# Plant Uptake of Dinitroaniline Herbicide-Related Nitrosamines

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Field experiments were conducted on the uptake and translocation of $^{14}C$-labeled *N*-nitrosodipropylamine (NDPA) and *N*-nitrosopendimethalin (NP) is soybeans grown in Matapeake silt loam amended with labeled NDPA and NP. Soils, beans, and plant parts were combusted to determine total $^{14}C$ and were also extracted and/or steam distilled. Extracts and distillates were analyzed for nitrosamines by a thermal energy analyzer (TEA) and for labeled materials by thin-layer chromatography–autoradiography and liquid scintillation. Residues of NP and NDPA in beans were not detected by $^{14}C$ or TEA. No $^{14}C$ residues from NDPA were detected in other plant parts. Low levels of $^{14}C$ were found in plants grown in NP amended soil; however, residues of NP were not identified.

One potential route of human exposure to pesticides and their byproducts is by human consumption of plant products that have taken up the compounds from soils. The disclosure that measurable amounts of nitrosodialkylamines were detected as impurities in four commercially available herbicides (Ross et al., 1977) prompted research on the environmental fate of these impurities. A concentration of 154 ppm *N*-nitrosodipropylamine (NDPA) was detected in formulated 2,6-dinitro-*N*,*N*-dipropyl-4-(trifluoromethyl)benzenamine (trifluralin). Subsequently Bontoyan et al. (1979) detected *N*-nitrosopendimethalin [*N*-(1-ethylpropyl)-*N*-nitroso-3,4-dimethyl-2,6-dinitrobenzenamine, NP] in the secondary amine herbicide pendimethalin [*N*-(1-ethylpropyl)-3,4-dimethyl-2,6-dinitrobenzenamine]. This nitrosamine had previously been reported by the manufacturer during registration. Since some of the herbicides in which nitrosamines were detected are preplant, soil-incorporated compounds, the possibility existed that the nitrosamines could be absorbed from the soil during the growing season and be present in residues in the harvested plant.

There is limited available information on the uptake of nitrosamines from soils. Dressel (1976a), who applied *N*-nitrosodimethylamine (NDMA) and *N*-nitrosodiethylamine (NDEA) to soil, reported rapid degradation within 12 days. When these compounds were added to soil fertilized with up to 120 kg of N/ha, concentrations up to 12.3 ppb NDMA and 13.8 ppb NDEA were detected in wheat and 11.0 and 13.0 ppb NDMA and NDEA, respectively, in barley. Similar results (Dressel, 1976b) were reported for vegetables (spinach, lettuce, carrots, and tomatoes) grown in soils receiving NDMA and NDEA and high N concentrations. The nitrosamines absorbed by plants disappeared rapidly. Wheat was also examined for nitrosamines by Sander et al. (1973) in soils receiving heavy doses of N fertilizer and either of three secondary amines (dimethylamine, *N*-methylaniline, or *N*-methyl-*N*-benzylamine), but no nitrosamines were detected. Subsequent studies by Sander et al. (1975) reported several nitrosamines could be removed from water by cress, but that plant residues rapidly decreased when nitrosamine-containing water was replaced with noncontaminated water.

Since human exposure to nitrosamines as contaminants in certain herbicides is important in assessing risk, experiments were initiated on the soil persistence, plant uptake, and residues of NDPA and NP in soybean plants grown under field conditions.

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## MATERIALS AND METHODS

**Chemicals.** Preparation of $^{14}C$-labeled NDPA and NP *N*-(1-ethylpropyl)-*N*-nitroso-3,4-dimethyl-2,6-dinitrobenzenamine-*1-ethyl*-$^{14}C$] have been described elsewhere (Oliver et al., 1979). Both compounds were assayed for purity by thin-layer chromatography and found to contain no major (<3%) impurities. Dichloromethane (DCM) solutions of both NDPA and NP were stored in the dark to avoid photoalteration.

**Soybeans and Soils.** Williams variety soybean [*Glycine max* (L.) Merr. var. Williams maturity group III, 90% germination] were obtained from the Cell and Nitrogen Fixation Laboratory at Beltsville. New samples of Matapeake (Typic Hapludults) silt loam, obtained from the Agricultural Research Center, Beltsville, MD, has soil properties as follows: pH 5.5, organic matter 1.5%, and sand, silt, and clay contents of 38.4, 49.4, and 17.2%, respectively.

A range of concentrations of NDPA and NP were established in soils, using the $^{14}C$-labeled materials as the only source of nitrosamines. Nitrosamines in the herbicide formulation were applied to soils to give rates corresponding to concentrations of 0, 0.1, 1, 10, and 100 ppb of NDPA or NP in the soil. The NDPA was applied in a formulation of trifluralin and at a rate corresponding to 2 ppm of the herbicide. The NP was applied alone. The labeled nitrosamines were applied to a 500-g portion of soil. After the solvent had evaporated, the treated soil was added to 8500 g of untreated soil and then thoroughly mixed in a tumbler for about 1 h. Soils treated at each level were subdivided into 1.5-kg samples (six replications) and placed in 15-cm plastic pots. From previous experiments on the volatilization of NDPA from soil (Oliver, 1979), it appeared likely that a few percent of the NDPA may have been lost during drying and mixing, and a few more percent was presumably lost by volatilization after the soil was added to the pots. The loss has not been quantitated. Three seeds were added to each pot and the pots were moved to the field on July 1, 1977. The pots were buried in the soil at the surface level and, when the seedling emerged, each pot was thinned to one plant. Plants were watered and hand weeded as necessary. After 110 days, the pots were recovered and moved to the greenhouse where beans, pods, upper leaves, lower leaves, upper stems, lower stems, and roots were collected. In order to ensure adequate sample sizes, replicate plant parts and soils were combined and mixed. The air-dried plant parts were ground in a Wiley mill and submitted for chemical analysis.

**$^{14}C$ Analysis.** Radioactivity in plant parts and soil was determined by combustion analyses. Air-dried samples of soil and plant material (ca. 0.3–0.5 g) were oxidized in a Packard Tri-Carb Model 306 sample oxidizer, $^{14}CO_2$ was collected in a basic trapping solution, and radioactivity was measured by liquid scintillation counting.

For extraction analyses, 25 g of dried stems from NP-treated plants were ground and homogenized with 150 mL of ethyl acetate and then with 150 mL of methanol. The solutions were independently filtered and concentrated in vacuo and made up to 2.0 and 3.0 mL, respectively. Ten percent of each solution was added to $Al_2O_3$ in a combustion boat and oxidized as described above.

For steam distillation analyses, 50 g of ground soybeans was homogenized with 125 mL of $H_2O$ in a Polytron homogenizer. The homogenate was transferred, with added $H_2O$, to a 1-L flask. To this flask was added 8 g of $Ba(OH)_2$ and 30 mL of 1.8 N KOH, then the mixture was steam distilled until 300 mL of distillate was collected. The distillate was extracted with DCM (4 × 20 mL), the DCM solution was washed with 6 N HCl and then with 5 N KOH. The solution was dried over $MgSO_4$, concentrated to ca. 5 mL in a Kuderna-Danish (K-D) evaporator, then further concentrated to ca. 0.3 mL in a micro version of the K-D evaporator. The amount of $^{14}C$ recovered was measured by liquid scintillation counting. A spiked sample of soybeans containging *N*-nitrosodipropylamine showed a 73% recovery.

**GLC–TEA Analyses.** The isolation procedure for the volatile nitrosamine, NDPA, resembled that used for the $^{14}C$ method, except *N*-nitrosoethylpropylamine was used as an internal standard and a column chromatographic cleanup step was added after the DCM was concentrated to 1.0 mL. Anhydrous pentane was used to assist in the quantitative transfer of the sample to a 14.5 × 250 mm water-cooled chromatographic column containing 5 g of silica gel. The column was first washed with 200 mL of pentane–DCM (3:1, v/v), and the eluate was discarded, then eluted with 125 mL of anhydrous ether–DCM (3:7 v/v). This eluate was colleced, concentrated to 1.0 mL, and analyzed by gas–liquid chromatography (GLC) interfaced directly with a thermal energy analyzer (TEA). The GLC equipped with a 230 cm × 3 mm o.d. column packed with 15% Carbowax 30 M-TPA on 60-80 mesh Gas-Chrom P. The GLC operating conditions were as follows: injector temperature, 200 °C; column programmed from 130 to 220 °C at 4 °C min; and argon carrier flow rate, 84 $cm^3$/min. The retention time of NDPA was 8.2 min.

**LC–TEA Analyses.** A 50-g aliquot of the ground soybean sample was spiked with 1 mL of a 0.25 μg/mL *N*-nitroso-*N*-methyl-2,6-dinitrobenzamide solution (NMB equivalent to 5 ppb), and the sample was thoroughly mixed and allowed to stand several hours. The sample was homogenized in a Virtis apparatus with 100 mL of $CH_3CN$ for 3 min, then decanted through a vaccum filtration setup using Whatman No. 1 filter paper. The sample was homogenized again in 100 mL of $CH_3CN$ and then the entire mixture was filtered. The $CH_3CN$ solution was passed through 35 g of anhydrous sodium sulfate, which was held in a 60-mL coarse fritted glass funnel, into a 500-mL, round-bottom flask. The $CH_3CN$ was removed on a rotary evaporator using a water bath maintained at 35 °C. The sample was taken up in 5 mL of hexane and applied to a 100 × 300 mm column containing 3.5 g of prewashed Florisil. The Florisil was first washed with $CH_3N$, then hexane and DCM, dried overnight in an oven at 120 °C, and deactivated with $H_2O$ (6%). The column was washed with 100 mL of hexane and the sample eluted with 100 mL of a mixture of DCM–hexane (3:1, v/v). The solvent was again removed on a rotary evaporator using a water bath kept at 35 °C. The residue was taken up in DCM and passed through a 0.45-μm millipore filter. (The filter had been prewashed with DCM.) The sample was transferred to a K-D evaporator tube and the DCM solution reduced to 1.0 mL under a stream of N. Chromatographic peaks obtained from the samples were compared with the standard nitrosamines to determine their precise retention time and quantitation.

Table I. High-Pressure Liquid Chromatograph Operating Conditions

column: 50 cm SI 10 (10 μm silica) column in series with a 25 cm, 5 μm Spherisorb (5 μm silica)
mobile phase: hexane-$CH_2Cl_2$-$CH_3CN$ (75:25:2, v/v) isocratic
flow rate: 1 mL/min
temperature: ambient
sample size: 100 μL
TEA Model 502 operating conditions:
  helium and oxygen flow to give a chamber pressure of 0.5 mm
  furnace temperature: 530 °C
  photomultiplier tube again (calibration): 2.0
  cold traps: dry ice-acetone
  attenuation: X 8
minimum detectability: 1 ng/100 μL injection (equivalent to 10 ng in 1.0 mL or 0.2 ppb in a 50-g sample)
elution times: NP, 8.6 min; NMB, 20.0 min.

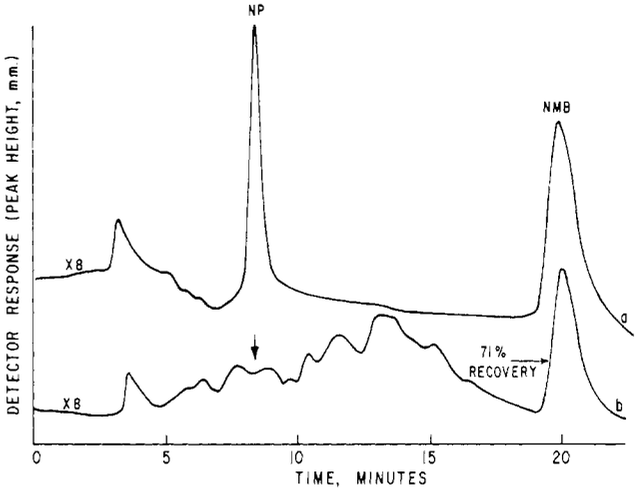


**Figure 1.** LC–TEA chromatogram of 100-μL injection of (a) 0.25 ng/μL NP and NMB standards in $CH_2Cl_2$ and (b) extract from soybeans (100 ppb soil NP) with 5 ppb NMB internal standard added.

The conditions for quantitations are as follows: A Waters Model 6000A solvent delivery system LC equipped with a U6K injector was interfaced directly with a thermal energy analyzer (TEA) Model 502. The operating conditions for the high-pressure liquid chromatograph are shown in Table I. A LC–TEA chromatograph of NP and *N*-nitroso-*N*-methyl-2,6-dinitrobenzamide (NMB) are shown in Figure 1.

## RESULTS AND DISCUSSION

The residues of NDPA and NP in various plant parts and soil are shown in Table II. Only the results from the soils receiving the highest treatments (100 ppb) are shown since in all other samples (i.e., from 0.1, 1, 10 ppb treatments), the concentrations of NDPA and NP were below the level of detection by combustion analyses (1 ppb). Soybeans from NDPA treated soils were also analyzed by steam distillation and GLC–TEA. No residues of NDPA

Table II. Radioactivity[a] in Soybean Plant Parts and Soil after Growing Plants in Soils Treated with 100 ppb of *N*-[$^{14}$C]Nitrosodipropylamine (NDPA) and *N*-Nitrosopendimethalin (NP)

| plant part or soil | treatment | |
|---|---|---|
| | NDPA | NP |
| soil | 28.6 | 71 |
| roots | 7.9[b] | 59.3 |
| lower stems | ND[b] | 14.4 |
| upper stems | ND | 6.9 |
| lower leaves | ND | 9.8 |
| upper leaves | ND | 3.0 |
| soybeans | ND | ND |

[a] Determined by $^{14}$C combustion analysis. [b] None detectable at 1 ppb by $^{14}$C combustion analysis.

Table III. GC-TEA Analysis of Soybeans from Plants Grown in Soils Containing Various Concentrations of *N*-Nitrosodipropylamine (NDPA)

| NDPA concn in soil, ppb | sample | NDPA concn[a] in soybean, ppb | NEPA,[b] % recov |
|---|---|---|---|
| 100 | 1 | ND[c] | 89 |
| | 2 | ND | 99 |
| 10 | 1 | ND | 94 |
| | 2 | ND | 99 |
| 1 | 1 | ND | 96 |
| | 2 | ND | 99 |

[a] Minimum detectability = 0.35 ppb. [b] 5 ppb *N*-nitrosoethylpropylamine added as an internal standard. [c] None detected.

were detected by steam distillation–$^{14}$C analysis with a limit of sensitivity of 0.01 ppb or GLC–TEA analysis at a limit of sensitivity of 0.35 ppm. Since the volatility of NP is low, NP samples could not be steam distilled.

Results of the GLC–TEA analyses are shown in Table III. Residues of NDPA could not be detected in soybeans from plants growing in soils receiving 100, 10, or 1 ppb NDPA. Soybeans spiked at a 5-ppb level of *N*-nitrosoethylpropylamine yielded an average recovery of 96%. A survey of NP residues in soybeans from plants grown in soils containing 0–100 ppb NP, using LC–TEA, also revealed no residues of NP. The minimum detectable level was 0.2 ppb with a soybean sample of 50 g. An average recovery of 80% was obtained in untreated soybeans spiked with 5 ppb NP. Samples grown in untreated soil with 5 ppb NMB added as an internal standard gave an average recovery of 78%.

The lower stems and lower leaves from soybeans grown in soils amended with 100 ppb NP were extracted and analyzed by TLC. Identification of products was difficult because of (1) low levels of radioactivity in the plant parts, (2) low extractability of the radioactivity, and (3) coextraction of considerable amounts of plant material that hindered chromatography. Within the limits of detection, none of the radioactivity migrated from the origin of the TLC plates, using solvents commonly used for TLC of NP. Thus, no NP was detected in any plant parts.

After the beans had been harvested, soils (two 500-g samples) containing NP residues were extracted with methanol (1500 mL/500-g soil sample). The extract was reduced in volume under N and chromatographed on silica-gel-coated TLC plates eluted with benzene. The $^{14}$C recovered by the methanol extraction, based on the amount originally added to the soil, was as follows: 100 ppb (32%), 10 ppb (25%), 1 ppb (38%). Thus about one-half of the $^{14}$C in soil (Table II) was in a form that was not extracted by methanol. The TLC plate showed a major $^{14}$C spot at $R_f$ 0.29 and very minor spots at $R_f$ 0.06 and at the origin, but the latter amounted to less than 1% of the $^{14}$C recovered on the plate. The stability of NP in soils has been reported (Oliver et al., 1979). As Table II indicates, NP was not rapidly degraded in soils and 71% of the original added $^{14}$C was recovered after 110 days in the soil.

The NDPA rapidly disappeared in the Matapeake soil, and only 28.6% of the $^{14}$C originally applied was recovered by combustion analysis. The methanol extract was concentrated and then partitioned between dichloromethane and water. The dichloromethane solution, after washing with aqueous HCl and NaOH, contained 1.6% of the $^{14}$C initially incorporated in the soil. Thin-layer chromatography revealed a single faint spot with a $R_f$ value corresponding to NDPA. The rapid degradation of NDPA would seem to be consistent with other dialkyl nitrosamines (Dressel, 1976a). We have also observed rapid metabolism of NDPA in soil biometer studies (Oliver et al., 1979).

Bontoyan et al. (1979) reported NDPA concentrations in several trifluralin samples in the range of 6–150 ppm and a NP concentration in a single pendimethalin sample of 102–104 ppm. Thus the nitrosamine level at our highest application rate was >300 times higher than would be encountered by normal use of the respective herbicides.

## LITERATURE CITED

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