# Metabolism of Isopropyl 3-Chlorocarbanilate by Soybean Plants

Gerald G. Still* and Eugene R. Mansager

Root-treated soybean plants absorb, translocate, and metabolize $^{14}C$-labeled isopropyl 3-chlorocarbanilate (CIPC-$^{14}C$ or chlorpropham-$^{14}C$). Polar products and insoluble residues are rapidly formed in the roots. Insoluble residues are not found in the shoots. Time-course experiments showed that CIPC was lost from the treating solution by volatilization. Pulse time-course experiments demonstrated a precursor–product relationship between CIPC, polar products, and insoluble residues. Polar metabolites are not translocated once they are formed in either root or shoot tissues. The radiocarbon distribution patterns of phenyl-$^{14}C$ and isopropyl-$^{14}C$ labeled CIPC were identical in all comparative experiments. Polar metabolites and insoluble residues from plants treated with isopropyl-$^{14}C$ labeled CIPC were hydrolyzed and steam distilled. The isolated alcohol was identified as 2-propanol. All of the radiocarbon in the soluble metabolites was recovered as 2-propanol. These data demonstrate that the CIPC carbanilate bond was not cleaved and that the isopropyl group was not altered by the plant.

For over 20 years isopropyl 3-chlorocarbanilate (chlorpropham or CIPC) has been used as a highly selective preemergence and early postemergence herbicide which effectively controls many annual grassy and broad leaf species. In spite of the many years of agricultural acceptance and much research effort, relatively little is known about its biochemical selectivity.

CIPC metabolism by soils has been reported by Kaufman (1967). Kearney (1966) demonstrated the conversion of CIPC-$^{14}C$ to $^{14}CO_2$ in soil perfusion columns. A soil microbial enzyme has been shown to hydrolyze CIPC to yield 3-chloroaniline (Kearney, 1965). Grunow *et al.* (1970) studied the metabolism of CIPC in rats and isolated and characterized the metabolites found in urine. The most important catabolic products of CIPC metabolism were found to be the hydroxylation of the para position of the aromatic nucleus, hydrolysis of the carbamate bond, and oxidation of the 2-propanol ester side chain. These metabolites were found in the urine as sulfate and glucuronide conjugates.

The plant metabolism of CIPC has been reported by several investigators (Hodgson, 1967; Prendeville *et al.*, 1968; James and Prendeville, 1969). Hodgson (1967) reported the cleavage of CIPC and the formation of polar metabolites by root-treated soybean plants. Prendeville *et al.* (1968) and James and Prendeville (1969) demonstrated the formation of water-soluble metabolites as a result of plant metabolism. However, their polar metabolites were shown to be β-glucosides of a modified CIPC molecule in which there was no cleavage of the carbamate bond. They concluded that the water-soluble metabolite from foliar treated smartweed, pigweed, tomato, and parsnip resulted from modification of the 2-propanol ester portion of the CIPC molecule.

This paper describes time-course studies that demonstrate a precursor product relationship between CIPC, the polar metabolites, and the insoluble residual materials. Analysis of the polar metabolites and the insoluble residual materials indicated that neither the 2-propanol ester moiety nor the carbamate bond of CIPC was altered by root-treated soybean plants.

Plant Science Research Division, ARS, United States Department of Agriculture, Metabolism and Radiation Research Laboratory, State University Station, Fargo, North Dakota 58102

## EXPERIMENTAL

**Plant Material and Treatment.** Soybeans [*Glycine max* (L.) Merr.] variety Hawkeye seeds, were germinated between moist paper towels at 25° C in the dark. The 4-day-old seedlings were transferred to pint jars (two seedlings/jar) containing Hoagland's solution and grown in an environmental chamber (Still, 1968). A 14-hr day at 27° C and a 10-hr night at 23° C was used with a relative humidity of approximately 54% with $1.72 \times 10^4$ one time at the leaf surface. The plants were allowed to grow until the third trifoliate was approximately one-half expanded (usually 5 to 6 days after being placed in the environmental chamber) when the time-course experiments were initiated. This consisted of replacing the pint jar with 400 g of one-third strength Hoagland's solution, which included the radio-labeled substrate. The long-term time-course experiment was carried out without any change of the aerated substrate solution and with only the addition of distilled water to maintain the nutrient solution level. Both of the pulse time-course experiments (short-term and long-term) were initiated in the same fashion and terminated by replacing the nutrient solution with a jar of fresh Hoagland's solution. In the long-term pulse experiment after the CIPC-$^{14}C$ treatment the soybean plants were transplanted into vermiculite and subirrigated with one-third strength Hoagland's solution. In all cases, upon initiation of the treatment period, 400-g samples of substrate solution were assayed for radiocarbon content. This procedure was repeated at the termination of the experiment to quantitate the loss of CIPC-$^{14}C$ either to the plants or through volatilization of the substrate from the aqueous solution.

At the termination of each time segment of the time-course experiments the plants were removed from the nutrient solution, the roots blotted to remove excess moisture, and sectioned. In the long-term time-course and the short-term pulse experiments the plants were divided into the roots and shoots. In the long-term pulse time-course experiment the plants were sectioned to yield root, primary leaf, stem sections 1 and 2, trifoliates 1 through 8, the pods, and the seeds. Stem section 1 is defined as the stem present during CIPC-$^{14}C$ treatment, and stem section 2 is the stem that emerged after removal of the $^{14}C$ substrate. In all experiments, the plant sections were freeze-dried and stored dry at freezer temperatures until analysis. In each experiment, at least four plants (two jars) were sampled at each harvest. The radiochemical content of each plant section was assayed.

Table I. CIPC-Phenyl-$^{14}C$ Inventory in Soybean

| Days treatment | Percent of total $^{14}C$ in system: Root plus shoots | Nutrient solution | Plant tissue plus nutrient solution | Volatility study, nutrient solution |
|---|---|---|---|---|
| 0 | 0 | 100.0 | 100.0 | 100.0 |
| 1 | 19.8 | 78.4 | 98.2 | |
| 2 | 25.0 | 61.1 | 86.1 | |
| 3 | 33.3 | 57.3 | 90.6 | 88.7 |
| 4 | 57.1 | 20.3 | 77.4 | |
| 6 | 49.7 | 20.8 | 70.5 | 65.4 |
| 8 | 49.7 | 10.2 | 59.9 | |
| 10 | 57.4 | 6.3 | 63.7 | 61.4 |
| 12 | 53.3 | 7.3 | 60.6 | |
| 14 | 64.6 | 1.9 | 66.5 | |
| 16 | 55.4 | 1.3 | 56.7 | 47.5 |

**Extraction of Plant Material.** The extraction procedure used was a modification of the methods described by Bligh and Dyer (1959). Plant material (200 mg, dry weight) was mixed at high speed in an Omnimixer for 5 min (with the cup cooled in an ice bath) with 20 ml of extraction solution, methanol:chloroform:water (2:1.0:8). At the end of the 5-min mixing period the contents of the cup were filtered through Whatman #4 and the filter paper contents were extracted in the same fashion with a second 20-ml portion of the extracting solution. After a second 5-min mixing the contents were again filtered and the filtrates combined in a separatory funnel. Chloroform (10 ml) and 10 ml of water were added to the separatory funnel, which yielded a two-phase system. On occasion the sharpness of the interface between the solutions could be enhanced by the addition of small amounts of methanol. The chloroform layer and the polar (methanol–water) layer were assayed for radiocarbon. The insoluble filtered material was dried and combusted by the oxygen flask technique for radiocarbon assay.

**Radiochemical Assays.** Quantitative measurements of radiocarbon were made on a liquid scintillation spectrometer. All samples were counted as homogeneous solutions in either toluene or alcohol toluene scintillation cocktails. Each sample was corrected for quenching by external standardization. Radiochemical assay of CIPC-$^{14}C$ treating solutions was accomplished by transferring the treating solution into a 500-ml liquid–liquid extractor and extracting for 48 hr with ethyl ether. The ether extract was assayed by liquid scintillation spectrometry.

All particulate samples, such as the insoluble residues and the isopropyl benzoates, were dried and combusted using the oxygen combustion flask method described by Kelly *et al.* (1961) as modified by Oliverio *et al.* (1962). The $^{14}CO_2$ from these combustions was assayed by liquid scintillation spectrometry.

**Purification of CIPC Radiochemical Substrate.** CIPC-phenyl-$^{14}C$ and CIPC-2-isopropyl-$^{14}C$ were purified by preparative thin-layer chromatography. The impure substrate was streaked on 250-$\mu$ silica gel HF thin layers and developed to 15 cm with benzene:acetic acid (50:4). The CIPC and other impurities were visualized in bands under ultraviolet light and $^{14}C$ bands were located by use of a windowless Geiger counter. The CIPC band was scraped from the plates, eluted from silica gel with solvent, and concentrated. To confirm the substrate purity two-dimensional thin-layer chromatography on silica gel was employed, using first ethyl acetate:acetic acid:water (23:1:1) and second benzene:acetic acid (50:4). In all cases a single spot was observed after autoradiography before the substrate was considered chemically pure.

The radiochemical purity was assayed by caustic hydrolysis, steam distillation, and assay of either the 3-chloroaniline or the 2-propanol portions of the molecule. In all cases the substrates were found to be specifically labeled in either the phenyl nucleus or the alcohol ester moiety.

The specific activity of CIPC-phenyl-$^{14}C$ was 1.27 mCi/mmol and CIPC-2-isopropyl-$^{14}C$ was 1.13 mCi/mmol. Where comparative experiments between specifically labeled CIPC-$^{14}C$ were required, cold substrate was added to the CIPC-phenyl-$^{14}C$ to yield a specific activity of 1.13 mCi/mmol.

CIPC-$^{14}C$ was dissolved in chloroform and layered on the bottom of carboys under water. The chloroform was removed by a stream of air at room temperature with stirring. All of the experiments were performed using 400 g of specifically labeled substrate solution for each pint jar, with two plants in each jar. The concentrations of radio-labeled CIPC used were from 0.3 $\mu$Ci (0.266 $\mu$mol) to 0.5 $\mu$Ci (0.443 $\mu$mol) per 400 ml of Hoagland's solution ($0.67 \times 10^{-6}$ to $1.11 \times 10^{-6}$ *M*, CIPC). The water solution of substrate was prepared as Hoagland's solution by addition of the appropriate salts just prior to execution of the experiment. The specific activity was determined for each aqueous solution preparation.

## RESULTS

**Long-Term Time-Course.** Long-term time-course experiments were designed to determine the rate of CIPC-$^{14}C$ absorption from nutrient solution into the root and translocated into shoot tissues of soybean plants. The percentage loss of CIPC-$^{14}C$ from the nutrient solution was determined in concurrent volatility experiments, so a complete inventory of radiocarbon could be obtained. Table I reports the results of a 16-day experiment. Plants were supplied with CIPC-phenyl-$^{14}C$ for 16 days. During this period, radiocarbon levels in the plant tissue increased with a concomitant decrease of radiocarbon in the nutrient solution to 1.3% at 16 days. Volatility studies show that the radiocarbon in the nutrient solution decreased to 47.5% of the original concentration at the end of 16 days. This loss of CIPC-$^{14}C$ from the Hoagland's solution agrees well with Parochetti and Warren (1966) and the Battelle-Northwest report (1968). At 3 days, 33% of the total radiocarbon was incorporated into the plant tissues, with 57% remaining in the nutrient solution. The volatility study showed a loss of approximately 10%, which is in good agreement with the total recovery of 91% found in the entire system.

**Short-Term Pulse Time-Course.** CIPC-Phenyl-$^{14}C$ and CIPC-2-isopropyl-$^{14}C$ were supplied to soybean plants for 16 hr, at which time the radio-labeled feeding solutions were replaced with fresh nutrient solution. At the end of 24 hr the fresh nutrient solution was replaced and assayed for radiocarbon. Both the phenyl- and 2-isopropyl-labeled substrates behaved identically in the treating solutions and the plant tissues. As an example of the movement of CIPC-$^{14}C$ in the short-term pulse experiments, an inventory of the CIPC-phenyl-$^{14}C$ treated tissue is reported in Table II. At the end of the 16-hr feeding period 20% of the radiocarbon was not accounted for. This loss was due to volatility as substantiated in the previous volatility experiments. Between 16 and 24 hr there was $50.8 \times 10^3$ dpm of CIPC leached from the roots into the nutrient solution. This may be explained by the movement of CIPC-$^{14}C$ from the free

Table II. Inventory of CIPC-Phenyl-$^{14}C$

Soybean root-treated 16 hr, dpm × $10^3$

| Hr | Hoagland's solution CIPC-$^{14}C$ | Plants Root plus shoot | $^{14}C$ not accounted for dpm | $^{14}C$ not accounted for % of total |
|---|---|---|---|---|
| 0[a] | 1550.0 | 0.0 | 0.0 | 0.0 |
| 16[b] | 1063.8 | 174.0 | 312.2 | 20.1 |
| 24[b] | 50.8 | 108.3 | 14.9 | 8.6 |
| 40 | | 103.4 | | |
| 48 | | 95.7 | | |
| 64 | | 92.5 | | |

[a] Extraction and radiochemical assay of the zero-time sample yielded 100% recovery of CIPC-$^{14}C$ added to the system. [b] The CIPC-phenyl-$^{14}C$ Hoagland's solution was removed and replaced with fresh Hoagland's solution after 16 and 24 hr.

spaces of the root tissue back into the nutrient solution, as described by Moody *et al.* (1970). The second nutrient solution sample also showed the same volatility effects observed in the previous sample and accounted for an 8.5% loss during this 8-hr period. The movement of radiocarbon from the plant tissues to the nutrient solution was also reflected by the decrease in the radiocarbon content of the plant tissues with time. The extraction technique separated the unaltered CIPC-$^{14}C$ into the chloroform fraction, the water-soluble metabolites into the polar fraction, and left the insoluble residues. Table III reports the data from soybean plants treated with either CIPC-phenyl-$^{14}C$ or CIPC-2-isopropyl-$^{14}C$. A comparison of the distribution of radiocarbon in the root and shoot extracts shows little variation between the specifically-labeled substrates. This close similarity between the alcohol and phenyl-$^{14}C$ labeled substrates indicated that the carbamate bond of CIPC-$^{14}C$ was not cleaved by soybean plants. If the CIPC-$^{14}C$ molecule remained intact in the short-term pulse experiments, we may analyze our data as a summation of several experiments. Figure 1 is an average of several short-term pulse experiments using either CIPC-phenyl-$^{14}C$ or CIPC-2-isopropyl-$^{14}C$ as substrate. From 0 to 16 hr the nutrient solution was labeled. After this time CIPC-$^{14}C$-free nutrient solution was used. The clear areas of Figure 1 represent light periods and the crosshatch areas between 16 and 24 hr and 40 to 48 hr represent dark periods. Analysis of the data

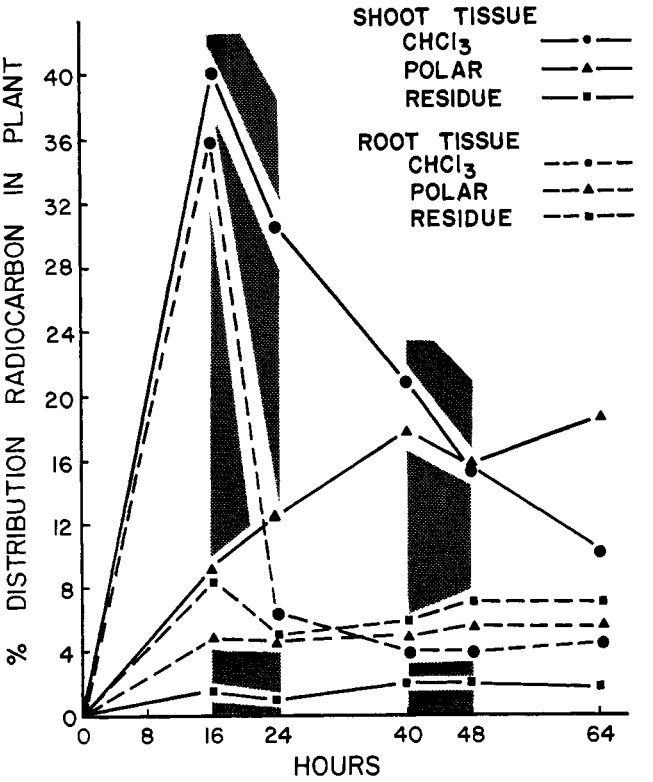


**Figure 1. Short-term pulse time-course. The distribution of $^{14}C$ between CIPC-$^{14}C$, polar metabolites, and insoluble residues (Bligh–Dyer extractions) in soybean root and shoot tissues. The data are an average of several experiments using either CIPC-phenyl-$^{14}C$ or CIPC-2-isopropyl-$^{14}C$ as substrate. One hundred percent is based on the $^{14}C$ content in root and shoot tissues at the end of the treating period**

from root tissue reflects the loss of CIPC-$^{14}C$ to the nutrient solution between 16 and 24 hr (Table II). After 24 hr a precursor–product relationship was evident between the water-soluble and insoluble residual products. The highest content of $^{14}C$-insoluble residual products appeared in the root tissues.

Shoot tissues showed a similar precursor–product relationship between 16 and 64 hr with the disappearance of the CIPC-$^{14}C$ and the appearance of the polar metabolites.

Table III. Percent Distribution of Radiocarbon in Bligh–Dyer Extracts and Residues of Soybean Plants, Root-Treated 16 hr with CIPC-$^{14}C$

| Hr | Root $CHCl_3$ | Root Polar | Root Res. | Shoot $CHCl_3$ | Shoot Polar | Shoot Res. | Summation of groups[a] Root | Shoot | Root plus shoot | Nutrient[b] solution |
|---|---|---|---|---|---|---|---|---|---|---|
| **CIPC-phenyl-$^{14}C$** | | | | | | | | | | |
| 16 | 33.1 | 4.5 | 5.9 | 44.7 | 10.4 | 1.4 | 43.5 | 56.5 | 100.0 | |
| 24 | 7.9 | 4.7 | 4.8 | 31.3 | 12.6 | 0.9 | 17.4 | 44.8 | 62.2 | 37.8 |
| 40 | 6.1 | 5.7 | 5.5 | 22.1 | 18.0 | 1.9 | 17.3 | 42.0 | 59.3 | 2.9 |
| 48 | 5.4 | 5.4 | 7.1 | 16.0 | 18.2 | 2.8 | 17.9 | 37.0 | 54.9 | 4.4 |
| 64 | 5.9 | 5.7 | 6.7 | 10.5 | 22.3 | 2.2 | 18.3 | 35.0 | 53.3 | 1.6 |
| **CIPC-2-isopropyl-$^{14}C$** | | | | | | | | | | |
| 16 | 41.3 | 5.5 | 4.0 | 40.2 | 8.1 | 0.9 | 50.8 | 49.2 | 100.0 | |
| 24 | 5.1 | 5.8 | 4.2 | 35.0 | 12.8 | 0.9 | 16.1 | 48.6 | 64.7 | 35.3 |
| 40 | 2.9 | 5.6 | 5.3 | 19.7 | 20.0 | 2.2 | 13.8 | 41.9 | 55.7 | 9.0 |
| 48 | 3.0 | 6.3 | 6.2 | 15.6 | 16.7 | 1.5 | 15.5 | 33.8 | 49.3 | 6.4 |
| 64 | 3.6 | 6.0 | 6.5 | 11.2 | 21.2 | 1.5 | 16.1 | 33.9 | 50.0 | 0.0 |

[a] $^{14}C$ incorporation at 16 hr is equal to 100% radiocarbon content of the plants. [b] Nutrient solution assayed for $^{14}C$ concentration to show loss is due to volatility.

Table IV. Percent Distribution of Radiocarbon Incorporated into Soybean Plants Pretreated for 3 Days with CIPC-phenyl-$^{14}C$ or CIPC-2-isopropyl-$^{14}C$

| Plant segment assayed | Days after pretreatment | | | | | | | |
|---|---|---|---|---|---|---|---|---|
| | 0 | | 3 | | 11 | | 28 | |
| | Phenyl | Isopropyl | Phenyl | Isopropyl | Phenyl | Isopropyl | Phenyl | Isopropyl |
| Root | 34.3 | 33.7 | 40.3 | 33.3 | 38.8 | 30.9 | 34.8 | 36.0 |
| Primary leaf | 10.5 | 13.9 | 23.7 | 21.6 | 12.1 | 21.0 | 21.0 | 27.9 |
| Stem 1 | 18.5 | 17.4 | 12.2 | 11.4 | 11.7 | 13.9 | 10.8 | 12.8 |
| Stem 2 | | | | | 0.3 | 0.4 | 0.2 | 0.0 |
| Trifoliate 1 | 11.9 | 14.0 | 13.7 | 17.6 | 16.3 | 16.1 | 16.8 | 11.1 |
| Trifoliate 2 | 20.0 | 17.2 | 7.0 | 9.5 | 13.7 | 12.3 | 8.3 | 5.0 |
| Trifoliate 3 | 4.2 | 3.3 | 2.1 | 5.5 | 5.4 | 4.2 | 5.0 | 2.8 |
| Trifoliate 4 | 0.3 | 0.2 | 0.4 | 0.7 | 1.0 | 0.7 | 1.6 | 3.3 |
| Trifoliate 5 | | | 0.3 | 0.2 | 0.4 | 0.2 | 0.6 | 0.4 |
| Trifoliate 6 | | | | | 0.0 | 0.0 | 0.4 | 0.2 |
| Trifoliate 7 | | | | | 0.0 | 0.0 | 0.1 | 0.1 |
| Trifoliate 8 | | | | | | | 0.0 | 0.0 |
| Seed pod | | | | | | | 0.0 | 0.0 |
| Fruit | | | | | | | 0.0 | 0.0 |

Table V. Average Total Dpm $\times 10^4$ Found in Soybean Segments, Root-Treated CIPC-phenyl-$^{14}C$ or CIPC-2-isopropyl-$^{14}C$ for a 3-Day Pretreatment[a]

| Plant segment assayed | Days after pretreatment | | | |
|---|---|---|---|---|
| | 0 | 3 | 11 | 28 |
| Root | 20.40 | 16.75 | 16.22 | 15.20 |
| Primary leaf | 7.40 | 10.35 | 7.48 | 7.80 |
| Stem 1 | 10.75 | 5.40 | 5.87 | 5.10 |
| Stem 2 | | | 0.16 | 0.05 |
| Trifoliate 1 | 7.85 | 7.18 | 7.50 | 5.82 |
| Trifoliate 2 | 11.10 | 3.80 | 6.03 | 2.74 |
| Trifoliate 3 | 2.25 | 1.77 | 2.25 | 1.64 |
| Trifoliate 4 | 0.18 | 0.24 | 0.42 | 1.12 |
| Trifoliate 5 | | 0.12 | 0.15 | 0.22 |
| Trifoliate 6 | | | 0.00 | 0.12 |
| Trifoliate 7 | | | 0.00 | 0.04 |
| Trifoliate 8 | | | | 0.00 |
| Seed pod | | | | 0.00 |
| Fruit | | | | 0.00 |

[a] All dpm data from oxygen combustion of each sample.

However, only a very small amount of insoluble residue formed when compared to root tissue. In root tissue, light had no effect upon the metabolism of CIPC. For photosynthetic tissue, the slope of the CIPC-$^{14}C$ disappearance curve appeared to be a function of light or dark. The significance of this observation is not known, but could have implications with regard to the coupling of the catabolism of CIPC and photosynthesis.

**Long-Term Pulse Time-Course.** These experiments were designed to follow the movement of CIPC-$^{14}C$ from the root into the shoot tissues and to determine if there was movement of the water-soluble materials from the leaf tissues into the newly emerging leaf tissues or the fruits of the soybean plant. Soybean roots were exposed to either CIPC-phenyl-$^{14}C$ or CIPC-2-isopropyl-$^{14}C$ both at 1.13 mCi/mmol (0.5 $\mu$Ci or 0.443 $\mu$mol CIPC-$^{14}C$/two plants/400 ml Hoagland's solution) for a 3-day pretreatment period at which time the nutrient solution was exchanged with fresh nutrient solution. The movement of the radiocarbon throughout the plant was followed by sampling the plants at 0 days (end of 3-day feeding period), 3, 11, and 28 days after the removal of the labeled substrate from the nutrient solution. At harvest the plants were sectioned into root, primary leaf, trifoliates 1 through 8, pod and seed, and stem section 1 and stem section 2. Each plant segment was assayed separately. The percentage distribution of radiocarbon incorporated into the soybean plants is found in Table IV. As was found with the short-term pulse experiments there was no difference between the specifically labeled CIPC-$^{14}C$ substrates. This indicates that the carbamate bond is not cleaved as a result of soybean metabolism.

Because the radioactivity from both specifically labeled substrates was absorbed and translocated identically, and because both substrates had the same specific activity, the radiocarbon distribution is reported as an average of both labeled substrates (Table V). The small decrease in root radiocarbon content of the roots with increasing posttreatment time reflects the movement of CIPC-$^{14}C$ from the root into the fresh nutrient solution (0 day to 3-day posttreatment) and/or the translocation to the aerial portions of the plant. After the 3-day posttreatment period the root, primary leaf, and stem segments reached a constant radiocarbon content, suggesting that the labeled material must be incorporated into the tissues, reducing the mobility of radiocarbon. Trifoliates 1 through 3 generally decreased in radiocarbon content throughout the experiment with the concomitant increase in radiocarbon in trifoliates 4 through 7. The absence of radiocarbon label in trifoliate 8 and in the pod and seed tissues indicates that there must be little translocation of radiocarbon from the older tissues into the newly emerging trifoliates or fruit tissue.

To determine the chemical character of the radiocarbon components found in each plant segment, at each posttreatment sampling the radio-labeled tissues from CIPC-phenyl-$^{14}C$ or CIPC-2-isopropyl-$^{14}C$ treated tissues were extracted as before. The CIPC-phenyl-$^{14}C$ and the CIPC-2-isopropyl-$^{14}C$ distribution patterns were identical so the data were combined and normalized (Table VI). At 28 days root tissues had the largest percent of radiocarbon found in any of the plant parts (Table V). From Table VI it is seen that the radiocarbon in root was principally in the polar metabolites and the insoluble residues. The greatest amount of activity in the insoluble residue was found in root tissues, while the trifoliate leaves were void of any $^{14}C$ labeling in this fraction. As was seen in the short-term pulse experiment, a precursor–product relationship exists between parent CIPC (chloroform extract), the polar metabolites (water–methanol extract), and the solid residues in root, primary leaf, and stem tissues. The same precursor product relationship exists in the trifoliate tissues with the exception that the

Table VI. Percent Distribution of Radiocarbon in the Bligh–Dyer Extraction of Soybean Plant Segments, Pretreated for 3 Days with Either CIPC-phenyl-$^{14}C$ or CIPC-2-isopropyl-$^{14}C$. Data Normalized to 100%

| | Chloroform extract days posttreatment | | | | Polar extract days posttreatment | | | | Solid residue days posttreatment | | | |
|---|---|---|---|---|---|---|---|---|---|---|---|---|
| | 0 | 3 | 11 | 28 | 0 | 3 | 11 | 28 | 0 | 3 | 11 | 28 |
| Root | 3.4 | 0.4 | 0.0 | 0.0 | 3.7 | 6.3 | 4.7 | 3.8 | 1.9 | 2.7 | 4.2 | 5.0 |
| Primary leaf | 0.8 | 0.0 | 0.0 | 0.0 | 4.0 | 4.6 | 4.8 | 4.1 | 0.0 | 0.2 | 0.0 | 0.7 |
| Stem 1 | 2.0 | 0.2 | 0.0 | 0.0 | 1.5 | 3.2 | 3.1 | 3.2 | 0.0 | 0.2 | 0.4 | 0.2 |
| Stem 2 | | | N[a] | N | | | N | N | | | N | N |
| Trifoliate 1 | 2.1 | 0.4 | 0.0 | 0.0 | 1.8 | 3.4 | 3.7 | 3.7 | 0.0 | 0.0 | 0.0 | 0.0 |
| Trifoliate 2 | 2.0 | 0.9 | 0.0 | 0.0 | 1.0 | 2.2 | 3.0 | 3.0 | 0.2 | 0.0 | 0.0 | 0.0 |
| Trifoliate 3 | 0.6 | 0.3 | 0.0 | 0.0 | 0.7 | 0.9 | 1.1 | 1.1 | 0.1 | 0.0 | 0.0 | 0.0 |
| Trifoliate 4 | 0.0 | 0.0 | 0.0 | 0.0 | 0.2 | 0.2 | 0.3 | 0.3 | 0.0 | 0.0 | 0.0 | 0.0 |
| Trifoliate 5 | | N | N | N | | N | N | N | | N | N | N |
| Trifoliate 6 | | | 0.0 | N | | | 0.0 | N | | | 0.0 | N |
| Trifoliate 7 | | | 0.0 | N | | | 0.0 | N | | | 0.0 | N |
| Trifoliate 8 | | | | 0.0 | | | | 0.0 | | | | 0.0 |
| Pod | | | | 0.0 | | | | 0.0 | | | | 0.0 |
| Seed | | | | 0.0 | | | | 0.0 | | | | 0.0 |

[a] N = The tissue sample did not contain enough radiocarbon to carry out the Bligh–Dyer extraction and assay.

photosynthetic tissues do not form the insoluble residues. At the end of the feeding period (0 days posttreatment) parent CIPC-$^{14}C$ was found in all of the tissues with the exception of the expanding trifoliate 4. The data for the 3-day posttreatment sample demonstrate the movement of CIPC-$^{14}C$ from the root to the foliar tissues. In root and primary leaf tissue the polar metabolites increase in amount to a maximum between 3 and 11 days and then decrease, with a concurrent increase in the amount in the insoluble residues.

Data (Table VI) from trifoliate tissues support the argument for the movement of CIPC into the polar metabolite pool. At the 0 day sample time, trifoliates 1 through 3 contain 4.7% of the radiocarbon as CIPC-$^{14}C$ and 3.5% as polar metabolites. During the next 3-day period there was a 2.9% decrease in the CIPC-$^{14}C$ concentration with a 3.0% increase in the polar metabolites in these leaves. By the end of the next 8-day period, the CIPC-$^{14}C$ concentration had reached 0 (−1.6%) and the polar metabolite pool was increased by 1.3% which remained constant through the next 17-day period. The close correlation between the CIPC-$^{14}C$ catabolism and polar metabolite metabolism is in good agreement with the precursor–product hypothesis.

The polar metabolite concentration never decreased in the trifoliate leaves during the time course. If these polar metabolites were mobile and could be translocated to the newly-emerging tissues, one would expect to find a decrease in the concentration of the water-soluble metabolites in these leaves. Therefore, the radiocarbon contribution found in trifoliates 5 through 7 must result from translocation of CIPC-$^{14}C$ from the root or stem tissues and not from mobilization of 'radio-labeled materials from the trifoliate tissues. This conclusion is also supported by the absence of radiolabel in the fruit tissues.

**Isolation of 2-Propanol-$^{14}C$ from Bligh–Dyer Water-Soluble Metabolites and the Insoluble Residues.** The data from the pulse experiments indicated that the CIPC carbamate bond was not cleaved in soybean. The question remains whether the chloroaniline moiety or the 2-propanol ester half of the carbanilate is altered to yield the polar and insoluble metabolites. Bligh–Dyer purified polar metabolites and insoluble residue metabolites from tissues root-fed CIPC-phenyl-$^{14}C$ and CIPC-2-isopropyl-$^{14}C$ were subjected to caustic hydrolysis and steam distillation. Those tissues fed CIPC-phenyl-$^{14}C$ yielded no steam distillable materials from either the polar metabolites or the insoluble residue.

Tissues from plants treated with CIPC-2-isopropyl-$^{14}C$ yielded nearly quantitative recovery of radiocarbon. Preliminary investigations led us to believe that this distillable radiocarbon was 2-propanol-$^{14}C$. To substantiate this assumption the unknown steam distillable radiocarbon sample was diluted with 2-propanol. The alcohol was twice distilled, the boiling range of 79.0 to 80.0° C collected, and its specific activity determined. The purified 2-propanol was derivatized with 3,5-dinitrobenzylchloride (Cheronis *et al.*, 1965) and the benzoate recrystallized to constant specific activity. The radio-labeled 2-propanol from CIPC polar metabolites yielded an alcohol distillate with a specific activity of 560.0 dpm/mmol. The constant specific activity of the benzoate derivative was found to be 568.8 dpm/mmol. The 2-propanol distillate from the CIPC-insoluble residue was found to have a specific activity of 111.0 dpm/mmol and the benzoate derivative yielded a specific activity of 105.0 dpm/mmol. Because the recovery of radiocarbon was nearly 100% from the hydrolysis and steam distillation of the CIPC-2-isopropyl-$^{14}C$ incorporated material, and because of the close correlation between the specific activities of the diluted alcohols and their 3,5-dinitrobenzoates, it was concluded that the unknown radiocarbon-labeled compound was 2-propanol. This confirmed the fact that the 2-propanol moiety of CIPC was not metabolically altered in the formation of either the water-soluble metabolites or the insoluble residues.

## DISCUSSION

Several investigators have reported the movement of CIPC into seeds (Rieder *et al.*, 1970) through excised petiole sections (Taylor and Warren, 1970). Prendeville *et al.* (1968) showed that CIPC moved in intact plants, but that it did not move basipetally to any degree. Data presented here support these conclusions and confirm not only that the parent CIPC molecule is translocated acropetally but that the polar metabolites are immobile and not translocated from the site of their anabolism. This is evident in the data for trifoliate-8 and for the seed and pod tissues which contained no radioactivity (Table VI). If the polar metabolites of CIPC were translocatable, they would be expected to move into these tissues.

Gard *et al.* (1959) demonstrated the rapid degradation of parent CIPC. Hodgson (1967) reported that CIPC was

cleaved into a water-soluble aniline-containing fraction and an isopropyl fraction, either or both of which could be further metabolized or conjugated by soybean plants. This observation was contrary to the finding of Prendeville *et al.* (1968), who showed that foliar-applied CIPC yielded water-soluble metabolites that were not a result of cleavage of the CIPC molecule, but were conjugates of the intact carbanilate. In further studies James and Prendeville (1969) isolated water-soluble metabolites from smartweed, pigweed, tomato, and parsnip that contained the basic CIPC structure. These metabolites were $^{14}C$-labeled irrespective of whether the CIPC-$^{14}C$ treatment was phenyl- or 2-isopropyl-labeled. The data presented from our pulse time-course studies support the conclusions of Prendeville and James and are in contradiction to the report that CIPC was hydrolzyed by soybean plants. If hydrolysis had occurred and 2-propanol was available to the plant system, one would expect that the alcohol moiety would be metabolized to $CO_2$. Kearney (1966) demonstrated in soil perfusion experiments that CIPC-$^{14}C$ was converted to $^{14}CO_2$ with the alkyl ester moiety appearing prior to the evolution of $^{14}CO_2$ from the carbanilate ring carbons. Prendeville *et al.* (1968) supplied CIPC-2-isopropyl-$^{14}C$ to roots and were not able to detect any appreciable evolution of $^{14}CO_2$. Considering the number of years that this radiocarbon-labeled compound has been studied in plants, one would expect evidence to support the contention that $CO_2$ could be evolved from the 2-propanol ester moiety of CIPC. The total absence of evidence for $^{14}CO_2$ evolution indicates that this carbanilate molecule is not cleaved during plant metabolism.

The formation of polar metabolites from CIPC and 4-chloro-2-butynyl-3-chlorocarbanilate (barban) has been described (Hodgson, 1967; Prendeville *et al.*, 1968; James and Prendeville, 1969; Riden and Hopkins, 1962). The quantitative data from our time-course studies give an inventory of the distribution of CIPC-$^{14}C$ throughout the duration of the experiments. These data show the existence of the precursor product relationship between CIPC, polar metabolites, and the insoluble residual materials. We did not detect any labeled insoluble residues in soybean leaves and neither were they detected in smartweed after foliar treatment (Prendeville *et al.*, 1968).

The polar metabolites from CIPC and barban were reported to yield 3-chloroaniline upon chemical or enzymatic degradation (Riden and Hopkins, 1962; James and Prendeville, 1969). James and Prendeville (1969) concluded from their data that the *N*-hydroxy derivative of CIPC, reported by Baskakov and Zemskaya (1959), could not be a component of their polar metabolites. However, they reported that their water-soluble metabolite must result from the alteration of the 2-propanol moiety of CIPC. From our experiments with root-treated soybean, we found that CIPC-2-isopropyl-$^{14}C$ yielded either a polar metabolite or an insoluble residue. The isolation and characterization of 2-propanol-$^{14}C$ from both the polar and the insoluble residue fractions demonstrates that the alcohol ester moiety of the carbamate is not altered and is present in both of these metabolic pools.

The inability to steam distil the radiocarbon from the CIPC-phenyl-$^{14}C$ labeled analog is indicative of an amphoteric hydrolysis product. This suggests that the most likely biological alteration is an hydroxylation or oxidation of the CIPC phenyl nucleus. The character of the soybean water-soluble and insoluble metabolites will only be defined by isolation and characterization of the intact metabolites.


## ACKNOWLEDGMENT

The authors wish to thank PPG Industries for their contribution of isopropyl-3-chlorocarbanilate-$^{14}C$.


## LITERATURE CITED


Baskakov, Yu. A., Zemskaya, V. A., *Fiziol. Rast.* **6,** 67 (1959).
Battelle-Northwest, Annual Report to USDA, Alterations of the Persistence of Herbicides in Soils, Water, and Plants, Richland, Washington, Dec. 1968, pp 27–30.
Bligh, E. G., Dyer, W. J., *Can. J. Biochem. Physiol.* **37,** 911 (1959).
Cheronis, N. D., Entrikin, J. B., Hodnett, E. M., Semimicro Qualitative Organic Analysis, Interscience Publishers, New York, N. Y., 1965, p 486.
Gard, L. N., Ferguson, Jr., C. E., Reynolds, T. L., J. AGR. FOOD CHEM. **7,** 335 (1959).
Grunow, W., Böhme, Chr., Budczies, Babette, *Food Cosmet. Toxicol.* **8,** 277 (1970).
Hodgson, R. H., Abstr. 1967 Meeting Weed Sci. Soc. Amer., Washington, D.C., Feb. 1967, p 65–66.
James, C. S., Prendeville, G. N., J. AGR. FOOD CHEM. **17,** 1257 (1969).
Kaufman, D. D., J. AGR. FOOD CHEM. **15,** 582 (1967).
Kearney, P. C., *Advan. Chem. Ser.* **60,** 250 (1966).
Kearney, P. C., J. AGR. FOOD CHEM. **13,** 561 (1965).
Kelly, R. G., Peets, E. A., Gordon, S., Buyski, D. A., *Anal. Biochem.* **2,** 267 (1961).
Moody, J., Kust, C. A., Buchholtz, K. P., *Weed Sci.* **18,** 214 (1970).
Oliverio, V. T., Denham, C., Davidson, J. D., *Anal. Biochem.* **4,** 188 (1962).
Parochetti, J. V., Warren, G. F., *Weeds* **14,** 281 (1966).
Prendeville, G. N., Eshel, Y., Jones, C. S., Warren, G. F., Schreiber, M. M., *Weed Sci.* **16,** 432 (1968).
Riden, J. R., Hopkins, T. R., J. AGR. FOOD CHEM. **10,** 455 (1962).
Rieder, G., Buchholtz, K. P., Kust, C. A., *Weed Sci.* **18,** 101 (1970).
Still, G. G., *Plant Physiol.* **43,** 543 (1968).
Taylor, T. O., Warren, G. F., *Weed Sci.* **18,** 64 (1970).



*Received for review March 24, 1971. Accepted April 21, 1971. Presented in part at the Fourth Great Lakes Regional Meeting, American Chemical Society, Fargo, N.D., June 18, 1970. Mention of a trademark or proprietary product does not constitute a guarantee or warranty of the product by the U.S. Department of Agriculture and does not imply its approval to the exclusion of other products that may also be suitable.*