

groundwater:  $\text{Ca}^{2+}$ ,  $\text{Mg}^{2+}$ ,  $\text{Fe}^{2+}$ , and  $\text{Fe}^{3+}$ , among others. The same ions are coordinated by glufosinate. Some of these complexes are thermodynamically quite stable, as evidenced by the negligible conductivities in aqueous solution. Nevertheless, at low concentration none of the glufosinate complexes examined precipitate, but remain in solution. It is uncertain whether any complexation occurs at the still lower concentrations that would be likely in groundwater, since equilibrium constants for glufosinate complex formation have not been measured.

If some complexation does occur, whether this yields an increased risk or is in fact beneficial, depends on many factors involving a comparison of properties of the metal complexes with those of free glufosinate: transport properties through, and interactions with, soils, mammalian toxicities, and degradation mechanisms, to name a few. However, to the extent that the metal complexes remain intact in dilute solution, deactivation of both herbicidal and zootoxic activity seems likely, provided that some of the more stable complexes are also kinetically inert. On the other hand, kinetically labile metal complexes may provide a transport mechanism within the plant that enhances herbicidal activity.

#### ACKNOWLEDGMENT

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**Registry No.** Mg, 7439-95-4; Cu, 7440-50-8; Ni, 7440-02-0;  $\text{Fe}^{2+}$ , 15438-31-0;  $\text{Fe}^{3+}$ , 20074-52-6;  $[\text{Mg}(\text{gluf})]$ , 121098-49-5;  $[\text{Cu}(\text{gluf})]$ , 121098-50-8;  $[\text{Ni}(\text{gluf})(\text{H}_2\text{O})]$ , 121098-51-9;  $\text{K}_{0.6}(\text{NH}_4)_{0.5}[\text{Cr}(\text{gluf})_2]$ , 121124-92-3;  $[\text{Co}(\text{gluf})_2]$ , 121124-93-4; Cr, 7440-47-3; Co, 7440-48-4.

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## Uptake and Metabolic Fate of Indole in Soybeans Grown in Hydroponic Solutions and Soil<sup>1</sup>

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Indole, a neutral aromatic heterocycle, was studied to determine its rate and patterns of uptake as well as its metabolic fate in soybeans grown in solution culture and soil. Indole was rapidly accumulated from amended nutrient solutions with an apparent  $K_s = 0.304$  mM and  $V_{\text{max}} = 86.9$   $\mu\text{g}$  of indole/h per g fresh weight root. Free indole, however, was no longer detected inside the plant within 48 h following exposure. Radiochromatography and additional analysis indicated that the material was not volatilized from the foliage but instead was rapidly converted to tryptophan and subsequently metabolized. Soil studies indicated that >80% of the amended indole was bound in a nonextractable form within 24 h. Uptake of label by soybeans from [<sup>14</sup>C]indole-amended Hagerstown soil, a high clay-low organic matter soil, was 3-7 times greater than from Palouse soil, a low clay-high organic matter soil. Analysis of the plant tissues indicated label distribution patterns of the Hagerstown plants were similar to those of the [<sup>14</sup>C]indole-amended plants grown in solution culture, while the Palouse plants resembled those grown in [<sup>14</sup>C]tryptophan-amended solution culture. The higher organic matter content of the Palouse soil may have contributed to increased metabolic transformation of the bioavailable form of the indole, thus altering the uptake rates.

Growing concern has emerged over the uncontrolled release of organic chemicals and their potential health and

environmental effects as they migrate through soils to surface or ground waters. Terrestrial plants are capable of intercepting these materials via direct root uptake (Chaney, 1983) and are an important link between these soil mobile waste forms and the food web leading to man. It is, therefore, important to consider those processes that affect the potential of terrestrial plants to accumulate, modify, or recycle organic xenobiotics back into the en-

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vironment. However, very little is known concerning the structural and chemical requirements for the root absorption of organic residues; similarly, the recognized plant availability of tightly bound soil residues (Ashton and Crafts, 1981) remains unclear.

Once organic xenobiotics are transported across root membranes, their potential impact on food chains will depend on their spatial partitioning within the plant and, most importantly, their chemical fate. Hatzios and Penner (1982) described some of the metabolic transformations possible in plants for this range of chemical structures. These transformations include hydroxylation, methylation, hydrogenation, conjugation, and partial to complete degradation of the residue.

Rates of plant root absorption have previously been shown to range from 13  $\mu\text{g/g}$  of fresh weight root per day from solutions containing 10 ppm aniline to 180  $\mu\text{g/g}$  of fresh weight root per day from hydroponic solutions containing 50 ppm phenol (Cataldo et al., 1987). Once accumulated, the metabolic fate of either phenol or aniline appeared to depend on whether the xenobiotic was chemically similar in structure to metabolites for which pathways and processes are already present for anabolism, catabolism, or detoxification. For example, exogenously applied phenol was shown to be rapidly incorporated into metabolites with a significant fraction (35%) of the absorbed carbon respired (Cataldo et al., 1987). Casterline et al. (1985) reported that 48% of the total radioactivity from soybeans given [ $^{14}\text{C}$ ]pentachlorophenol was distributed into unidentifiable metabolites in the aqueous fraction. Aniline has been shown to be sequestered via conjugation with plant metabolites; only a small fraction is oxidatively decomposed (Cataldo et al., 1987). While quinoline undergoes some metabolic modification following absorption, about 75% of the absorbed material persists as quinoline (Cataldo et al., 1987). In addition, a substantial fraction of absorbed anthracene and quinoline was shown to be reemitted from leaves to the atmosphere (Edwards et al., 1982; Cataldo et al., 1987). Durmishidze et al. (1974) reported that in seven plant species exposed to sterile solutions of 3,4-benzpyrene-1,2- $^{14}\text{C}$  the majority of absorbed radioactivity was located in the organic acids. Edwards et al. (1982) showed that soybeans accumulate [ $^{14}\text{C}$ ]anthracene from nutrient solutions, soil, and the atmosphere surrounding foliage; in a later study using bush beans, Edwards (1986) showed that 90% of the absorbed anthracene was metabolized.

This study addresses the root absorption and chemical/metabolic fate of a neutral aromatic heterocycle, indole, by hydroponically grown and soil-grown soybeans (*Glycine max* L. Merr.). Indole was selected for study for two reasons: It is similar in size, but different in basicity, to quinoline, and it represents a metabolic intermediary in tryptophan synthesis, thus providing a basis for comparing the metabolic fate of quinoline and indole. The plant-based processes investigated include the (1) regulation of xenobiotic uptake by the roots; (2) patterns of accumulation and partitioning between the roots, stems, and leaves; (3) extent to which indole is released to the atmosphere (foliar volatilization); and (4) extent to which indole may undergo metabolic transformation or accumulation in modified chemical forms.

## MATERIALS AND METHODS

**Plant Uptake Experiments (Solution Culture).** Soybean (*G. max* L. Merr., var. Williams '82) plants were grown in solution culture under previously described growth conditions (Cataldo et al., 1983). The 21–24-day-old plants were transferred to beakers containing 500 mL of nutrient solution, containing 0.37 MBq/plant of indole-benzene-ring-UL- $^{14}\text{C}$  (specific activity 457.3

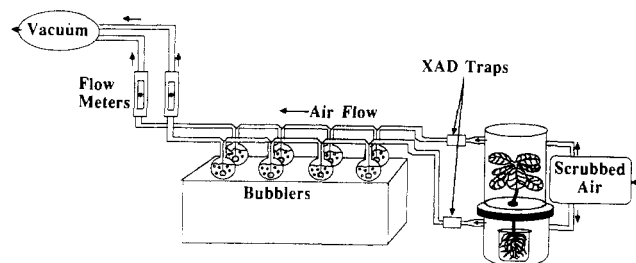
MBq/mmol; Lamplighter, Columbus, OH) and either 1, 5, 10, 25, or 50 mM of carrier indole (Aldrich Chemical Co., St. Louis, MO) as indicated. Plants were incubated in these solutions for 2 or 24 h depending on the concentration used and experimental needs. Following incubation, the plants were removed and the roots briefly (<15 s) washed in 80% methanol to remove surface-sorbed indole, following which the roots were quickly blotted dry, weighed, diced, and frozen in less than 1 min. It is believed that potential damage from the methanol was minimized in this fashion while the removal of extraneous surface indole was maximized. The fine and fibrous roots were then removed weighed, diced, and frozen in powdered dry ice. The remainder of the plant was further divided into stem (including petioles), primary leaves, first trifoliates, and remaining leaves and processed in the same manner. These parts were subsequently subsampled and oxidized to  $\text{CO}_2$  in a Packard Model 306 oxidizer (Packard Instrument Co., Downers Grove, IL), the  $\text{CO}_2$  trapped in scintillation fluid and counted. Data are expressed as micrograms of indole taken up per hour per gram fresh weight root.

Long-term solution experiments were conducted over a 1-week period, where the solutions and plants were analyzed for indole and label as given below at 24-h intervals and the [ $^{14}\text{C}$ ]indole was replaced at 48-h intervals.

Additional correlative experiments conducted included 72-h solution culture exposures of 50 ppm solutions of L-[U- $^{14}\text{C}$ ]tryptophan (0.37 MBq/plant, specific activity 20.6 GBq/mmol; New England Nuclear, Boston, MA) plants, and solutions were analyzed as described below.

**Partitioning/Metabolic Fate.** Hydroponically grown plants were exposed to  $^{14}\text{C}$ -labeled indole solutions in growth chambers for times and at concentrations as described in the text. Indole and its metabolites were assayed by three different procedures. In the first procedure, plant tissue (0.5–3 g) was placed into a Sorval tissue grinder together with 18 mL of methylene chloride, 10 mL of water, and about 1 g of dry ice immediately following labeling. The mixture was ground for 2 min, the organic layer was then transferred to a Corex centrifuge tube, and an additional 5 mL of methylene chloride was added to the grinder and ground for another 2 min. The second organic layer was then decanted. The combined organic layers were centrifuged at 1100g for 10 min, and the clarified organic layer was removed and evaporated under a stream of nitrogen. Two milliliters of *n*-heptane was then added and the solution subsampled for scintillation counting. The solution was poured over a Waters Florisil Sep-Pac solid absorbant bed, using an additional 1 mL of *n*-hexane as rinse. Elution was carried out with 4 mL of acetonitrile. The volume of the acetonitrile eluate was brought to 4.0 mL and the solution subsampled for scintillation counting. The acetonitrile solution was analyzed for indole by HPLC using the procedure described below. Recovery studies were performed by adding 42  $\mu\text{g}$  of radiolabeled indole to 3-g samples of exposed leaves. Recovery of radiolabel in the acetonitrile fraction was  $63.55 \pm 16.82\%$  ( $n = 4$ ); recovery of indole by HPLC analysis was  $53.62 \pm 1.86\%$  ( $n = 4$ ). Detection limits were estimated at less than 1  $\mu\text{g/g}$  of tissue. The recoveries are acceptable based on the rapidity and extent of indole metabolism.

In the second procedure, indole-exposed soybean tissue (1–3 g) was ground in a Sorvall tissue grinder with 15 mL of methanol for 3 min and the mixture transferred to a Corex centrifuge tube and centrifuged at 3000g for 20 min. The methanol solution was evaporated to 2 mL under a stream of nitrogen, filtered through a  $\text{C}_{18}$  Sep-Pac, and rinsed with an additional 1 mL of methanol, and the volume was reduced again to 2 mL. The solution was subsampled for scintillation counting and sufficient sample (20–80  $\mu\text{L}$ ) injected into the HPLC to place at least 2000 dpm on the analytical column. Chromatography was carried out with use of the equipment described above for HPLC assay of indole, using a linear 1 mL/min gradient of methanol/water, starting at 100% water and reaching 100% methanol in 45 min. Fractions were collected in scintillation vials every 0.5 min and counted, and a chromatogram was constructed with a graphing program. Quantitation of chromatographic components was determined by the sum of the dpm under peaks; total recovery of radiolabel was generally over 90%. Recovery studies were performed by adding 20- $\mu\text{g}$  samples each of indole and tryptophan to samples of soybean leaves and root. Mean recoveries from leaves (indole,



**Figure 1.** Diagram of gas sampling system used to determine loss of [ $^{14}\text{C}$ ]indole and  $^{14}\text{CO}_2$  from roots and shoots of a soybean plant whose roots are in a 50 ppm solution of [ $^{14}\text{C}$ ]indole.

$64.7 \pm 4.2\%$ ; tryptophan,  $73.0 \pm 4.7\%$ ;  $n = 3$ ) were indistinguishable from those from roots (indole,  $68.8\% \pm 17.8$ ; tryptophan,  $74.6 \pm 9.8\%$ ;  $n = 3$ ). Thus, the means from all six determinations were used to correct for recovery (indole,  $66.8\%$ ; tryptophan,  $73.8\%$ ).

The third was a modified procedure of Dickson (1979), where 0.5 g of fresh tissue was sampled the protein extracted with a 0.04% protease (w/v) (Sigma Chemical Co., St. Louis, MO) in 50 mM *N*-(2-hydroxyethyl)piperazine-*N'*-2-ethanesulfonic acid (HEPES) buffer, pH 7.4, for 24 h at 30 °C, and the starch extracted with 0.1% (w/v) amyloglucosidase (Sigma) in 20 mM 2-(*N*-morpholino)ethanesulfonic acid (MES) buffer, pH 4.5, for 48 h at 45 °C. The final pellet (insoluble fraction) was then oxidized as described above and counted. All liquid scintillation samples were counted with appropriate quench correction on a Beckman Model 9800 liquid scintillation spectrophotometer (Beckman Instruments, Irvine, CA).

Additional aliquots from the basic fraction were freeze-dried, brought to a 0.5-mL volume with a lithium citrate buffer, pH 2.2, and either directly processed through a Glenco Model MM amino acid analyzer (Glenco Scientific, Houston, TX), operated in the physiological fluid mode with Pico-Buffer System IV (Pierce Chemical Co., Rockford, IL), or hydrolyzed first with HCL or mercaptoethanesulfonic acid. Fractions were collected from the analyzer effluent and counted by liquid scintillation. Peaks were identified with known unlabeled and  $^{14}\text{C}$ -labeled amino acid standards.

**Analysis of Plant Exposure Media by HPLC.** Plant hydroponic solutions and soil extracts were analyzed for indole and tryptophan by injection into a Waters Model 680/510 dual-pump liquid chromatographic system using a Model 710B autoinjector (Waters Associates, Milford, MA). Elution over the 20-cm Waters  $\text{C}_{18}$   $\mu$ -Bondapak column was accomplished isocratically with a 50/50 v/v mixture of methanol and water at 1 mL/min. Responses from the Model 481 spectrophotometric detector were recorded and integrated with a Hewlett-Packard Model 3390A integrator/recorder (Hewlett-Packard, Avondale, PA). Results were quantified by comparison with standards in methanol.

**Plant and Soil Volatilization Experiments.** A 28-day-old solution grown soybean plant was placed in a beaker containing 500 mL of [ $^{14}\text{C}$ ]indole-amended nutrient solution (50 ppm, 0.37 MBq). The beaker was placed into the bottom compartment of a chamber consisting of two lexan cylinders, with a midplate, effectively separating the root and shoot (Figure 1). The entire assembly was then placed into a growth chamber with the same growth conditions as given above.

Over a 72-h period each cylinder received its own air supply (0.2 L/min) and was vented through a series of four gas traps, each containing 10 mL of 3 N NaOH. The NaOH traps were changed twice each 24-h period (during the middle of the light and dark periods) by removing the solution, taking a volume, subsampling for liquid scintillation ( $^{14}\text{CO}_2$ ), and replacing it with fresh NaOH. At the same time, packed columns (1.0  $\times$  15 cm) of methanol-washed and dried XAD resin were placed in the gas trains ahead of the traps for 2-h periods to trap organic compounds. Following the sampling periods, the columns were eluted (3 times void volume) with 100% methanol, a volume was taken, and the eluent was subsampled for liquid scintillation and HPLC analysis as described above. Control experiments confirmed that the NaOH would not absorb indole or related compounds and that the XAD resin would not trap  $^{14}\text{CO}_2$ . The experiment was

replicated twice and the data were expressed both as milligrams of indole equivalents per hour and as percent total applied label.

The volatilization of indole or decomposition products and  $^{14}\text{CO}_2$  derived from soil amended indole were evaluated by a similar gas sampling system. Soils amended with 50 ppm indole and incubated for 30 days were used to evaluate the extent of oxidative decomposition of bound indole. Each soil was brought to moisture and placed in a closed metal chamber attached to the gas-trapping apparatus described above. Filtered air was drawn over the pots at 250 mL/min, and the  $\text{CO}_2$  and indole/volatile products were sampled in the same manner as above for 7 days.

**Indole Uptake by Plants from Soils.** Two soil types were employed: (1) a Palouse silt loam, an A horizon Pacific Ultic Haploxeroll from eastern Washington; (2) a Hagerstown silty clay loam, a BT horizon Typic Hapludalf from central Pennsylvania. Soils had pHs of 5.6 and 5.46, clay contents of 22% and 34%, and silt contents of 74% and 62% for the Palouse and Hagerstown soils, respectively. Five-hundred-gram pots of soil, plus 100 g of washed sand in the Hagerstown pots, were prepared at 23% moisture and amended with either 0, 1, 10, or 50 ppm [ $^{14}\text{C}$ ]indole (0.37 MBq/pot), nine pots per concentration, and placed in the growth chamber under the conditions given above. Soybean seeds were planted in three pots of each concentration at 0, 30, and 90 days after indole amendment. Soil pots were brought to weight (23% moisture w/w) three times weekly with distilled water.

Following germination, plants were thinned to two per pot. Plants were allowed to grow for 30 days in the growth chamber, and then they were gently removed from the pots, the roots washed free of soil with distilled  $\text{H}_2\text{O}$ , and the plants processed as described above. The frozen tissues were either directly weighed and oxidized or freeze-dried, weighed, and oxidized with the  $^{14}\text{C}$  trapped and counted as described above. Selected plants were also extracted as described in procedure 3 above. Data are expressed as indole equivalents per gram dry weight of tissue.

**Indole Depuration in Soils and Extraction.** Soils prepared and maintained in the manner described above were also sampled for indole depuration and partitioning. Four soil cores (1  $\times$  4 cm) were taken from pots at all four concentrations and both soil types at 0, 2, 8, and 24 h and at 2, 3, 7, 21, 30, 60, and 90 days. Care was taken during sampling to select areas at least 3 cm away from previous sampling sites. Three of the cores were weighed and extracted initially by the addition of 10 mL of distilled  $\text{H}_2\text{O}$ ; they were then vortexed for 2 min and centrifuged at 10000g for 10 min, the supernate (water fraction) was decanted, a volume was taken, and an aliquot was counted by liquid scintillation. The pellet was then sequentially extracted in a similar manner with 10 mL of 100% methanol (methanol fraction) and 10 mL of 2 N  $\text{NH}_4\text{OH}$  (organic fraction). The final pellet was dried under a stream of  $\text{N}_2$  gas and oxidized and counted as described above to obtain a measure of the bound fraction. Data are expressed as percent total  $^{14}\text{C}$  or microgram indole equivalents per gram dry weight. The fourth core sample was weighed and subdivided, with half oxidized directly to determine total  $^{14}\text{C}$  and the other half dried to determine percent moisture for dry weight corrections. The water fraction was further subjected to HPLC analysis as described for nutrient solutions above to determine indole or indole derivative content.

In those studies where the effects of soil microbial activity on the insolubilization of indole was investigated, soils were subjected to steam sterilization for 30 min at 138 kPa, followed by filter-sterilized indole (0.2  $\mu\text{m}$ ) amendment using sterile technique and subsequent extraction.

**Soil Nitrogen-Plant Interactions.** Six pots of each soil type were prepared at 10 ppm [ $^{14}\text{C}$ ]indole as described above. All pots were brought to weight with distilled  $\text{H}_2\text{O}$  three times weekly, while three pots of each soil type were given an additional 5 mL of 0.625 M  $\text{NH}_4\text{NO}_3$  once a week to inhibit nitrogen fixation. Two weeks after indole amendment, all pots were seeded. The plants were thinned to three per pot after 10 days and the remaining plants grown for 30 days. At that time they were harvested and processed as described in the soil experiments above.

## RESULTS

**Plant Uptake of Indole from Solutions and Metabolic Fate.** In the solution cultures, soybeans rapidly

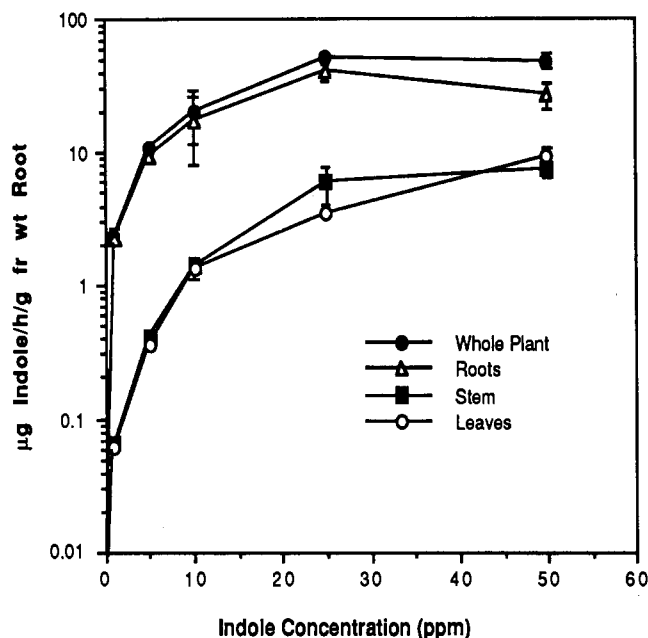


Figure 2. Uptake rates for [ $^{14}\text{C}$ ]indole from solution culture by soybeans. Data are averages of three plants  $\pm$  SD.

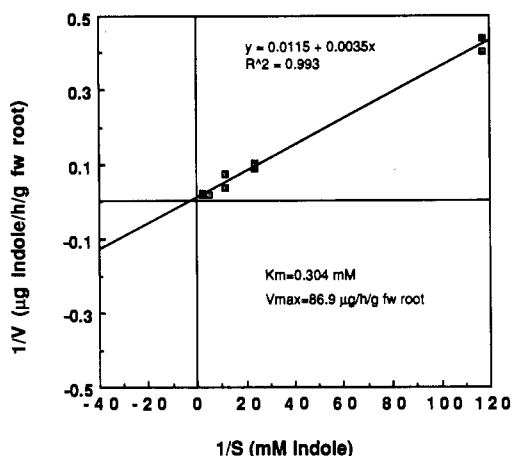


Figure 3. Double-reciprocal plot of whole plant indole uptake rate ( $\mu\text{mol/h}$  per g of fresh weight root) vs indole concentration (mmol) in soybeans.

accumulated [ $^{14}\text{C}$ ]indole (Figure 2). While a major portion of the labeled material, based on total  $^{14}\text{C}$ , was taken up and retained by the roots, approximately 10% of the total plant accumulation was quickly transported to the stem and leaves over a 24-h absorption period. Whole plant and root accumulation appeared to saturate at approximately 25 ppm indole. However, a redistribution of the label continued, resulting in a final shoot accumulation of 17% of the total plant label. At comparable concentrations, the rate of indole uptake was higher than that reported for aniline and quinoline but less than that reported for phenol (Cataldo et al., 1987). Lineweaver-Burke plots for the concentration-dependent uptake of indole for the whole plant (Figure 3) show  $K_m$  values for indole of 0.30 mM and  $V_{\text{max}}$  of 87  $\mu\text{g}$  of indole/h per g of fresh weight root.

Preliminary studies were performed to determine the chemical fate of indole following accumulation. Plants were exposed to solutions containing 50 ppm of [ $^{14}\text{C}$ ]indole or unlabeled indole for 24 h and roots and shoots analyzed immediately for indole. Indole was found in both soybean roots and shoots at concentrations of  $32 \pm 6$  and  $22 \pm 5$   $\mu\text{g/g}$  of tissue, respectively. However, these concentrations accounted for only 46% and 56% of the total radioactivity

Table I. Comparison of Concentrations of Indole and Tryptophan in Soybean Tissue Samples from Plants Exposed to 50 ppm Solutions of [ $^{14}\text{C}$ ]Indole for 24 h As Determined by LC/Diode Array UV Detection and LC/Radioassay

sample/tissue	tryptophan, $\mu\text{g/g}$		indole, $\mu\text{g/g}$	
	radioassay	UV	radioassay	UV
root 1	75.8	74.7	21.8	17.8
root 2	66.3	57.3	26.7	30.3
shoot 1	58.1	68.0	8.8	nd <sup>a</sup>
shoot 2	98.7	103.3	21.9	nd

<sup>a</sup> Not determined because of interference from other plant components.

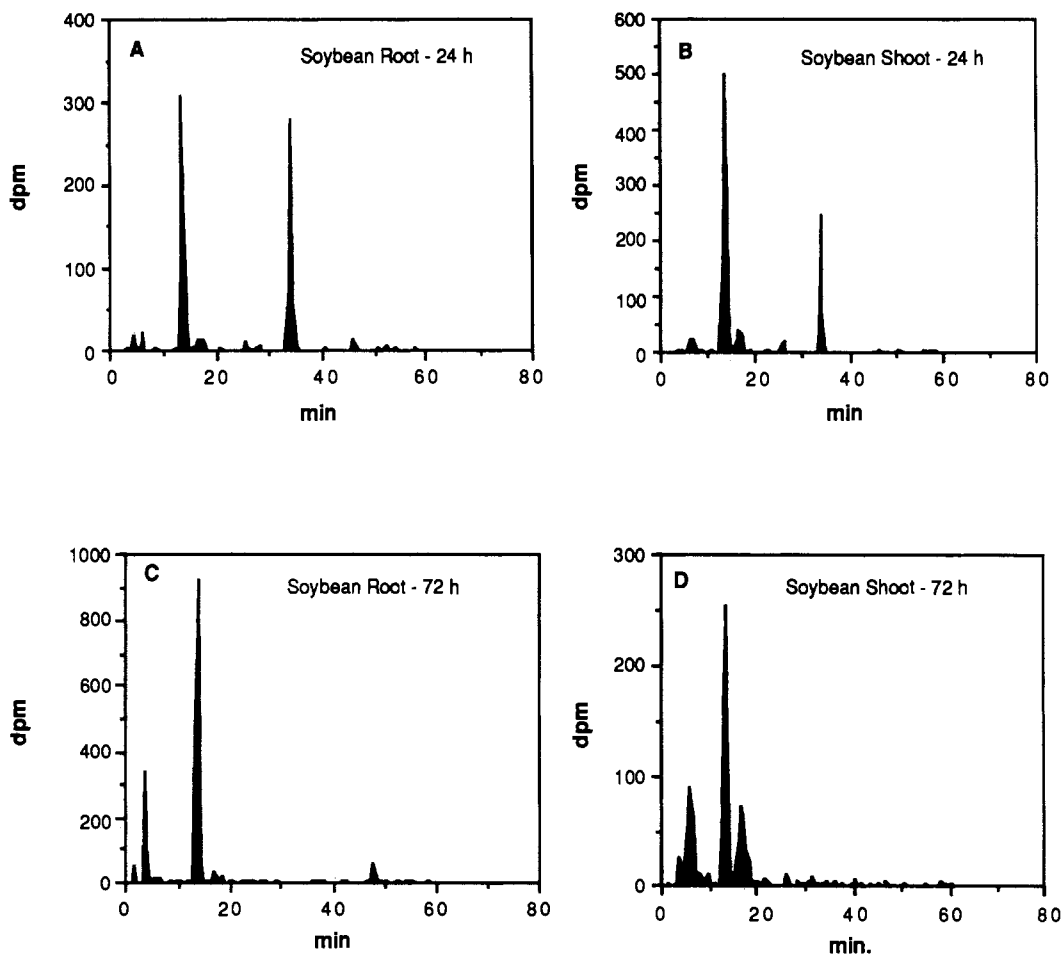
Table II. Concentrations of Indole and Tryptophan in Methanol Extracts from Two Soybean Plants Exposed to 50 ppm Solutions of [ $^{14}\text{C}$ ]Indole over Time As Determined by Radiochromatography

compound	tissue, <sup>a</sup> $\mu\text{g/g}$				
	root	stem	leaves	total shoot <sup>b</sup>	
24-h Uptake					
indole	21.8, 26.7	c	c	8.8, 21.9	
tryptophan	75.8, 66.3	c	c	58.1, 98.7	
other <sup>d</sup>	4.7, 9.6	c	c	10.3, 3.0	
72-h Uptake					
indole	0.0, 0.0	0.0, 0.0	0.0, 0.0	0.0, 0.0	
tryptophan	126.6, 142.7	82.3, 108.2	17.8, 13.1	38.2, 38.6	
other	35.7, 37.6	8.3, 8.1	9.5, 12.2	9.1, 11.1	

<sup>a</sup> Values are for duplicate plant samples. <sup>b</sup> Shoot values for 72-h exposure were calculated from stem and leaf data. <sup>c</sup> Stem and leaf samples combined for 24-h exposure tests. <sup>d</sup> Nonidentified label containing peaks.

found in the extract. When the total label extracted is considered, based on [ $^{14}\text{C}$ ]indole equivalents, the roots should have contained  $79 \pm 10$   $\mu\text{g/g}$  of indole equivalents and the shoots  $39 \pm 16$   $\mu\text{g/g}$ , respectively. Further extraction of these same tissues with an additional 50 mL of methanol increased the extracted [ $^{14}\text{C}$ ]indole equivalent values to  $93 \pm 9$   $\mu\text{g/g}$  for the roots and  $68 \pm 28$   $\mu\text{g/g}$  for the shoots; however, no increase in extractable HPLC-identified indole was evident. These results therefore indicated that indole was extensively altered or metabolized in both the root and shoot of soybean plants after as little as 24-h exposure.

To investigate the initial fate of absorbed indole, radiochromatography was performed on the methanol extracts of soybean tissues after the plants were exposed to 50 ppm indole solutions for 24 and 72 h. The chromatograms obtained (Figure 4A,B) revealed that, after 24 h, both indole and another major constituent, which eluted at an earlier retention time, were present in the root and shoot. Analysis by LC/diode array detection of the retention time and ultraviolet spectra showed that the unidentified constituent was tryptophan. Comparison of indole and tryptophan concentrations for the 24-h samples from two plants, as determined by radioassay and UV detection in root and shoot tissue extracts, indicated that little if any of these two compounds was being produced by the plant; all that was extracted had been absorbed or metabolized from the radiolabeled indole (Table I). After 72 h of exposure (Figure 4C,D), indole was no longer detectable while tryptophan remained the major constituent. However, apparent degradation and/or incorporation of the indole and/or tryptophan into other extractable unidentified components occurred both in the roots and particularly in the shoots. The actual concentrations of indole, tryptophan, and other metabolites in the plant extracts for these experiments as determined by radio-



**Figure 4.** Radiochromatograms of soybean tissues after solution exposure to 50 ppm indole: A, root after 24 h; B, shoot after 24 h; C, root after 72 h; D, shoot after 24 h. Retention times: tryptophan, 13.5 min; indole, 34 min.

chromatography are given in Table II.

It was also noted at this time that a concomitant loss of indole but not radioactivity had occurred from the root bathing solutions. To determine the reason for this discrepancy, plants were exposed to 40 ppm indole solutions and sampled as shown in Figure 5. The root bathing solutions were analyzed for indole and radioactivity at 12- and 24-h intervals and replaced with freshly prepared solutions of the same concentration and activity at 48-h intervals. Although plant-to-plant variations were evident over the first 24 h of the experiment, a decline in the amount of both indole and label was observed, with indole showing the greater loss (Figure 5A). However, after 48 h, while there was no detectable indole or other identifiable compound left in the solution, approximately half of the label remained (Figure 5B). The subsequent changes of fresh solutions at 48 and 96 h exhibited a similar loss (within 24–48 h) of detectable indole. The loss rate of label remained constant over this period as well. Analysis of the plants accounted for most of the  $^{14}\text{C}$  taken up from the solution, but again there was no detectable indole.

The total  $^{14}\text{C}$  recovered in the initial extraction experiments given above accounted for 30–40% of the total label taken up by the plants after 72 h. To further assess the partitioning of indole and possible metabolites, such as tryptophan, between the major soluble and insoluble carbon and nitrogen pools of the plant, additional extraction experiments were conducted using chemical, enzymatic, ion-exchange, and tissue oxidation methods. The results of these analyses for [ $^{14}\text{C}$ ]indole and [ $^{14}\text{C}$ ]tryptophan are given in Tables III and IV, respectively.

**Table III. Partitioning of [ $^{14}\text{C}$ ]Indole within a 21-Day-Old Soybean following a 72-h Exposure in a 50 ppm Indole/Hydroponic Solution (At the Conclusion of Experiment No HPLC-Detectable Indole Present in the Nutrient Solution)**

sample	plant segment, % total $^{14}\text{C}$ recovered			
	root	stem	node 1 (primary leaves)	node 2 (trifoliates)
total plant	70.72	13.35	0.76	15.16
fraction				
water/alcohol	13.73	35.58	18.62	26.22
chloroform <sup>a</sup>	7.14	2.40	1.44	2.88
protein	35.65	4.68	71.28	50.64
starch	5.07	6.31	2.04	9.42
insoluble	38.42	51.03	6.62	10.85
water/alcohol				
ion exchange				
neutral <sup>b</sup>	14.94	4.25	28.75	20.09
basic <sup>c</sup>	61.91	88.97	37.91	62.70
acid <sup>d</sup>	17.73	4.50	33.35	14.92
acid II <sup>e</sup>	5.42	2.28	0.00	2.29
total plant	620.2	117.1	6.6	138.2
dpm <sup>f</sup> ( $\times 10^4$ )				
total dpm	43.98	53.22	19.0	39.3
used for ion				
exchange <sup>g</sup>				
( $\times 10^4$ )				

<sup>a</sup>Lipids. <sup>b</sup>Sugars. <sup>c</sup>Amino acids. <sup>d</sup>Organic acids. <sup>e</sup>Sugar phosphates. <sup>f</sup>Total dpm in samples used to calculate percentages. <sup>g</sup>Total dpm eluted from columns used to calculate percentages.

Subsequent analysis of the water/alcohol component for all of the plant tissues by ion-exchange chromatography into acid, basic, and neutral fractions showed that the

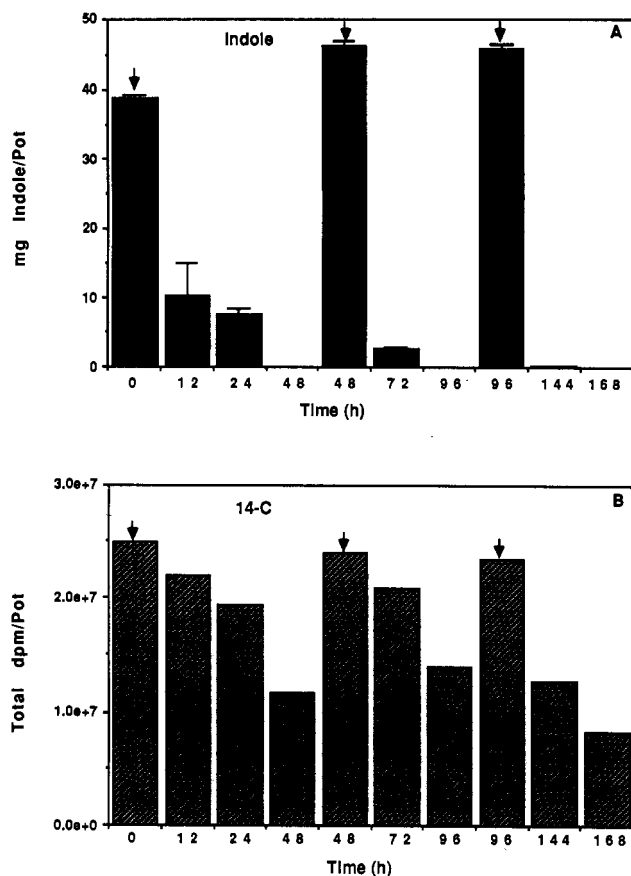


Figure 5. Average total indole (A) and  $^{14}\text{C}$  (B) in nutrient solution of soybean plants ( $n = 4$ ) at initial amendment of the solution with 40 ppm  $^{14}\text{C}$  indole (0.37 MBq) (time 0) and at 12-, 24-, or 48-h intervals thereafter. Solutions were replaced at 48-h intervals for first 96 h (arrows).

Table IV. Partitioning of  $^{14}\text{C}$  Tryptophan in a 21-Day-Old Soybean Plant Exposed to a 50 ppm  $^{14}\text{C}$  Tryptophan Solution for 72 h

sample	plant segment, % total $^{14}\text{C}$ recovered			
	root	stem	node 1 (primary leaves)	node 2+ (trifoliates)
total plant fraction	42.81	34.19	0.09	22.91
water/alcohol	21.52	17.11	58.32	5.91
chloroform <sup>a</sup>	4.34	0.00	0.00	0.00
protein	22.35	39.52	25.66	54.24
starch	20.97	19.56	0.0	8.06
insoluble	30.81	23.81	16.01	31.79
water/alcohol ion exchange				
neutral <sup>b</sup>	2.50	1.25	0.00	7.26
basic <sup>c</sup>	8.76	40.12	34.65	14.53
acid I <sup>d</sup>	80.45	54.33	39.60	53.27
acid II <sup>e</sup>	8.29	4.30	25.74	24.94
total plant dpm/ ( $\times 10^4$ )	80.8	33.9	2.4	25.4
total dmp used for ion exchange <sup>f</sup> ( $\times 10^4$ )	17.4	5.8	1.7	2.5

<sup>a</sup>Lipid. <sup>b</sup>Sugars. <sup>c</sup>Amino acids. <sup>d</sup>Organic acids. <sup>e</sup>Sugar phosphates. <sup>f</sup>Total dpm in samples used to calculate percentages. <sup>g</sup>Total dpm eluted from columns used to calculate percentages.

majority of the label was contained in the basic fraction (Table III). This was particularly evident in the stem, where 89% of the soluble label was associated with this fraction. Confirmation of the radiochromatography data

Table V. Distribution of  $^{14}\text{C}$  Indole in a Hydroponically Grown Soybean following a 72-h Exposure to an Approximately 50 ppm Solution (Plant Maintained under Normal Growth Conditions and in a Split Chamber That Separated the Root from the Shoot)

tissue/fraction	indole, mg	% total $^{14}\text{C}$
orig indole soln	23.900 <sup>a</sup>	100.00
plant tissue	5.680 <sup>b</sup>	23.78
root $\text{CO}_2$ <sup>c</sup>	0.560	2.37
shoot $\text{CO}_2$ <sup>c</sup>	0.029	0.12
root XAD <sup>d</sup>	0.012	0.05
shoot XAD <sup>d</sup>	0.003	0.01
sum	6.294	26.33
final indole soln	14.976 <sup>e</sup>	62.66
sum	21.27	88.99

<sup>a</sup>Based on HPLC analysis of the 500-mL initial solution. <sup>b</sup>Based on  $^{14}\text{C}$  indole equivalents from initial solution's specific activity. <sup>c</sup>Based on  $^{14}\text{C}$  indole equivalents from NaOH traps. <sup>d</sup>Based on  $^{14}\text{C}$  indole equivalents from XAD resin columns. <sup>e</sup>Based on  $^{14}\text{C}$  indole equivalents in root washes and remaining indole solution.

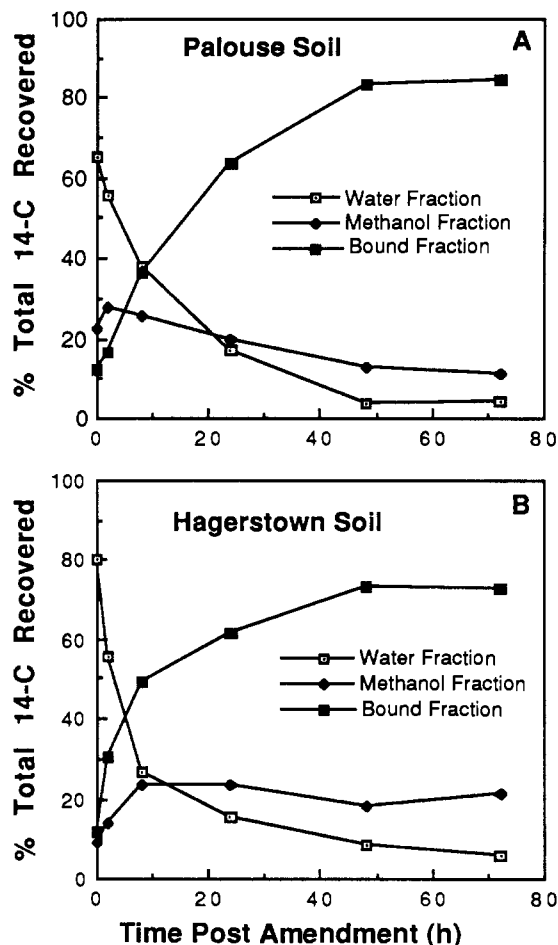
given above was obtained when aliquots of the basic fraction from these tissues were analyzed with an amino acid analyzer. The amino acid pattern observed was similar to that of untreated control plants with the exception of tryptophan, which was often 2–3 times higher than the level in control tissues (data not shown). Further, when fractions were collected from the analyzer eluate and counted, greater than 83% of the total label applied to the column was recovered in the tryptophan peak.

A comparative study on the uptake and partitioning of labeled tryptophan from solution culture at the same exposure concentration exhibited slight differences from that of indole. A higher percentage of the label was transported out of the roots to the stem and leaves over 72 h (Table IV). Although the partitioning of label into protein and insoluble (or storage) fractions was similar to indole, there was also an apparent metabolism of the amino acid to organic acids in all plant organs.

**Plant Volatilization.** Since free indole was not detected within the plant after a short time following exposure, it was important to determine whether indole or its metabolites may have been lost from the plant through transpiration or volatilization prior to any metabolic incorporation. The results of this type of analysis, obtained in an experiment using a single plant, provided a mass balance of the initial label supplied and are given in Table V.

After 72-h exposure the plant accumulated 26% of the total label present in the original solution. Of this, 90% was retained within the plant (70% in the roots, 15% in the stem, and 5% in the leaves). Of that label taken out of the solution by the plant and not retained, approximately 9% was lost via respiration ( $^{14}\text{CO}_2$ ) primarily from the root while the shoot lost less than 1%. The amount of recovered label trapped by the XAD resin was extremely low for both the shoot and the root. This indicated negligible direct loss of indole or its metabolites through volatilization from leaves to the atmosphere over the 72 h.

**Indole Availability to Plants Growing in Soil.** While soybeans were shown to be readily capable of accumulating indole from amended hydroponic solutions, it was also important to determine whether similar results would be obtained when the plants were grown in soil. Given the natural wide variations in soil types, the two soils chosen for this analysis, Palouse and Hagerstown, were selected for their differences in geographic origin and or-



**Figure 6.** Distribution of <sup>14</sup>C expressed as percent of total <sup>14</sup>C recovered from extraction of Palouse (A) and Hagerstown (B) soils between the water, methanol, and bound components over time following amendment of the soils with 50 ppm [<sup>14</sup>C]indole (0.37 MBq).

ganic carbon and clay contents.

For the plant to absorb indole from the soil, a soluble, available form must be present in the soil. Previous reports in the literature indicated a short half-life of 1 day for high concentrations (500 ppm) of indole in soil (Medvedev and Davidov, 1972). Our experimental observations indicated similar effects at lower concentrations. Following amendment to both soil types, at a soil concentration of 50 ppm, free (extractable) indole, as determined by percent of total [<sup>14</sup>C]indole extracted, was greatly diminished within 24–72 h (Figure 6). The percentage of label distribution observed at 72 h remained essentially unchanged for up to 120 days following amendment.

These results indicated that microbial incorporation, physical/chemical binding, and/or chemical transformation of the indole to a nonextractable form in soil rapidly occurred. When Hagerstown soil was sterilized prior to amendment, a decrease in the apparent rate of binding (a decrease in the amount of label in the bound fraction, an increase in the water) was observed up to 48 h following amendment (Table VI). There remained, however, a significantly large amount still bound after this brief time. In the nonsterile soil, direct microbial incorporation and transformation of indole into tryptophan or protein were apparently not significant factors in limiting the availability of indole. When samples of both soils that had been previously extracted with the same solvents were treated with a protease solution, only an additional 0.9% of the total label recovered was obtained.

**Table VI.** Partitioning of <sup>14</sup>C Applied as 50 ppm [<sup>14</sup>C]Indole in Sterilized and Nonsterilized Hagerstown Soil over 48 h

sterility	fraction, <sup>a</sup> % total <sup>14</sup> C ± variance			
	water	methanol	NH <sub>4</sub> OH	bound
0 h				
sterile <sup>b</sup>	87.49 ± 0.22	6.30 ± 1.10	0.19 ± 0.15	6.02 ± 1.10
non-sterile	80.59 ± 1.76	3.93 ± 3.60	1.90 ± 1.2	13.58 ± 3.28
48 h				
sterile	18.27 ± 1.59	24.34 ± 2.45	8.72 ± 0.06	48.67 ± 4.11
non-sterile	7.34 ± 2.75	17.09 ± 1.65	4.95 ± 0.87	70.62 ± 3.52

<sup>a</sup> N = 2. <sup>b</sup> Autoclaved for 30 min at 138 kPa.

**Table VII.** Plant Dry Weight for 30-Day-Old Soybean Plants Grown in Soils Amended with Different Concentrations of [<sup>14</sup>C]Indole

concn, ppm	days seeded postamendment, av g dry wt ± SD (N = 6)		
	0	30	90
	Palouse		
1	1.590 ± 0.45	1.767 ± 0.53	1.339 ± 0.19
10	1.505 ± 0.16	1.674 ± 0.18	1.353 ± 0.51
50	1.309 ± 0.32	1.503 ± 0.20	1.478 ± 0.14
controls	1.176 ± 0.15	1.514 ± 0.21	1.191 ± 0.07
	Hagerstown		
1	0.833 ± 0.12	0.936 <sup>a</sup>	1.093 <sup>a</sup>
10	0.816 ± 0.07	0.906 <sup>a</sup>	1.200 <sup>a</sup>
50	0.845 ± 0.11	0.800 <sup>a</sup>	1.443 <sup>a</sup>
controls	0.712 ± 0.23	0.685 <sup>a</sup>	0.933 ± 0.09

<sup>a</sup> N = 2.

The residual sorbed indole was strong enough to further resist 24 h of Soxhlet extraction with a 50/50 chloroform/methanol mixture.

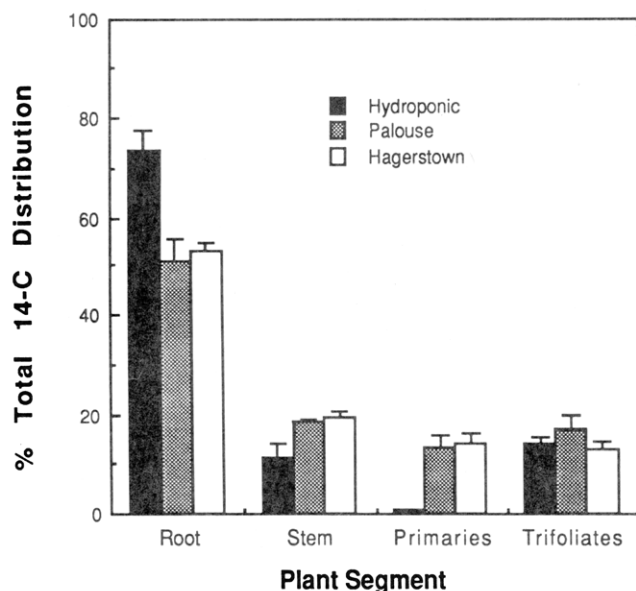
#### Plant Uptake and Partitioning of Indole from Soil.

Although apparently bound to the soil, the indole was available for plant uptake for extended periods of time following its initial amendment.

In studies where 1, 10, and 50 ppm indole was amended to soil and seeded at 0, 30, and 90 days postamendment, only the 50 ppm treatment seeded at day 0 exhibited a 10–20% reduction in germination rate for both soil types. There were no differences apparent in the plant dry weight at any of the seeding dates. There instead appeared to be a slight increase in most cases in the average dry weight of the plants over those of the controls (Table VII).

Plants grown in the Hagerstown soil also appeared to exhibit no significant declines in shoot dry weight of exposed and control plants at any indole concentration, although a slight increase in dry weight was observed over time (Table VII). However, it was also noted that the average dry weights of the Hagerstown plants, both exposed and controls, were frequently less than those of the Palouse (Table VII). Slight physical differences were also apparent, with the leaves of plants grown in the Hagerstown soil appearing somewhat smaller and lighter green than those grown in the Palouse soil for the first 15 days after germination. However, at the conclusion of the individual growth periods (30 days), they appeared to be identical in coloration to those grown on the Palouse.

Plant partitioning patterns based on total percent recovered <sup>14</sup>C after 30 days of growth in soils amended with 50 ppm [<sup>14</sup>C]indole and planted immediately after amendment were generally similar to that observed in plants from the 50 ppm 72-h hydroponic studies (Figure 7), except for primary leaves. Distribution patterns of the label within the plants grown in the two types of soil differed slightly (Table VIII) with more of the material



**Figure 7.** Comparison of [<sup>14</sup>C]indole partitioning patterns in different tissues. Expressed as percent total <sup>14</sup>C recovered in soybean plants exposed to 50 ppm [<sup>14</sup>C]indole (0.37 MBq) growing in either solution culture (72-h exposure) or in Palouse or Hagerstown soil (30-day exposure).

**Table VIII.** Partitioning of [<sup>14</sup>C]Indole in 30-Day-Old Soybean Plants Grown in Palouse or Hagerstown Soil Amended with 50 ppm [<sup>14</sup>C]Indole

fraction	plant segment, % total <sup>14</sup> C recovered			
	root	stem (primary leaves)	node 1 (trifoliates)	node 2
	Palouse			
whole plant	55.0	16.9	13.0	15.1
water/alcohol	30.8	12.9	25.0	9.7
chloroform	6.0	2.6	1.0	2.4
protein	7.2	13.6	14.5	28.1
starch	7.1	5.0	13.7	12.8
insoluble	48.9	65.9	45.8	47.0
	Hagerstown			
whole plant	66.7	13.6	11.6	8.1
water/alcohol	44.7	38.6	32.4	21.3
chloroform	7.2	4.1	5.0	1.6
protein	9.1	26.4	34.7	60.4
starch	4.8	6.0	4.9	7.7
insoluble	34.2	24.9	47.9	9.0

found in the insoluble fraction in the Palouse grown plants and a higher distribution going to the protein and water/alcohol fractions in the Hagerstown grown plants (Table VIII). Both soil-grown plants differed from the solution-grown plants (Table III), particularly in regard to the percentage of label in protein within the roots. These differences in partitioning patterns between the soil- and solution-grown plants, based on percent distribution, could be attributed in part to the nature of the label application, effectively a steady-state exposure for the soils and a pulse for the solution grown plants.

Absolute partitioning values for these experiments (expressed in indole equivalents based on <sup>14</sup>C concentrations per gram of dry weight) in roots and shoots (leaves plus stem) of the hydroponically grown plants were 12.6 and 5.21 μg/g, respectively, after 72 h. However, for 30-day-old Palouse and Hagerstown soil grown plants, root [<sup>14</sup>C]indole equivalent concentrations were 45.4 and 150 μg/g, while shoot concentrations were 49.1 and 216 μg/g, respectively. The differences between the totals for the two soil types and the higher total accumulation by the shoots of the

**Table IX.** Average <sup>14</sup>C Content per Gram Dry Weight of Soil for Palouse and Hagerstown Soils Sampled over 120 Days following an Initial Amendment with 50 ppm [<sup>14</sup>C]Indole

soil type	day postamendment, <sup>a</sup> total KBq/q dry wt			
	0	30	60	120
Palouse	2.28 ± 0.46	1.43 ± 0.41	1.72 ± 0.40	1.02 ± 0.08
Hagerstown	1.97 ± 0.61	1.39 ± 0.11	1.35 ± 0.04	1.43 ± 0.17

<sup>a</sup> Mean ± SD, N = 3.

Hagerstown soil grown plants were significant.

Further differences between the soil types emerged when tissue [<sup>14</sup>C]indole equivalent distribution patterns were determined based on planting time and the indole concentration amended to the soil. The results given in Figure 8 show that total plant accumulation remained proportional to soil indole concentration for plants grown on both soils. Indole distribution patterns remained constant at all concentrations in the Palouse soil plants and, although reduced in amount, retained a similar pattern for each of the later planting dates.

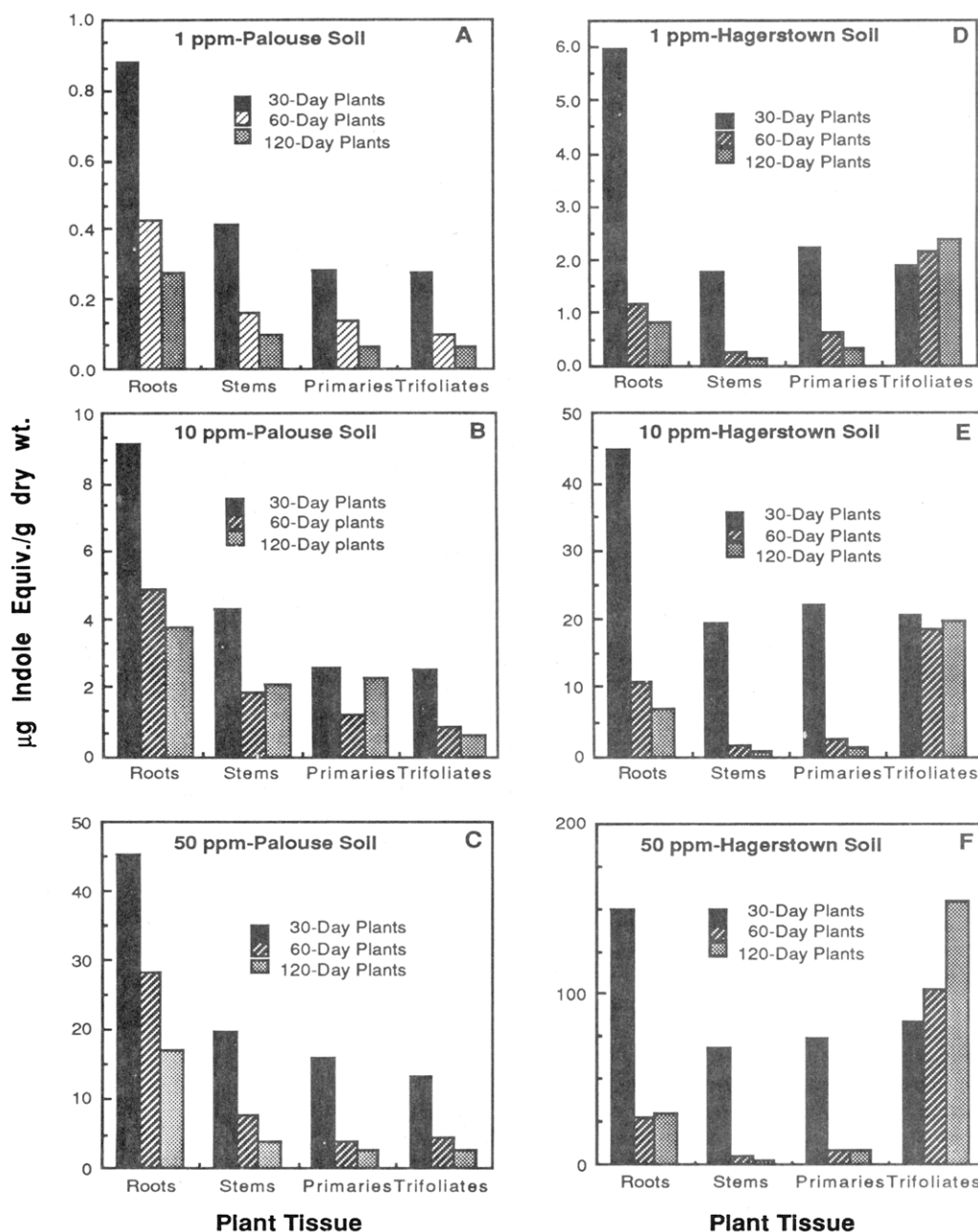
The concentrations of indole derived <sup>14</sup>C in plants grown in Hagerstown soil and seeded at 0 days (day 30 plants) were 3–7 times higher than those grown in the Palouse (Figure 8). This difference was similar following a 30- or 90-day delay period to planting (day 60 and 120 plants, respectively). A distinctive shift in partitioning was observed from the roots to the shoots, particularly the new trifoliates of the Hagerstown plants. This shift increased with exposure concentration.

The observed differences in label uptake could not be explained by variations in plant dry weights, since the Palouse dry weights averaged only 30–40% higher than the Hagerstown grown plants both in control and treated plants (Table VII). Further, these apparent differences may have been decreasing with time (Table VII). Therefore, the possibilities that some factor affecting the availability of the material either within the plants themselves (at the roots) or within the soil were investigated.

Of the soybeans grown on the two soils, only those grown on the Hagerstown had nodules and therefore had a slightly different root morphology. The Palouse soil, as most of the soil from Washington state, is reported to be deficient in *Rhizobium japonicum*, essential for nodulation in soybeans (D. Bezdicek, Washington State University, personal communication; Bezdicek et al., 1978). Nodulation and its accompanying nitrogen metabolic pathways may affect the relative root uptake of N-containing compounds (Pate, 1980) such as indole and could account for some of the observed differences between the Hagerstown- and Palouse-grown plants. To assess the potential impact of nodulation on [<sup>14</sup>C]indole uptake and distribution, plants were grown in Hagerstown and Palouse soil with and without added nitrate, to inhibit nodule formation in Hagerstown grown plants. The results, given in Figure 9, indicate that while there was an inhibitory effect from added N in the Palouse grown plants, a slight enhancement was evident with the Hagerstown plants and the differences in total uptake by the roots between the two soil types remained.

Significant differences in the total amount of label present within the two soil types were not evident (Table IX) at each of the planting dates except at 120 days. Further, while the Palouse showed the greater loss over time, the differences were not sufficient to explain the uptake results. When amended nonseeded pots were placed in the volatility chamber 45 days after amendment and sampled over 7 days, the rate of loss of <sup>14</sup>CO<sub>2</sub>





**Figure 8.** Absolute partitioning values ( $\mu\text{g}$  indole equivalents/g dry weight) of  $^{14}\text{C}$  indole in tissues of plants grown in Palouse (A–C) and Hagerstown (D–F) soils amended with indole concentrations of 1, 10, and 50 ppm. Plants were seeded in pots at 0, 30, and 90 days after amendment and harvested at 30, 60, and 120 days, respectively.

from the pots averaged  $0.95 \pm 0.2\%$  of the total label in the pot per day for the Palouse soil and  $0.61 \pm 0.25\%$  of the total label per pot for the Hagerstown soil. The XAD-trapped label, which was not indole as determined by HPLC, averaged  $0.04\text{--}0.09\%$ /day for both soils, with no significant difference apparent between them. These loss rates would not have been constant over the entire length of the experiment but indicate the greater loss of label from the Palouse may have been caused in part by microbial activity.

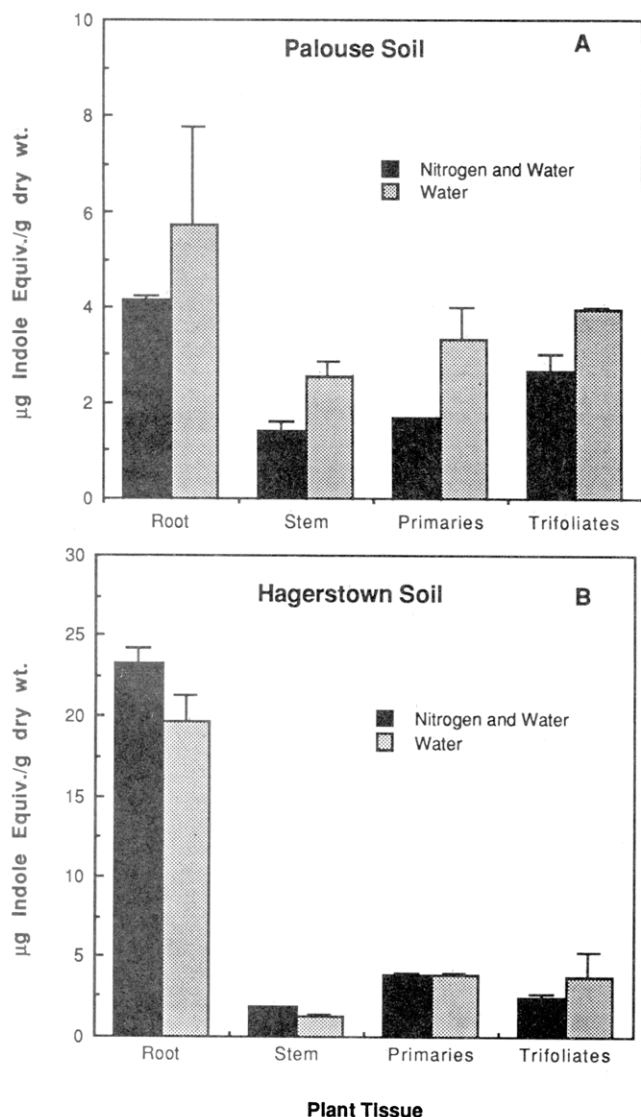
Evidence that a different form of the material was taken up by plants grown on the two soil types was suggested initially when the overall partitioning patterns were considered as described above (Table VIII). This hypothesis was also supported when their water/alcohol extracts were processed further and compared by ion-exchange fractionation (Table X). The partitioning patterns observed in the plants grown in the Palouse soil resembled those found in plants exposed to tryptophan, while those from

**Table X.** Distribution of  $^{14}\text{C}$  in Ion-Exchange Fractions from Roots of Soybean Plants Grown in either 50 ppm  $^{14}\text{C}$  Indole- or  $^{14}\text{C}$  Tryptophan-Amended Solution Culture for 72 h and in 50 ppm  $^{14}\text{C}$  Indole-Amended Palouse or Hagerstown Soil for 30 Days (Data Percentages of Total Label Recovered in the Ion-Exchange Fractions)

ion-exchange fraction	growth media, % total label recovered from ion-exchange column			
	indole soln culture	tryptophan soln culture	Palouse soil	Hagerstown soil
neutral <sup>a</sup>	14.94	2.50	14.09	10.17
basic <sup>b</sup>	61.91	8.76	13.07	62.27
acid I <sup>c</sup>	17.33	80.45	52.85	26.01
acid II <sup>d</sup>	5.42	8.29	19.99	1.55

<sup>a</sup> Sugars. <sup>b</sup> Amino acids. <sup>c</sup> Organic acids. <sup>d</sup> Sugar phosphates.

the Hagerstown soil plants resembled those from plants exposed to indole. However, subsequent chromatographic analysis, performed on methanol and  $\text{NH}_4\text{OH}$  extracts from both soil types, failed to provide direct supporting



**Figure 9.** Partitioning of indole equivalents/gram dry weight  $\pm$  SD ( $N = 6$ ) of tissue as determined by  $^{14}\text{C}$  in plants grown for 30 days in Palouse (A) or Hagerstown (B) soil amended with 50 ppm  $^{14}\text{C}$  indole (0.37 MBq). Plants were given either distilled water plus extra nitrogen (5.0 mL of 0.625 M  $\text{NH}_4\text{NO}_3$  each week) (+N) or distilled water alone (-N).

evidence for this idea when neither indole nor tryptophan could be identified.

## DISCUSSION

**Plant Uptake of Indole from Solutions and Metabolic Fate.** Indole, as an exogenous xenobiotic and potential pollutant, was readily available for root absorption from both nutrient solutions and soil, even though apparently tightly bound to the soil. The rapid uptake by the root and the subsequent tissue distribution patterns of  $^{14}\text{C}$  derived from  $^{14}\text{C}$  indole indicated that either a specific carrier(s) for either indole or a structurally analogous molecule was (were) present within the root or that indole was quickly transformed prior to entry.

Extraction and analysis of the plant tissues showed that absorbed indole was accumulated as both soluble tryptophan and its polymers, protein and structural materials. Widholm (1974, 1981) has shown that cultured tobacco and carrot cells are capable of taking up indole and indole analogues and subsequently synthesizing tryptophan and corresponding tryptophan analogues. However, this uptake and synthesis has not been shown in intact plants.

The synthesis of tryptophan in plants and microorganisms is via the shikimic acid pathway (Mifflin and Lea, 1982), with the terminal step being catalysis by tryptophan synthase: 1-(indol-3-yl)glycerol 3-phosphate + L-serine  $\rightarrow$  L-tryptophan + D-glyceraldehyde 3-phosphate. The overall process may be considered as the sum of two separate reactions: (a) 1-(indol-3-yl) glycerol 3-phosphate  $\rightarrow$  indole + D-glyceraldehyde 3-phosphate and (b) indole + L-serine  $\rightarrow$  L-tryptophan +  $\text{H}_2\text{O}$ . Reactions a and b are known to be catalyzed by two active protein subunits of the tryptophan synthase enzyme molecule (Delmer and Mills, 1968). The component that catalyzes reaction b using free indole was thought to be of minor significance in plants and microorganisms because free indole was not usually observed in vitro (Gilchrist and Kosuge, 1980). More recent reports, however, indicate that while this pathway may be more important in yeast than was previously thought (Bailey and Turner, 1983), it has not been observed in higher plants. Higher plants were instead believed to quickly transform indole-containing compounds to indole alkaloids or to derivatives of indole 3-acetic acid (IAA), a primary plant hormone (Mazelis, 1980). In these experiments no IAA was detected when plant extracts were compared with known standards. Instead, free indole was found in freshly harvested roots and shoots in the plants exposed to the higher concentrations ( $>25$  ppm), which indicates that the molecule is capable of entering the symplastic compartment. However, the low indole levels detected in these plants, along with the rapid loss following exposure and the lack of direct release to the atmosphere through volatilization from the leaves, indicates that rapid metabolic processing of the material had occurred.

It was not possible to determine whether the specific site of action for the rapid uptake/chemical alteration of the indole from solution was located either at the root surface or within the root itself. Results indicating that roots were capable of modifying the free indole in the solution surrounding them, and that the rate of this modification increased with subsequent exposures, suggests that an inducible system may have already been present in or on the root, either in the microflora of the rhizosphere or in the epidermal tissue. External processing of all of the indole to tryptophan by rhizosphere microbial populations and tryptophan's subsequent absorption was discounted when the partitioning patterns for the two compounds were shown to differ over the same time period.

**Plant Uptake and Partitioning of Indole from Soil.** The processes involved become much more difficult to interpret in the soil/plant system. Even without plants in the two diverse soil systems chosen, free indole was rapidly altered or sorbed. This rapid and persistent sorption of indole to the soil was apparently modified in part by microbial activity in the Hagerstown soil and partly by possible physical and chemical phenomena.

The adsorption of the indole to the soil may have been caused by either physical binding (e.g., van der Waals forces, nonionic materials), chemical reactions (e.g., ion-exchange, protonation), hydrogen bonding, or the formation of coordination complexes. In compounds such as substituted phenols, hydrogen bonding has been implicated in sorptive strength (Boyd, 1982), while binding of nonpolar polynuclear aromatics such as anthracene, with high octanol/water partitioning coefficients, may be physical in nature (Sims and Overcash, 1983). Aliphatic amines, alcohols, and heterocyclic ring complexes have been observed to form coordinate complexes (Kaufmann, 1983). Indole, a neutral aromatic heterocyclic compound, may be bound in this manner, but determination of the

actual mechanism was not undertaken in this study.

This sorption of indole to soil did not prevent the plants from rapidly removing or desorbing the indole (indole-derived label) from both soils, where uptake was observed for at least 120 days after indole was amended to the soil. Further, there may be significant interactions between the plant root and the soil environment that can alter the rate of uptake and the fate of indole.

The organic content of the Palouse soil was higher than that of the Hagerstown (1.8% vs 0.41%). High organic carbon contents have been reported to correlate with high absorptive capacity and, therefore, high octanol/water partitioning capacity in soils (Sims and Overcash, 1983). The lower organic content in the Hagerstown soil may, therefore, have been influential in the apparent greater indole plant availability. This availability, coupled with the high rates of protein synthesis and storage in the foliage of soybeans (Franceschi and Giaquinta, 1983) at this time, may have accounted for the partitioning patterns observed in these plants.

The higher organic matter content of Palouse soil may also have led to increased metabolic transformation, or changes in the bioavailable form of indole. The correlation between the distribution patterns from tryptophan solution uptake studies and those of the Palouse-grown plants may indicate that the material available to the latter plants was in a form closer to tryptophan than its precursor indole.

#### CONCLUSIONS

It is apparent that exogenously supplied indole is readily able to be absorbed and metabolized by higher plants to form the amino acid tryptophan. This reflects the physiological ability/need for plants to sequester and process exogenous xenobiotics through normally available metabolic pathways. The modified chemical species, in this instance tryptophan, may then be partitioned within the plant along patterns that reflect the nitrogen nutritional status of the plant at that time.

It is also apparent that variations in uptake rate and partitioning of indole may occur between plants of the same species depending upon the soil conditions in which they are grown. This observation may be highly significant in any attempt to develop generalized models of xenobiotic behavior in the environment.

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**Registry No.** CO<sub>2</sub>, 124-38-9; indole, 120-72-9; L-tryptophan, 73-22-3.

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