

Dissipation of ^{14}C N-Nitroso-di-N-Propylamine from Field Soil and Residue Determinations in Field-Grown Soybeans

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ROSS et al. (1977) reported the detection of nitrosamines in certain herbicide formulations. Treflan[®], a widely used herbicide in cotton and soybeans, was found to contain approximately 150 ppm of N-nitroso-di-n-propylamine (NDPA). In order to investigate some of the possible consequences from the introduction of NDPA into the environment, a study was carried out to determine the rate of dissipation of ^{14}C NDPA from field soil and to assess the level of residues in soybeans (Glycine Max (L.) Merr. 'Calland') grown in NDPA-treated soil. For this purpose field soil was treated with ^{14}C NDPA at a level corresponding to an NDPA contamination level in Treflan[®] of 2275 ppm.

MATERIALS AND METHODS

Radiolabeled NDPA

NDPA labeled with ^{14}C in the 1-position was obtained from New England Nuclear, Boston, Massachusetts. The specific activity of this material was 28.08 $\mu\text{Ci}/\text{mg}$ (62,338 dpm/ μg). Autoradiographs prepared from thin-layer chromatograms showed a single radioactive zone.

Radiochemical Determinations

Plant tissue and soil were assayed for total radioactivity by combusting the samples to CO_2 and water in an electrically heated furnace and trapping the CO_2 in 10 ml of 30% ethanolamine in methyl cellosolve. This solution was then mixed with 10 ml of dioxane-methyl cellosolve scintillation fluid and the ^{14}C determined using a Packard, Model 3380, Tri-Carb Scintillation Counter. Counting efficiency was determined by internal standardization with ^{14}C toluene.

The radioactivity content of methanol extracts of soil was determined by direct counting of aliquots equal to 3 g of soil in dioxane-methyl cellosolve scintillator.

Treatment of Soil

The test plots, located at the Lilly Research Laboratories, Greenfield, Indiana, were 0.66 m^2 in area and were enclosed by a cylindrical metal ring. Two treated plots and two control plots

were used in the study. An application of ^{14}C NDPA equal to $571\text{ }\mu\text{g}/\text{m}^2$ of surface area was made to each of the treated plots by dissolving $377\text{ }\mu\text{g}$ of NDPA in 1000 ml of water and sprinkling the solution over the soil surface with a watering can. The NDPA was then incorporated into the top 8 cm of soil by mixing with a cultivation fork. Soybean seeds were planted eight days after NDPA application.

Soil Sampling

Soil samples consisting of 10-15 cores were taken to a depth of 8 cm with a Hoffer soil sampling tube having a cross-sectional area of 2.86 cm^2 . Samples were taken 2 hours, 8 days, and 49 days after application of NDPA. Soil cores from each plot were pooled to form a composite sample.

Extraction and Analysis of Soil Samples

Immediately after collection, the composited samples were shaken for 30 minutes in a closed container with 1000 ml of methanol. After standing for an additional 48 hours the samples were filtered and aliquots of the filtrates immediately removed for radioactivity determinations. The extracted soils from the 2 hour and 8 day samples were combusted to determine residual radioactivity. Radioactivity in the 49 day sample was determined by combustion without previous methanol extraction. The extract of the 2 hour sample was also analyzed directly for NDPA by Thermal Energy Analysis (TEA).

Plant Sampling

Soybean plants were removed from treated and control plots 21 and 49 days after treatment by cutting the stems at the soil surface. Six plants were removed from control plots and each of two NDPA-treated plots. The lower stem of each plant was rinsed with water to remove any adhering soil. The shoots were frozen in liquid nitrogen, ground, and stored frozen prior to analysis. Soybean seed, collected 119 days after the application of ^{14}C NDPA, was air-dried and ground in a Waring blender.

RESULTS AND DISCUSSION

Although $571\text{ }\mu\text{g NDPA}/\text{m}^2$ was initially applied, soil samples taken two hours after application indicated the presence of only $300\text{ }\mu\text{g NDPA}/\text{m}^2$ or 52.6% of the amount originally applied (Table 1). Volatilization of NDPA was undoubtedly responsible for this rapid loss of 47% of the applied NDPA. This observation corresponds with that of OLIVER (1978) who found that NDPA surface-applied to soil maintained in a closed container at 35°C to approximate mid-day soil surface temperatures was rapidly volatilized with 42% being lost during the first hour following application.

In the two hour samples approximately 92% of the radioactivity was extractable with methanol. Analysis of these extracts by TEA confirmed that all of the extractable radioactivity was present as NDPA.

TABLE 1
DISSIPATION OF ^{14}C NDPA FROM SOIL

Time After Application ¹	Total Radioactivity μg Equivalents of NDPA/m ²				% of Applied NDPA ³
	Methanol Extract	Residual In Soil ²	Total	Mean	
2 hours NDPA #1	278.5	22.6	301.1	300.6	52.6
NDPA #2	273.1	26.9	300.0		
8 days NDPA #1	12.9	80.6	93.5	96.3	16.9
NDPA #2	19.4	79.6	99.0		
49 days NDPA #1	--	--	67.3 ²	62.9	11.0
NDPA #2	--	--	58.1 ²		
Control	--	--	3.3 ²	--	--

¹Soil Samples taken to a depth of 8.0 cm.

²Determined by combustion analysis.

³571 μg NDPA/m² was applied to soil.

The level of soil radioactivity continued to decline and after 8 and 49 days had decreased to 16.9 and 11%, respectively, of the amount initially applied. At eight days only 16% of the soil radioactivity was extractable with methanol.

The total radioactivity in the shoot portion of immature soybean plants and seed produced from plants grown in NDPA-treated and untreated soil expressed as ppm NDPA equivalents is shown in Table 2.

While low levels of radioactivity (0.0001 to 0.00029 ppm) were found in immature plants, the levels of radioactivity in seed produced in NDPA-treated soil did not exceed levels in seed produced in untreated soil. Previous reports have demonstrated the absence of NDPA residues in soybeans grown in fields treated with NDPA-contaminated Treflan® when analyzed by TEA (WEST and DAY 1978), and in soybeans grown in pots containing NDPA-treated soil (KEARNEY et al. 1978).

TABLE 2
RADIOACTIVE RESIDUES IN SOYBEAN PLANTS AND SEEDS OBTAINED FROM
¹⁴C NDPA-TREATED SOIL, EXPRESSED AS PPM NDPA EQUIVALENTS

Plot Designation	Plants			Soybean Seed	
	21 Days After Treatment	49 Days After Treatment		119 Days After Treatment	
	ppm	ppm	ppm (95% UCL ¹)	ppm	ppm (95% UCL ¹)
Control	0.0001	0.00006	0.0008	0.00032	0.00059
NDPA #1	0.00021	0.00013		0.00024	
NDPA #2	0.00029	0.00010		0.00013	

¹The 95% upper confidence limit (UCL) = $X + t_{n-1, \alpha} / 2 S_x / \sqrt{n}$, where t is the value for six observations and $\alpha = 0.05$. Treated means must exceed the 95% UCL before a residue is present. No statistical analysis was made on 21-day plants.

The results of this study indicate that NDPA is rapidly dissipated from the soil primarily by volatilization; and that soybeans harvested from plants grown in such treated soil are free of residues of NDPA and NDPA transformation products. The amount of NDPA applied to the soil represented a NDPA contamination level in Treflan® 15 times greater than the level reported by ROSS *et al.* (1977) and 570 times greater than the concentration in Treflan® produced in 1977 (WEST and DAY 1978).

REFERENCES

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