Bull. Environm. Contam. Toxicol. 23,136-140 (1979)

Uptake and Transformation of ^{14}C N-Nitroso-di-N-Propylamine by Soybean Plants

D. F. Berard and D. P. Rainey

Agricultural Biochemistry, Lilly Research Laboratories, Division of Eli Lilly and Company, Greenfield, Ind. 46140

Trifluralin (α,α,α-trifluoro-2,6-dinitro-N,N-dipropyl-p-toluidine), a widely used herbicide in cotton and soybeans, has been reported (ROSS et al, 1977) to contain N-nitroso-di-n-propylamine (NDPA) as a contaminant. In order to investigate some of the possible consequences from the introduction of NDPA into the environment, a study was carried out to assess the uptake and transformation of NDPA by soybean plants. Young soybean plants could potentially be exposed to NDPA since trifluralin is incorporated into the soil prior to planting. The uptake of N-nitroso-dimethylamine by agronomic crops was reported by DEAN-RAYMOND and ALEXANDER (1976).

In this study bare-rooted soybean (Glycine Max (L.) Merr. 'Calland') plants were placed in a nutrient solution containing ^{14}C NDPA. This route of exposure was chosen as the best method for determining the maximum NDPA absorption potential of soybean plants. The rate of dissipation of soil-incorporated ^{14}C NDPA and the results of residue determinations in mature soybeans from plants grown in NDPA-treated soil are reported in a separate communication.

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 μCi/mg (62,338 dpm/μg). Autoradiographs prepared from thin-layer chromatograms showed a single radioactive zone.

Plant Absorption Study

Soybean plants were grown for ten days in sand flats under greenhouse conditions. The plants were removed from the flats and transferred to brown bottles containing 40 ml of nutrient solution (HOAGLAND and ARNON 1950) and 6.72 μg (0.17 ppm) ^{14}C NDPA. Two plants were placed in each container and a cotton plug was inserted to support the plants and reduce evaporation. The bottles were placed in a laboratory fume hood with auxilliary lighting supplied by 'Vita-Lite' fluorescent lights (Duro-Lite Lamps, Inc., Fair Lawn, New Jersey). The light intensity was 1.076 klux measured at the hood base with the lights suspended 38 cm above the base. During

0007-4861/79/0023-0136 $01.00

the course of the study water was added to the bottles when the volume of the nutrient solution had decreased to 1-2 ml.

Plants were removed from the nutrient solution after 1, 2, 3, 5, and 7 days exposure. Each sample consisted of four plants. After rinsing the roots with water, each plant was separated into root and shoot portions which were frozen prior to radiochemical analysis. The transformation of ^{14}C NDPA by soybeans was investigated using plants that had been exposed to the treatment solution for seven days.

Radiochemical Determinations

Plant tissue was assayed for total radioactivity by combusting 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 the nutrient solutions and plant extracts was determined, where possible, by direct counting of an aliquot in an appropriate scintillator. When extracts were too highly colored for direct counting, aliquots were pipetted into combustion boats, allowed to air dry, and combusted using the procedure described above for plant samples.

Quantitative TLC analyses were accomplished by scraping zones into counting vials, eluting the adsorbent with 1 ml of methanol, and counting directly in a toluene-based scintillator.

Thin-Layer Chromatography

The separation of NDPA from metabolites was accomplished on Brinkman Silica Gel G plates containing a fluorescent indicator. Samples suspected of containing NDPA were chromatographed in a solvent system consisting of hexane:diethyl ether:methylene chloride (40:30:20). Samples containing polar metabolites were chromatographed in acetone:chloroform (80:20). ^{14}C NDPA reference standard was spotted on thin-layer plates adjacent to the sample to locate the parent compound zone. Radioactive zones were visualized by exposure of plates to Kodak medical X-ray film. Thin-layer plates with film were stored at -20°C to reduce volatilization of NDPA and metabolites. The R_f of NDPA was 0.44 on silica gel plates developed in the hexane:diethyl ether:methylene chloride solvent system.

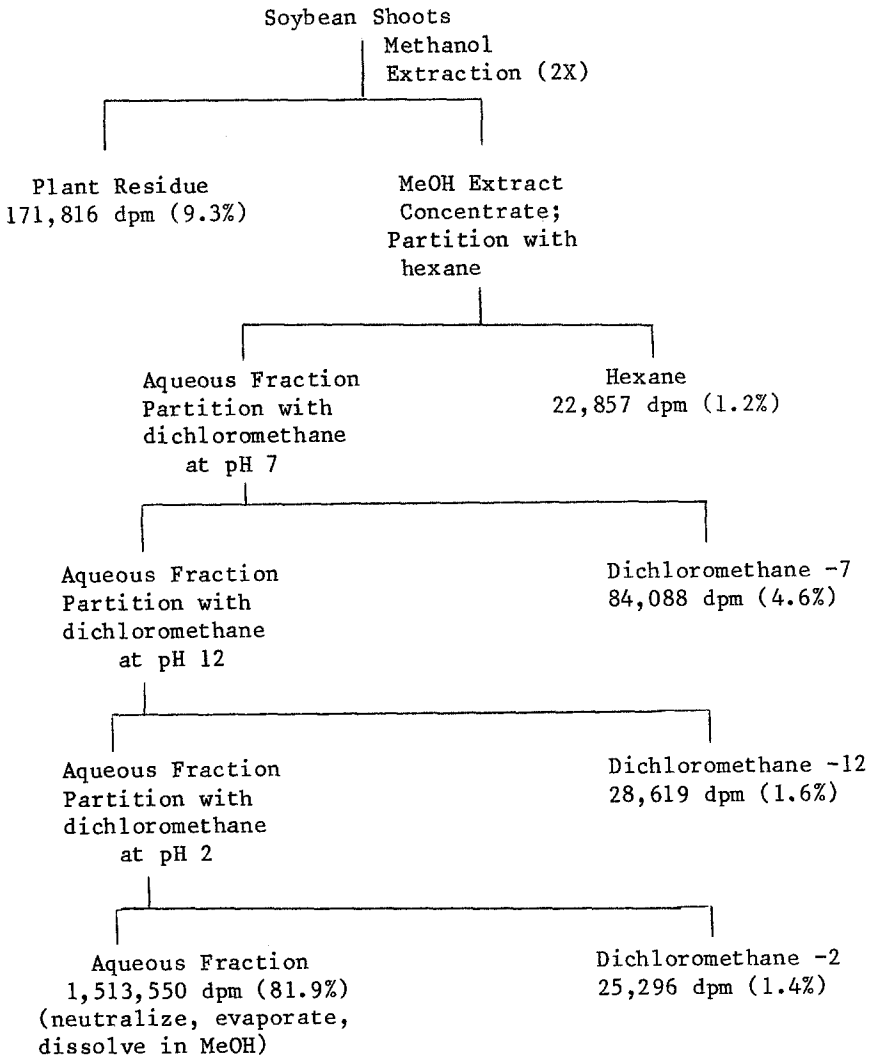
Characterization of Radioactivity in Soybean Shoots

One hundred grams of soybean shoots were extracted by blending twice with methanol (Figure 1). The plant residue was collected

by filtration and combusted to determine the amount of residual radioactivity. The methanol was removed from the extract under vacuum and the remaining aqueous phase extracted three times with hexane. Extraction of the aqueous phase was continued by partitioning three times each with dichloromethane at pH 7, 12, and 2.

FIGURE 1

Distribution of radioactivity in soybean shoots exposed to a 0.14 ppm concentration of ^{14}C NDPA in nutrient solution for seven days. Sample consisted of 100 grams of shoots containing 29.6 µg equivalents of NDPA.



RESULTS AND DISCUSSION

The uptake and translocation of NDPA radioactivity from nutrient solution by soybean plants, expressed as μg equivalents of NDPA/g fresh weight, is indicated in Table 1. NDPA was absorbed from the solution by soybean roots with the concentration increasing from 0.145 μg/g after a one day exposure to a maximum of 0.427 μg/g at five days exposure. Absorbed NDPA radioactivity was translocated into the shoot portion of the plants with the maximum concentration of 0.443 μg/g observed after seven days exposure.

TABLE 1
Uptake and Translocation of Radioactivity by Soybean Plants Exposed to a 0.17 ppm Concentration of ^{14}C NDPA in Nutrient Solution

Treatment Duration (Days)	μg equivalents of NDPA/ g fresh wt.[1]	
	Roots	Shoots
1	0.145±0.012	0.155±0.010
2	0.277±0.051	0.197±0.030
3	0.325±0.028	0.245±0.027
5	0.427±0.082	0.358±0.011
7	0.344±0.018	0.443±0.052

[1]Mean and statistical error of four replicates. Values obtained by combustion analysis.

The distribution of radioactivity in the methanol extract of soybean shoots from plants exposed to ^{14}C NDPA for seven days is indicated in Figure 1. The majority of the radioactivity (81.9%) was found to be highly polar in nature and remained in the aqueous fraction. The extracted plant tissue also contained a significant amount of residual radioactivity (9.3%). The hexane extract, the three dichloromethane extracts, and the final aqueous fraction after evaporation and dissolution in methanol were subjected to quantitative thin-layer analysis. Only the hexane and dichloromethane-7 fractions contained radioactivity which corresponded with the ^{14}C NDPA standard and the percentage of radioactivity in these fractions which corresponded to NDPA was 62.1 and 7.0%,

respectively. This represented a total of 0.3 μg of NDPA. Since this quantity of NDPA was isolated from 100 g of shoots the NDPA concentration in shoots after the seven day exposure period would have been 0.003 μg/g fresh weight.

Since the aqueous fraction contained 81.9% of the total radioactivity in soybean shoots, additional solvent extractions were performed in an attempt to further characterize this radioactivity. The aqueous fraction was partitioned with butanol both before and after hydrolysis in 2N hydrochloric acid. However, no additional radioactivity was removed by the butanol treatment indicating that the transformation products of NDPA are extremely water soluble. Identification of these transformation products has not been achieved.

The results of the uptake and transformation study indicate that soybean plants possess the capability of absorbing NDPA; however, extensive transformation of absorbed NDPA to highly water soluble products occurs rapidly and virtually quantitatively.

REFERENCES

DEAN-RAYMOND, D. and M. ALEXANDER: Nature 262, 394 (1976).

HOAGLAND, D. R. and D. I. ARNON: Univ. Calif., Agric. Exp. Stn. Circ., 343, 32 pp. (1950).

ROSS, R. D., J. MORRISON, D. P. ROUNBEHLER, S. FAN, and D. H. FINE: J. Agric. Food Chem. 25, 1416 (1977).