

The effectiveness of the initial crop extraction procedure was also examined by successively (exhaustively) extracting the filter cake from a treated sample with fresh portions of the methanol–sulfuric acid solution by shaking 15 min, filtering, and washing the filter cake to 100-mL volume. The original and two successive extractions were analyzed as individual samples. Data produced over a 5-year period indicate that dinoseb residues in field treated soybean and pea forage were 90–99% removed in the original extraction.

Standard curves of peak height vs. concentration were prepared each day and used to quantitate the residue in treated samples. Using the Barber Colman instrument, the curve could always be drawn smoothly through all points in the range of 0.005 to 0.10 μg/mL, but the curvature and slope did vary with time as the detector lost sensitivity and required higher polarizing voltage. The detector could be cleaned manually or by high temperature (400 °C), after which former conditions would return. The Tracor linearized detector system has been much more stable to dinoseb, permitting the use of the same standard curve for several days. Sensitivity has remained essentially the same for 4 years, without cleaning the detector. The detector has been continually operated at 350 °C.

Confirmation of dinoseb methyl ether can be made by gas chromatographing the sample on a column more polar than DC-200, such as OV-17. An alternative approach is p value determination, which utilizes the solubility of the molecules under observation in two differing solvents. Once the treated sample in trimethylpentane (or hexane) has been chromatographed, the solution is shaken with 60:40 acetonitrile/water and the trimethylpentane layer is rechromatographed. The p value is calculated by the equation of Bowman and Beroza (1966).

The determination is performed in duplicate on the treated sample and a suitable recovery sample, and perhaps a standard. It is important that the solvent system composition during partitioning be very similar, or the solubility of the dinoseb methyl ether will be altered in an uncalibrated manner, leading to erroneous conclusions.

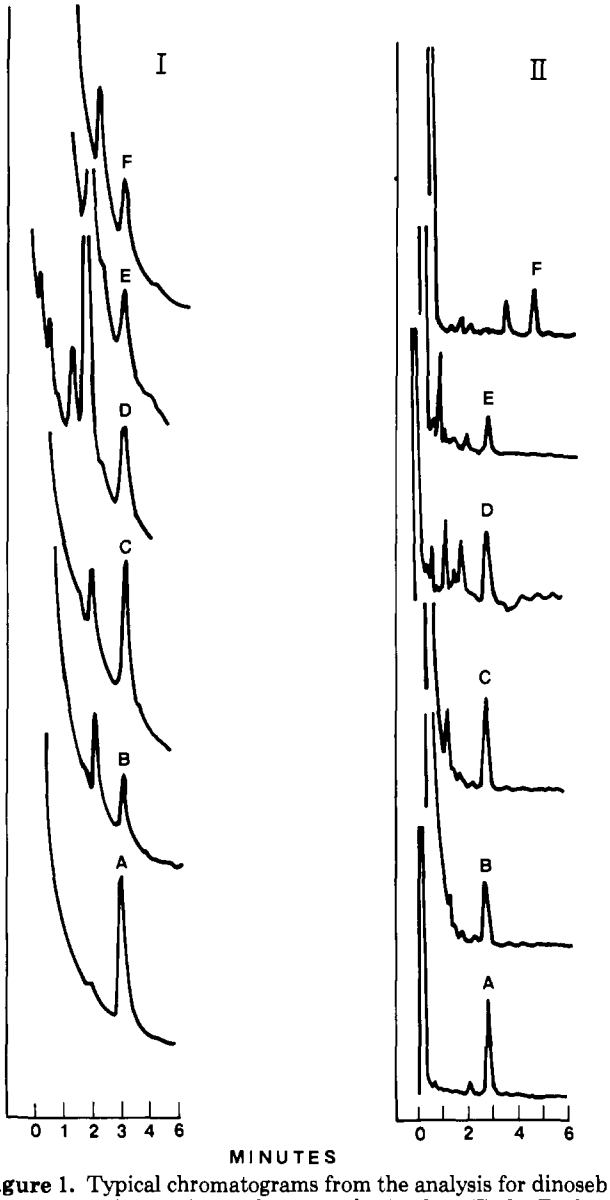

Figure 1. Typical chromatograms from the analysis for dinoseb (t_R = 3–4 min) in various substrates obtained on (I) the Barber Colman GC equipped with a ^{90}Sr ECD: (A) soil + 1 ppm, (B) wheat grain + 0.05 ppm, (C) green pea forage + 0.1 ppm, (D) soybean stover + 0.1 ppm, (E) peanut meats + 0.05 ppm, (F) grapes + 0.05 ppm and (II) the Tracor GC equipped with a ^{63}Ni ECD: (A) soil + 0.1 ppm, (B) corn grain + 0.05 ppm, (C) corn forage + 0.1 ppm, (D) soybean stover + 0.1 ppm, (E) peanut meats + 0.05 ppm, (F) potatoes + 0.05 ppm.

A modification was made during the analysis of corn and peanuts, which are oily substrates. Normally, the ether extraction of dinoseb from the methanolic extract is carried out in the presence of acid. However, if the extract is made alkaline with 15 mL of 1 N sodium hydroxide or sodium acetate, cleanup is improved and recovery is comparable with the general expectation of the method. When the modification was attempted for soil, recovery decreased significantly.

During the course of analysis of soil, it is desirable to determine the moisture content of each sample so that residues found can be normalized. Values reported in the literature will be more meaningful if they can be related on a "dry-weight" basis. Moisture in the soil samples used in recovery determinations can be neglected, but a correction for moisture content should be made for all other

determinations.

SUMMARY

Residues of dinoseb have been determined in many substrates to a lower limit of sensitivity of 0.05 ppm. Recovery experiments have been performed validating the method in nearly every crop for which there is an established EPA tolerance. An average percent recovery in the high 80's can be expected from the method when performed by a qualified analyst. Utilizing adsorption onto alumina and the selectivity and sensitivity of the electron-capture detector, quantities of 20 pg of dinoseb methyl ether and less have been quantitated in the presence of substrate extract.

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Routine Determination of Mirex and Photomirex in Fish Tissue in the Presence of Polychlorinated Biphenyls

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A procedure for the routine determination of mirex and photomirex in fish tissue is described which provides rapid analysis and confirmation using conventional gas chromatographic/electron capture detection (GC/ECD) methods. Coeluting intereferences (i.e., polychlorobiphenyls, PCB's) are nitrated allowing for simple separation from mirex analogue by column chromatography. In chinook salmon tissue (*Oncorhynchus tshawytscha*), PCB removal averaged 78% and mirex and photomirex recoveries were 91 and 86%, respectively. The method has been used successfully for trace analysis of mirex levels as low as 100 pg.

The pesticide chemical mirex was first discovered in fishes from Lake Ontario by Kaiser (1974). His discovery of mirex (dodecachlorooctahydro-1,3,4-metheno-2*H*-cyclobuta[*cd*]pentalene) was based on computerized mass spectral analysis indicating the presence of a *m/e* 272 $[C_5Cl_6]^+$ ion, an ion not seen in polychlorobiphenyl (PCB) mass spectral fragmentation patterns. Kaiser's work indicated that conventional gas chromatographic/electron capture detection (GC/ECD) analysis of fish samples gave misleading results with respect to trace contaminants because PCB's and mirex have overlapping retention times. Many laboratories had probably been misinterpreting mirex as a part of a PCB isomer peak. Similar situations had occurred previously with PCB interference in GC/ECD analyses of dichlorodiphenyltrichloroethane (DDT) and other related chlorinated pesticides (Reynolds, 1969; Bonelli, 1971; Gustafson, 1970).

The discovery of mirex in Lake Ontario stimulated research by various academic groups and regulatory agencies. Several analytical methologies for GC/ECD analysis of mirex were developed; many were modeled on PCB/chlorinated pesticide column chromatographic separation techniques (Reynolds, 1969; Armour and Burke, 1970; Holden and Marsden, 1969). Although high yields and separation efficiencies were reported for these methods, the procedures were not entirely satisfactory. Reproducibility was difficult to maintain in both adsorbent and solvent systems (Berg et al., 1972; Holdrinet, 1974; Task Force on Mirex, 1977).

Further analytical complications arose with the discovery in fish tissue of a new group of degradation products of mirex (Hallett et al., 1976). Photomirex (8-monohydromirex), with concentrations as high as 50% of the reported mirex values (TFM 1977), also coelutes with PCB's, making analysis by conventional GC/ECD techniques difficult.

A simple analytical procedure for separating mirex and photomirex from PCB's is needed for rapid routine assessment of the environmental impact of these persistent pesticides. Initially, our objective was to determine the concentrations of mirex in Lake Ontario fish and to determine where mirex accumulates within the fish. To accomplish this, we developed and report on a routine analytical procedure for mirex and photomirex. Also, our preliminary results on pesticide distribution within fish are presented.

One general approach to the problem of separating mirex and photomirex from PCB's is to chemically alter the PCB's by perchlorination or nitration and change their chromatographic behavior. Perchlorination converts all PCB isomers to the decachlorobiphenyl isomer by means of antimony pentachloride. In routine GC analyses, decachlorobiphenyl elutes sufficiently beyond mirex, thereby yielding adequate separation. The second type, nitration,

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