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Uptake of Tri-*p*-cresyl Phosphate in Soybean Plants

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Triaryl phosphate esters are widely used in consumer and industrial products as flame retardants. They are also used as plasticizers in polyvinyl chloride plastic materials and in hydraulic fluids (Midwest Research Institute 1979). In addition, the esters are used as lubricant additives, wood preservatives, and adhesives.

One of the esters of particular interest in tricresyl phosphate (TCP). The commercial production volume of this compound was estimated to reach about 20 million pounds by 1982 (Chemical Marketing Reporter 1978). TCP has been on the market for many years and has been found in fish downstream from manufacturing sites (Lombardo and Egry 1979). The results of several studies of the fate and metabolism of TCP in the environment and in sewage sludge have been published (Howard and Deo 1979; Ku and Alvarez 1982; Pickard et al 1975; Saegar et al 1979; Wageman et al 1974); however, relatively little work has been reported on plant uptake of TCP and on its metabolism within plant tissues. Plants are known to take up, translocate, accumulate, and metabolize certain chemicals that are not essential for plant growth and development.

Because of the possible release of TCP to the environment, this study was undertaken to determine the uptake and translocation of TCP by soybean plants, using pure tri-p-cresyl phosphate (TpCP) as a model compound. We wished to learn the propensity of TpCP to move into the food crops from the soil. This study was not concerned with phytotoxicity, but with the possibility of foods becoming contaminated with TCP through the use of sludge or wastewater on agricultural lands.

MATERIALS AND METHODS

TpCP (reagent grade) was purchased from Eastman Kodak Co. (Rochester, NY). Florisil, PR grade, 60-100 mesh, was obtained from Floridin Co. (Berkeley Springs, WV) and was activated by heating in an oven at 130°C for several days before use.

Gas chromatographic (GC) data were obtained by using a Varian 3700 gas chromatograph (Varian Instrument Group, Palo Alto, CA) equipped with a flame photometric detector (FPD) operating in the phosphorus

mode. GC operating conditions were: 2 m X 2 mm glass column packed with 2% OV-101 on Chromosorb W(HP), injector, 250°C, column 240°C, helium carrier, 25 ml/min; and FPD 240°C with hydrogen, 140 ml/min, air no. 1, 80 ml/min, and air no. 2, 170 ml/min.

Low resolution electron ionization mass spectra were obtained at 70 eV with a Finnigan 3300 F quadrupole mass spectrometer equipped with a Model 9610 gas chromatograph (Finnigan/MAT, San Jose, CA). A 25 m X 0.2 mm i.d. open tubular fused silica capillary column coated with methyl silicone was used. Column oven temperature was programmed to run from 30 to 240°C.

The experimental conditions for growing soybean plants were described previously (Casterline et al 1984). Soil consisted of 73% sand, 17% silt, 8% clay, and 2% organic matter and was sterilized before use. A solution of TpCP in acetone was added to dry soil to give a concentration of 10 ppm. The soil was treated by pipetting the TpCP solution onto the surface of a preweighed quantity of soil spread out on a stainless steel pan. The solvent was allowed to evaporate and the soil was mixed thoroughly. The treated soil was placed in pots (2000 g per pot) and was covered with a 2-cm layer of untreated soil, in which the seeds germinated. Twenty-four soybean plants were inoculated by adding a suspension of Rhizobium japonicum 61A76 (a gift of Dr. J.C. Burton, Nitragin Co., Milwaukee, WI) to the soil when the seeds were planted. After 90 days of growth, the plants were harvested and stems, leaves, pods, and seeds were separated, weighed, and stored frozen at -30°C until analysis.

Each of the plant organs (5-10 g) was blended and extracted with one 50-ml and three 25-ml portions of 1:1 methylene chloride/ethyl ether mixture. Emulsion was reduced by centrifugation. The organic extracts were pooled and then extracted with three 25-ml portions of 1N NaOH to remove some of the materials which interfered with the gas chromatography. The organic solution was reduced to 5 ml by rotary evaporation and then further cleaned up on a 10 X 1.5 cm i.d. Florisil column. The column was eluted with 150 ml of 50% ethyl ether in petroleum ether. This cleanup procedure was the same as that used by Lombardo and Egry (1979) except that the 6 and 15% eluants were omitted. Treated and untreated samples of soil from pots in which plants were grown were screened through 0.417-mm wire mesh. The soil (5 g) was extracted by the same procedure used for plant tissues.

RESULTS AND DISCUSSION

The extraction and Florisil chromatographic cleanup procedures were tested for recovery of TpCP from leaf samples (approximately 5 g) of untreated (control) soybean plants fortified with TpCP in amounts ranging from 100 to 800 µg. The mean ± standard deviation for five separate determinations was 85.9 ± 2.9%. The GC/FPD calibration curve of TpCP showed linear responses between 2 and 80 ng of the compound injected.

Approximately 70% of the TpCP in the soil on which soybean plants were grown had disappeared within 90 days (at the time plants were harvested). The data in the distribution studies (Table 1) show that TpCP was taken up from the soil by the plant and assimilated into various organs except seeds. Because of possible metabolism and volatilization of the compound in the soil and the plant, the amount taken up by the plant could not be estimated. Positive identification of TpCP extracted from plant tissues was made by comparing the mass spectrum of the isolated compound with that of a standard. At the time of harvest, the amount of the TpCP per plant was 34 μg or 0.17% of the applied chemical. Of this total plant content, 74% was found in the stem, 24% in the leaves, and 2% in the pods.

TABLE 1. Levels of TpCP in Soybean Plants Grown 90 Days on Soil Containing TpCP[1]

		TpCP	
Organ	Total fresh weight (g) of organ/plant	Total content (μg)	Concentration in tissue (μg/g fresh wt. + S.D.)
Shoot	19.6	33.8	1.72
seed	3.0	N.D.[2]	N.D.
pod	5.4	0.5	0.09 ± 0.01
leaf	7.2	8.2	1.14 ± 0.09
stem	4.0	25.1	6.28 ± 1.06

[1]These data represent means of 7 plants.
[2]Not detected, <0.001 μg/g.

After the free TpCP was separated by organic extraction, the aqueous layers of plant organs were hydrolyzed with 6N HCl overnight at 80°C. No evidence of acid-released parent TpCP was obtained.

In summary, it has been demonstrated that soybean plants do take up and translocate TpCP. The TpCP content was highest in the stem. Leaves contained the next largest amount. The pods had the smallest amount and the seeds contained no detectable TpCP. Thus, if sludges and wastewaters are used on agricultural lands, the potential exists for contamination of leafy crops other than soybean plants with TCP and related chemicals.

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