terization of these radioactive eluates was not possible because of the low amount of $^{14}C$ incorporated per milligram of amino acid. Detection would require injection of 25–50 mg of derivatized material into the GC–TRAP if a single amino acid contained all the radioactivity.

## CONCLUSIONS

The results of our investigations strongly suggest that a significant amount of the aromatic moiety of DMA-$^{14}C$-2,4-D was completely degraded, probably by microorganisms and other factors within the pools. These $^{14}C$ fragments were then metabolically incorporated by bluegills during the 84-day exposure into specific natural biochemicals. This hypothesis is based on our preliminary in vitro and in vivo experiments which showed that 2,4-D was taken up by bluegills, but that radioactive metabolites could not be detected and are probably not produced by fish liver microsomes. However, in outdoor pools with other elements of the aquatic biotic community present, the fish took up significant amounts of radioactive materials, presumably $^{14}C$ fragments from [$^{14}C$]2,4-D. Portions of the $^{14}C$ intermediates were converted into fatty acids, probably through chain elongation, and some $^{14}C$-fatty acids were incorporated into triglycerides. Also, the fish apparently synthesized $^{14}C$-amino acids from $^{14}C$ intermediates and incorporated them into proteins. We were unable to detect $^{14}C$ intermediates in the degradation of [$^{14}C$]2,4-D in water or fish, which suggests rapid decomposition of the parent molecule in natural aquatic communities.

## ACKNOWLEDGMENT

We thank staff members Ginger Gibson, James Johnson, James Hogan, Paul Mehrle, and finally Ellis Graham of the University of Missouri Soils Department for technical assistance.

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Received for review June 13, 1977. Accepted November 2, 1977. Reference to tradenames does not imply government endorsement of commerical products.

# Residue Analysis of β-Naphthoxyacetic Acid and β-Naphthol by High-Pressure Liquid Chromatography

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A method is described for the rapid, accurate, and sensitive quantitative measurement of β-naphthoxyacetic acid and β-naphthol by high-resolution liquid chromatography. The method sensitivity is 0.05 ppm for both compounds. Recovery studies of β-naphthoxyacetic acid fortified in strawberries in concentrations ranging from 0.05 to 0.25 ppm averaged 90.4 ± 4.0%. Recovery studies of β-naphthol fortified in strawberries in concentrations ranging from 0.05 to 0.25 ppm averaged 93.8 ± 4.2%. The percent deviation of the chromatographic method was established for β-naphthoxyacetic acid by measurement of peak areas for concentrations of 1.5, 3.6, and 12 ng/20-μL sample loop as 6.3% deviation and for β-naphthol by measurement of peak areas for concentrations of 1.5, 3, and 6 ng/20-μL sample loop as 7.8% deviation.

The failure of certain types of fruit to set on the early flower clusters is a common complaint of California growers who produce for the spring and summer market. Poor fruit-set is usually ascribed to low night temperatures. In each spring-market area, after the danger of winter frost, there follows a period of 6 weeks to 3 months when day temperatures are conducive to good vegetative growth, but night temperatures may drop too low for proper fruit setting. Various plant growth-regulating hormone-type chemicals such as β-naphthoxyacetic acid, 4-chlorophenoxyacetic acid, and 2,4-dichlorophenoxyacetic acid have been investigated on strawberries and tomatoes (Mann and Minges, 1948) for promoting early fruit-set. In California, β-naphthoxyacetic acid is currently registered for use on tomatoes as a tomato blossom fruit-set and in Oregon it is used on a limited basis on strawberries to increase fruit size. The Environmental Protection Agency Compendium of Registered Pesticides lists tolerances, dosages, and limitations for use of β-naphthoxyacetic acid on pineapple, strawberries, and tomatoes (EPA Compendium of Registered Pesticides, 1968).

No residue method has been reported in the literature for β-naphthoxyacetic acid or β-naphthol on strawberries. Only a preliminary report has been presented by Davidson (1970) on a spectrophotofluorometric determination of β-naphthoxyacetic acid in tomato products.

The purpose of the present manuscript is to describe a sensitive residue method for the analysis of β-naph-

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0021-8561/78/1426-0452$01.00/0 

thoxyacetic acid on strawberries by high-pressure liquid chromatography. The method sensitivity is 0.05 ppm for both β-naphthoxyacetic acid and its postulated metabolite β-naphthol.

## MATERIALS AND METHODS

**Apparatus.** Tracor, Inc. Model 3200 liquid chromatograph equipped with a 970 variable wavelength ultraviolet absorbance detector and a Model 3000 chromatographic pump as component modules. The liquid chromatograph was also equipped with a Rheodyne Model 7120 syringe loading sample injector containing a 20-μL sample loop. The liquid chromatograph column was a prepacked Reeve Angel serial number 1F 1021 25 cm long 6.4 mm o.d. × 4.6 mm i.d. stainless steel tube packed with 3 g of Partisil O.D.S.

Polar planimeter sensitive to 0.01 $in^2$.

**Reagents.** All solvents were reagent grade freshly distilled before use.

Silica gel, grade 923, 100–200 mesh, specifications MIL-D-3716, was obtained from the Davidson Chemical Division, W.R. Grace and Co., Baltimore, Md., and was used as received from the manufacturer without further activation. Each batch should be carefully investigated for elution patterns of the compounds under investigation.

Diazald was obtained from the Aldrich Chemical Co., Inc., Milwaukee, Wis.

*Preparation of Alcohol-Free Ethereal Solution of Diazomethane.* Thirty-five milliliters of 2-(2-ethoxyethoxy)ethanol (Aldrich Chemical, Co., Inc., No. E455-0) and 20 mL of diethyl ether were added to a solution of 6 g of potassium hydroxide in 10 mL of water. This solution was placed in a 100-mL long-necked distilling flask fitted with a dropping funnel, efficient condenser, and a water bath at 70 °C. As the distillation of the ether started, a solution of 21.5 g of Diazald in approximately 200 mL of diethyl ether was added through the dropping funnel over 20 min. During the distillation the solution was continuously mixed with a magnetic stir-bar. The ethereal diazomethane was collected in two receiver flasks which were connected to the condenser in series and the second receiver contained 20 to 30 mL of ether. The inlet of the second receiver dipped below the surface of the ether and both receiver flasks were cooled to 0 °C. The rate of distillation should about equal the rate of Diazald addition, and when the dripping funnel was empty, 40 mL of ether was added slowly and the distillation continued until the distilling ether was colorless. The combined ethereal distillate contained about 3 g of diazomethane (Aldrich Chemical Co., Inc.). Diazomethane ethereal solution could be toxic and hazardous if not handled with caution. Diazomethane should be used behind a safety shield in a well ventilated hood and the ethereal solution stored in proper containers.

Florisil obtained from Floridin Co., Three Penn Center, Pittsburgh, Pa. and activated at 270 °C for 3 h after receiving from the supplier.

**Procedure.** *Extraction of the Strawberries.* Fifteen grams of strawberries was weighed into a stainless steel Waring blender cup and 80 mL of acetone containing 1 mL of *o*-phosphoric acid was added. The sample was blended at low speeds for 1 min and filtered through Whatman No. 41 filter paper into a 100-mL volumetric flask. Blender and filter paper were washed with three 5-mL portions of acetone, and the volume was adjusted to 100 mL with acetone.

*Partition of the β-Naphthoxyacetic Acid and β-Naphthol into Dichloromethane.* A 10-mL aliquot of the extraction solution was added to a 250-mL separatory funnel followed by 100 mL of a 10% (w/v) aqueous solution of sodium sulfate and 25 mL of dichloromethane. The mixture was shaken vigorously for 3 min and then allowed to stand for at least 10 min for layer separation. The lower dichloromethane layer was filtered through Whatman No. 1 filter paper into a 300-mL round-bottomed flask. The aqueous layer was reextracted twice with 25 mL of dichloromethane, and the organic phase was filtered and pooled with the first organic extract. Twenty-five milliliters of isooctane was added to the flask as a keeper and the solvent was evaporated to approximately 5 mL on a rotary evaporator in vacuo at 50–60 °C. This sample was cleaned up on silica gel.

*Sample Cleanup by Column Chromatography on Silica Gel.* To a 1.0 × 25 cm glass chromatographic column with a top reservoir of 50 mL was added a glass wool plug. Three grams of silica gel was slurried in 10 mL of dichloromethane and added to the column. The slurry container was washed four times with 10 mL of dichloromethane, and the washes were added to the column.

As the solvent drained near the top of the silica gel, a 500-mL round-bottomed boiling flask was placed under the column and the sample was added. The sample container was washed twice with 10 mL of dichloromethane, and the washes were added to the column. One hundred milliliters of dichloromethane was added to the column and collected. This eluate contained the β-naphthol fraction in the sample (fraction 1). The receiver was changed and the β-naphthoxyacetic acid was eluted from the column with 150 mL of a mixture of 5% methanol–95% dichloromethane (v/v) (fraction 2).

Fraction 1 (β-naphthol) was concentrated on a rotary evaporator in vacuo at 50 to 60 °C to approximately 2 mL, transferred quantitatively with three 3-mL rinses of isooctane to a 15-mL graduated centrifuge tube, and evaporated just to dryness under a stream of dry nitrogen.

The final volume was adjusted to 1 mL of 50% aqueous methanol prior to analysis by liquid chromatography.

To fraction 2 (β-naphthoxyacetic acid), 10 mL of isooctane was added and the sample was concentrated on a rotary evaporator in vacuo at 50 to 60 °C to approximately 2 mL. The sample was quantitatively transferred to a 15 × 150 mm test tube with three 5-mL rinses of dichloromethane, and the solvent was evaporated under nitrogen to approximately 1 mL and 0.1 mL of ethereal diazomethane solution was added. The sample was allowed to stand at room temperature for at least 15 min. The solvent was evaporated under nitrogen to approximately 0.5 mL, 5 mL of isooctane was added, and this fraction was cleaned up on a Florisil column.

*Fraction 2 Cleanup by Column Chromatography on Florisil.* To a glass column 1.0 × 25.0 cm was added a glass wool plug, 3 g of Florisil, and 0.5 g of anhydrous sodium sulfate. The column was washed with 50 mL of mixed pentanes and a 500-mL round-bottomed boiling flask was placed under the column as a receiver. The methylated fraction 2 in isooctane was added to the column followed by two 5-mL mixed pentane washes of the sample container. The sample was eluted from the column with 50 mL of mixed pentanes followed by 100 mL of a solution of 30% diethyl ether–70% mixed pentanes (v/v). The eluate was concentrated on a rotary evaporator in vacuo at 50 to 60 °C to approximately 2 mL and quantitatively transferred to a 15-mL graduated centrifuge tube with isooctane, and the solvent was evaporated just to dryness under nitrogen. The final volume was adjusted to 1 mL with a 50% aqueous solution of methanol for analysis by liquid chromatography.

Table I. Recovery Studies of β-Naphthoxyacetic Acid and β-Naphthol in Everbearer Strawberries by High-Pressure[a] Liquid Chromatography

| Sample | Fortified ppm | % recov rep. no. 1 | % recov rep. no. 2 | % recov rep. no. 3 | Mean % recov | SD, % |
|---|---|---|---|---|---|---|
| Strawberries + | 0.05 | 89.0 | 85.6 | 92.4 | 89.0 | ±3.4 |
| β-naphthoxyacetic | 0.10 | 92.8 | 86.8 | 95.0 | 91.5 | ±4.2 |
| acid | 0.25 | 94.6 | 91.2 | 86.0 | 90.6 | ±4.3 |
| Strawberries + | 0.05 | 91.8 | 97.6 | 91.1 | 93.5 | ±3.6 |
| β-naphthol | 0.10 | 93.7 | 97.0 | 105.1 | 98.6 | ±5.9 |
| | 0.25 | 91.5 | 90.4 | 85.6 | 89.2 | ±3.1 |

[a] Partisil O.D.S. (3 g) column, 25 cm long, 6.4 mm o.d. × 4.6 mm i.d. liquid chromatograph with variable wavelength detector set at 223 nm and mobile phase 48% methanol–52% water (v/v) operated at room temperature. Method sensitivity 0.05 ppm.

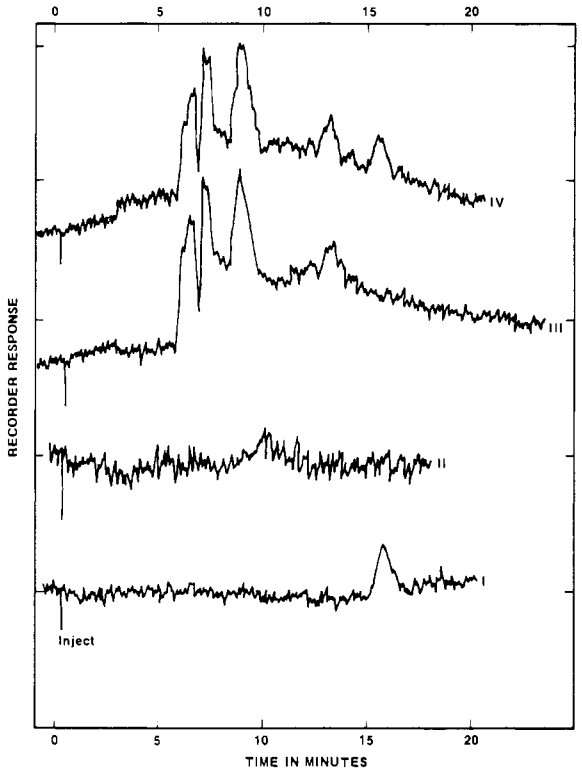


Figure 1. High-pressure liquid chromatograms of (I) 1.5 ng of methylated β-naphthoxyacetic acid standard; (II) 20 μL of a methylated reagent blank; (III) 30 mg of a methylated strawberry control; (IV) methylated sample containing 30 mg of a strawberry control plus 0.05 ppm of a β-naphthoxyacetic acid standard.

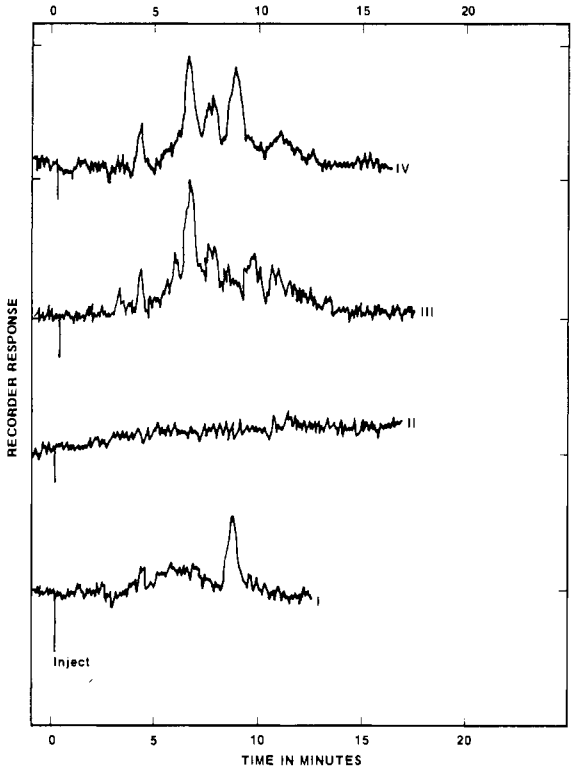


Figure 2. High-pressure liquid chromatograms of (I) 1.5 ng of a β-naphthol standard; (II) 20 μL of a reagent blank; (III) 30 mg of a strawberry control; (IV) 30 mg of a strawberry control plus 0.05 ppm of a β-naphthol standard.

**Analysis by Liquid Chromatography.** A Tracor liquid chromatograph equipped with a variable wavelength ultraviolet light detector set at 223 nm and containing a 25 cm long 6.4 mm o.d. × 4.6 mm i.d. column packed with Partisil O.D.S. was used. The mobile phase was 48% methanol and 52% water (v/v) with a flow rate of 72 mL/h with the high-pressure pump set at a stroke of 25 and a frequency of 50. Attenuation was set at 1. Quantitation of the sample peak areas relative to standards was by measurement of peak areas with a polar planimeter. All results were based on fresh weights with a method sensitivity of 0.05 ppm for the compounds analyzed.

## RESULTS AND DISCUSSION

Figure 1 shows chromatograms from the liquid chromatograph ultraviolet light detector for methylated β-naphthoxyacetic acid standard, reagent blank, strawberry control, and fortified strawberry control at the 0.05 ppm level. The standard chemical had a retention time of 15.4 min and the chromatogram peak was resolved from other peaks which were in the background from the strawberry sample.

Figure 2 shows chromatograms from the liquid chromatograph ultraviolet light detector for β-naphthol standard, reagent blank, strawberry control, and fortified strawberry control at the 0.05 ppm level. The standard chemical had a retention time of 7.5 min and the chromatogram peak was resolved from the other peaks which were in the background from the strawberry sample.

When five repetitive injections of β-naphthoxyacetic acid as the methyl ester at each of the following concentrations of 1.5, 3, 6, and 12 ng/20-μL sample loop were measured as peak areas, the average percent deviation was 6.3. When five injections of β-naphthol at concentrations of 1.5, 3, and 6 ng/20-μL sample loop were measured as peak areas, the average percent deviation was 7.8.

Table I shows recovery studies for methylated β-naphthoxyacetic acid and nonmethylated β-naphthol in Everbearer strawberries. When β-naphthoxyacetic acid was added to strawberries in triplication at concentrations of 0.05, 0.1, and 0.25 ppm and the samples were extracted and analyzed as described above, the mean percent recovery at each level was 89.0, 91.5, and 90.6, respectively. When β-naphthol was added to strawberries in triplication

at concentrations of 0.05, 0.10, and 0.25 ppm and the samples were extracted and analyzed as described above, the mean percent recovery at each level was 93.5, 98.6, and 89.2, respectively. Typical control values for both β-naphthoxyacetic acid and β-naphthol were <0.05 ppm.

Both β-naphthoxyacetic and β-naphthol can be analyzed in strawberries with precision, accuracy, and reproducibility at levels ranging from 0.05 to 0.25 ppm or higher by high-resolution liquid chromatography. The method is fast and accurate and the chromatographic resolution of the chemicals is good in the presence of strawberry extractives.

ACKNOWLEDGMENT

The authors wish to thank the Department of Pomology, University of California, Davis, for providing the strawberry plots.

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Received for review August 2, 1977. Accepted November 4, 1977.

# Assessment of Dipropylnitrosamine Levels in a Tomato Field Following Application of Treflan EC

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A tomato field was examined for residues of *N*-nitrosodipropylamine in air, soil, irrigation water, and crops following application of a herbicide formulation containing an *N*-nitrosodipropylamine impurity. No residues of *N*-nitrosodipropylamine were detected. Application of a general screening procedure for *N*-nitroso compounds in the irrigation water revealed no evidence for the presence of *N*-nitroso compounds in the water due to use of the *N*-nitrosamine-contaminated herbicide formulation.

The presence of carcinogenic nitrosamines in food has been studied since Barnes and Magee (1956) first associated acute liver necrosis with *N*-nitrosodimethylamine (NDMA). It is only recently, however, that pesticide application as a source of nitrosamines in food has been considered (Sen et al., 1975; Eisenbrandt et al., 1975; Wolf and Zepp, 1976; Elespuru et al., 1974). Although nitrosation of pesticide residues in the field has not been demonstrated, Schmeltz et al. (1977) postulated a pesticidal genesis for the nitrosodiethanolamine they found in tobacco. *N*-Nitrosamines have been found in pesticide formulations (Ross et al., 1977). Reported here is the examination of possible human exposure to dipropylnitrosamine (NDPA), resulting from application of one of these formulations, Treflan EC containing 154 ppm (w/w) NDPA (Treflan is a registered trademark of Elanco Products Company, Division of Eli Lilly and Company, for the emulsifiable concentrate formulation of α,α,α-trifluoro-2,6-dinitro-*N*,*N*-dipropyl-*p*-toluidine, trifluralin).

The study field, located in the Sacramento Valley approximately 2 miles north of Davis, Calif., was devoted to tomato cultivation with row irrigation. After the last manual cultivation, the field was treated with Treflan EC by soil incorporation. Treatment of the field was completed over a 2-day period. After treatment with Treflan EC, the field was plowed for row irrigation. Four days after Treflan EC treatment, irrigation was initiated. Treflan had been applied to the field in the two previous years.

## MATERIALS AND METHODS

The Thermal Energy Analyzer gas chromatograph (TEA-GC) was constructed from a Thermo Electron Model 661 single-column gas chromatograph interfaced to a Thermo Electron TEA Model 502 detector. An 8-ft stainless steel tube, 0.125 in. o.d. packed with 5% Carbowax 20M on Chromosorb W,HP was used as the chromatographic column. Argon was used as the carrier gas at a flow rate of 15 mL/min with a column temperature of 150 °C.

The high-pressure liquid chromatograph–Thermal Energy Analyzer (TEA-HPLC) was constructed from a high-pressure pump (Waters Associates, Model 6000A), an injector (Waters Associates, Model U6K), and a Thermo Electron TEA Model 502 LC detector fitted with the TEA-HPLC interface. A UV detector (Waters Associates, Model 440) was also used. Operating conditions on the HPLC were 2 mL/min of 1:1 dichloromethane (DCM) and *n*-hexane eluent with a μ-$NH_2$ column (Waters Associates).

Formulated Treflan was obtained from the applicator in the study field at the time of application. Authenticated NDPA was obtained from the U.S. National Cancer Institute. Solvents were obtained from Burdick and Jackson (Muskegon, Mich.) of a grade which had been distilled in glass.

The XAD-4 resin (Rohm & Haas Co., Philadelphia, Pa.) was cleaned prior to use by washing with aqueous sodium carbonate, followed by dilute hydrochloric acid. The resin was then subjected to 24-h sequential Soxhlet extractions with water, methanol, acetone, and DCM. The resin was dried at 100 °C for 1 h.

Air samples were collected by drawing air at a flow rate of 500 mL/min through a 0.5 in. o.d. by 7 in. long stainless steel tube which had been packed with XAD-4 resin. The sampling systems were battery operated. The valve used to regulate flow was calibrated with a Hastings Mass Flow Meter.

The XAD-4 resin was eluted with three sequential column volumes of DCM. The eluent was concentrated by solvent distillation at 55 °C using a Snyder column. Recoveries were performed by spiking the XAD-4 resin and drawing a typical sample volume through the resin. Recoveries at the 0.1 μg/$M^3$ level were 70%. Determi-

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