Similarities in the pyrolysis of natural lignin from rice roots and the synthetic lignin copolymers also suggest that the bonding of the chloroanilines may have been similar.

Mechanisms for the generation of soil organic matter have been described (Flaig, 1963). These mechanisms suggest that similar reactions take place between decomposed lignin and the amine groups of amino acids with the formation of humic substances in soil. As with lignin, investigations in soil organic matter present difficult experimental problems which have blocked rapid progress in this field. However, there is precedent for free radical and ionic intermediates which combine lignin and nitrogen components to form soil organic matter. These findings also support the conclusion that lignin, in addition to being an important structural material, also may serve as an effective reservoir for the storage of many aromatic materials in plants and soil biopolymers (Freudenberg and Neish, 1968; Flaig, 1963). Although our data do not exclude the possibility that the test chloroanilines are incorporated into lignin as inclusion products, the data suggest that the chloroaniline residues were associated with the lignin by a chemical bond.

It is the intent of the authors to share these results, pointing out the marginal successes, in an effort to stimulate research that may result in new tools and approaches so that more definitive data will be available in the future and that those social questions that arise due to the environmental involvement of these materials may be more intelligently assessed.

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Residue Analysis of Isopropyl *N*-(3-Chlorophenyl)carbamate in Fruits and Vegetables Using High-Performance Liquid Chromatography

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A high-performance liquid chromatographic method was developed for the determination of residues of isopropyl *N*-(3-chlorophenyl)carbamate (CIPC) in Monona potatoes at levels of 0.25–81 ppm and in beans, peas, and blueberries fortified at 0.25 ppm. CIPC was extracted with methanol, and the extract was cleaned up by being chromatographed through an acid alumina column. The average recoveries from all four commodities ranged from 64 to 102%. A study conducted to test 16 pesticides for possible interferences with CIPC demonstrated that none of the 16 cochromatographed. The lower limit of detection by using this method for beans, peas, potatoes, and blueberries is 0.12 ppm.

Isopropyl *N*-(3-chlorophenyl)carbamate (CIPC) is used primarily as a pre- and postemergence herbicide on a variety of fruit and vegetable crops and as a sprout inhibitor for potato tubers. Its varied use can be attributed to its several modes of action in plants such as the inhibition of shoot and root growth, particularly of the primary roots (Scott and Struckmeyer, 1955; Roberts, 1965; Eshel and Warren, 1967), inhibition of the photolytic activity of the chloroplasts (Moreland and Hill, 1959), inhibition of protein synthesis (Mann et al., 1965), and inhibition of mitosis

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(Ennis, 1949; Scott and Struckmeyer, 1955).

Knowledge of CIPC concentration in stored potatoes is of importance because sprouting could occur if the concentration in the peel falls below 20 ppm (Corsini et al., 1979). Because of the need to monitor concentrations of this chemical during potato storage and to determine concentrations in fresh and stored fruits and vegetables, a fast and accurate method for determination of CIPC residues is needed.

Present methods for the determination of these residues include colorimetric, infrared, and gas chromatographic (GC) procedures. The colorimetric methods involve hydrolyzing CIPC (acid or alkaline hydrolysis) to an aromatic amine which is then measured spectrophotometrically by complexing with the dyes *N*-(1-naphthylethylene)diamine dihydrochloride or N-(ethyl-1-naphthyl)amine (Montgomery and Freed, 1959; Ferguson and Gard, 1969; Ercegovich and Witkonton, 1972; Friestad, 1974). The problems with these methods are that some pesticides like the dimethylureas (monuron, diuron, etc.) interfere and that sample preparation is time consuming. The GC and infrared procedures also involve lengthy sample preparation and/or require derivatization or are not sensitive in the ppb range (Ferguson et al., 1963; Gutenmann and Lisk, 1964; Fishbein and Zielinski, 1965; VanVliet and Hertog, 1966; Corsini et al., 1978).

This paper describes a high-performance liquid chromatographic (HPLC) procedure that overcomes many problems inherent in the other methods. However, this method has not been tested for determining metabolites of CIPC, which can be determined by the hydrolysis method.

EXPERIMENTAL SECTION

Reagents. All solvents used were either certified A.C.S. grade (for extracting and dissolving the extracts) or HPLC grade (for the liquid chromatograph), both of which were obtained from Fisher Scientific Co. The water used was glass distilled.

The CIPC standard, 99% pure, was obtained from Sigma Chemical Co.

The acid alumina, Brockman Activity I, 80–200 mesh, was purchased from Fisher Scientific Co. and was used as received.

Liquid Chromatographic System. A Model ALC/GPC 244 high-performance liquid chromatograph containing a Model 6000-A pump, a U6K injector, and a Model 450 Schoeffel UV detector (Waters Associates) was used. The detector was set at 236 nm and 0.04 AUFS. A Houston Instruments dual-pen recorder, set at a chart speed of 0.4 in./min, recorded the detector signal.

Column. A Waters Associates 30 cm × 3.9 mm i.d. μBondapak C_{18} column (octadecyltrichlorosilane chemically bonded to a 10-μm Porasil packing) was used.

Mobile Phase and Flow Rate. The mobile phase was methanol–acetonitrile–water (35:35:30) at ambient temperature. The flow rate was 1.0 mL/min.

CIPC Treatment. Two weeks after harvest, CIPC at a dosage of 4 lb/240 000 lb of potatoes was injected into an air stream by using an aerosol generator. Recirculation of the CIPC occurred for 48 h.

Washing Procedure. Six tubers were scrubbed clean, without brushing, under running water and peeled. Four subsamples of peels, 50 g each, were removed and analyzed as described below.

Extraction. The washed tubers, as well as the peas, beans, and blueberries, 50-g portions, were extracted twice with two 100-mL portions of methanol in a Waring blender, 1-qt jar size, at high speeds for 5 min. The extract was vacuum filtered through Whatman No. 50 filter paper. The filtrate was placed in a 100-mL round-bottom flask and concentrated to ~30 mL by using a Buchi rotary evaporator with the temperature set at 40 °C. The concentrate was transferred to a 250-mL round-bottom flask by using three 15-mL aliquots of methanol and was concentrated to ~15 mL before being brought to volume in a 25-mL volumetric flask with methanol. For samples with higher concentrations of CIPC (ppm range), it was not necessary to concentrate the sample to such a small volume.

Table I. Recovery of CIPC Added to Untreated Monona Potatoes

section of tuber	no. of determn	CIPC added, ppm	CIPC recov, ppm	% recov	SD
peel[a,b]	5	81	83	102	1.9
peel[a,b]	5	19	19	100	3.0
peel[a,b]	5	1	0.83	83	3.7
peel[a,b]	5	0.25	0.20	80	2.5
flesh[b,c]	4	0.25	0.18	72	7.5

[a] Peel consisted of the skin and ~2–3 mm of the flesh. [b] Background levels for all peel and flesh studies were 0 at the 0.04 AUFS sensitivity setting. [c] Flesh consisted of the entire potato after the removal of the peel.

Sample Cleanup through Acid Alumina Column. A glass wool plug was placed in the bottom of a 10-mL disposable pipet which had the top 3 cm removed. The column was then dry packed with 4 cm^3 of acid alumina. The sample was passed through the column until the first milliliter was collected, a portion of which was injected into the HPLC.

HPLC Analysis. The response curve was determined by taking 0.5-, 1-, 2-, 3-, 4-, 5-, 8-, and 10-mL aliquots of a CIPC standard (concentration 10 ppm) and putting each aliquot in a 25-mL volumetric flask and bringing it to volume with methanol. The standards were then passed through an acid alumina column, as were the samples. Twenty microliters of each solution was then injected into the HPLC, and a curve of detector response vs. nanograms of CIPC was plotted. A sample volume of 6–20 μL was injected, depending upon the concentration of CIPC in the sample. Samples were quantified by comparison of the peak height with that of the standard curve since peak height vs. concentration was linear within the range of concentrations used in this study.

Testing for Interferences. Sixteen widely used pesticides which might possibly be found in conjunction with CIPC were chromatographed by using the same conditions as for CIPC. These pesticides were propham, monuron, diuron, pentachloronitrobenzene, 2,4-D, atrazine, simazine, carbaryl, carbofuran, promecarb, dicamba, prometryn, pirimicarb, prometon, propanize, and amitrole.

RESULTS AND DISCUSSION

Three vegetables and one fruit were used to determine recoveries of CIPC at residue levels. Potatoes were fortified at levels ranging from 0.25 to 81 ppm. These studies indicated that at higher concentrations, above 1 ppm, of CIPC, the recovery was 100% or better, while at lower residue levels, 1 ppm or below, recovery was less (Table I). Recovery studies with peas, beans, and blueberries, all fortified with 0.25 ppm of CIPC, gave recoveries similar to those for potatoes with 0.25 ppm added except for the beans for which nearly 100% of the CIPC was recovered (Table II). These lower recoveries may have been due to binding of the CIPC to the particular fruit or vegetable.

The chromatograms of these four commodities fortified with 0.25 ppm of CIPC and their blanks are shown in

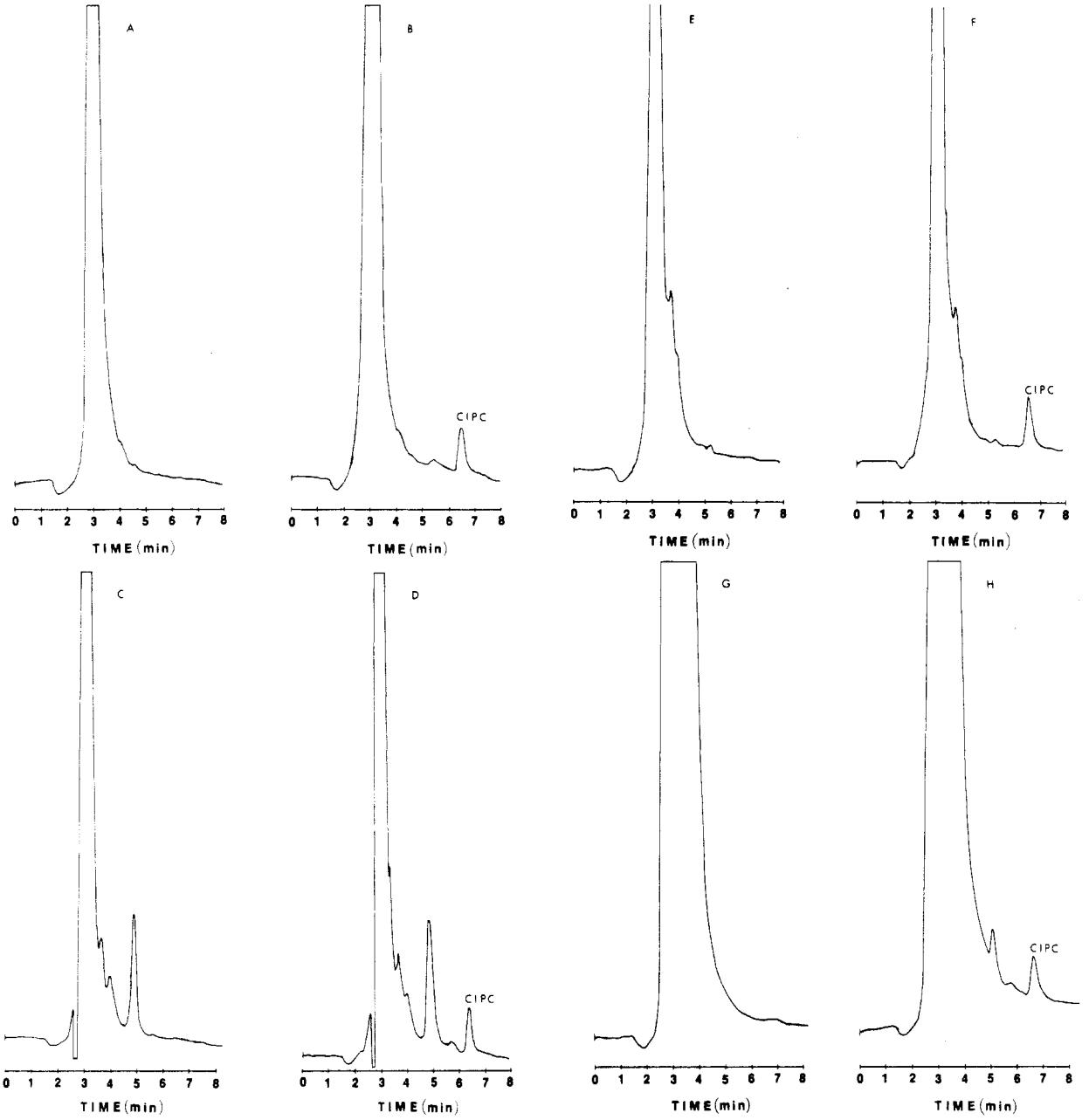

Figure 1. High-performance liquid chromatograms of extracts of four commodities fortified and unfortified with CIPC. (A) Potato blank. (B) Potatoes fortified with 250 ppb of CIPC. (C) Peas blank. (D) Peas fortified with 250 ppb of CIPC. (E) Green beans blank. (F) Green beans fortified with 250 ppb of CIPC. (G) Blueberries blank. (H) Blueberries fortified with 250 ppb of CIPC. Conditions: column, μBondapak C_{18}; mobile phase, methanol–acetonitrile–water (35:35:30); flow rate, 1 mL/min; column temperature, ambient; wavelength, 236 nm; absorbance full scale, 0.04; injection volume, 20 μL.

Table II. Recovery of CIPC Added to Various Commodities

sample	no. of determn	CIPC added, ppm	CIPC recov, ppm	% recov	SD
blueberries[a]	5	0.25	0.16	64	10.9
peas[b]	4	0.25	0.19	74	6.9
green beans[a]	5	0.25	0.24	97	7.4

[a] The background levels for these commodities at this sensitivity (0.04 AUFS) were 2.5 ppb. The answers have been corrected for this value. [b] The background level was 0 at the 0.04 AUFS sensitivity setting.

Figure 1. The CIPC elutes from the column in ~7 min with only slight interferences present in beans and blueberries (0.5 mm) as shown by the chromatograms of each blank. Even though the CIPC peak height is low thus contributing to possible error, the low peak height adds very little to the overall variation of this method. This was confirmed by injecting a bean sample with 0.25 ppm of added CIPC 6 times consecutively, for which a coefficient of variation of 6% was obtained and by injecting another bean sample, with 0.12 ppm of CIPC added, 6 times for which a 2.3% coefficient of variation was determined.

The lower limit of detection for this method with these commodities and the UV detector set at 0.04 AUFS was determined to be 0.12 ppm. Since the backgrounds are minimal at 0.04 AUFS, the detector can be set at 0.02 AUFS if lower limits of detection are needed. The 0.02 AUFS setting results in detection as low as 0.05 ppm on the four commodities. So that this could be tested further, a sample of beans fortified at 0.25 ppm and one not fortified were analyzed by using a UV setting of 0.02 AUFS. Beans were used since they gave one of the highest back-

Table III. Retention Times of Various Pesticides on C_{18} Using the Same Conditions as for CIPC

pesticide	retention time, cm
chlorpropham (CIPC)	6.55
propham	4.98
monuron	4.21
diuron	5.00
pentachloronitrobenzene	16.90
2,4-D	1.75
atrazine	4.95
simazine	4.40
carbaryl	4.35
carbofuran	4.21
promecarb	5.90
dicamba	1.80
prometryn	6.90
pirimicarb	5.00
prometon	4.41
propanize	5.81
amitrole	2.90

ground levels (0.5-mm peak height or 2.5 ppb) at 0.04 AUFS. The background level of the nonfortified samples of beans was 1.0 mm (peak height) or 5 ppb of CIPC. Although the noise level at the latter setting was slightly higher than at 0.04 AUFS, the coefficient of variation for six consecutive injections of the fortified bean sample was 3.2%. This demonstrates that acceptable chromatography was obtained with the lower sensitivity setting. If other commodities are analyzed for CIPC by using this method, their detection limits, which will depend upon the background levels encountered, will have to be determined.

A study was conducted to determine if it was sufficient to collect only 1 mL from the alumina column. It was shown that 1 mL of the standard or sample collected from the column gave an identical peak height to standard or sample not passed through the column when an equal amount of each was injected into the HPLC. The cleanup step then did not affect the concentrations of the standard or the samples and was used to eliminate early and late eluting peaks from the chromatogram.

Possible interferences from 16 pesticides were tested by injecting each into the HPLC using the same chromatographic conditions employed for CIPC. As shown in Table III, none of these pesticides had the same retention time as CIPC. Also, monuron and diuron, two arylurea herbicides, did not interfere with CIPC analysis on the HPLC as they interfere with some of the other methods. It is unlikely that any of the other arylurea herbicides would interfere either because of their structural characteristics which would make their retention times closer to those of monuron and diuron than to that of CIPC.

A study was conducted to show if CIPC would wash off potatoes under normal washing conditions, information which is valuable to both the consumer and the analyst. The CIPC concentration in the unwashed tubers was 45.0 ppm and in the washed tubers 40.4 ppm. Thus most of the CIPC was not removed by washing under these conditions. These data are in agreement with that of Koivistoinen and Karinpaa (1965), who demonstrated, when working with tomatoes and plums, that CIPC residues were easily removed by washing immediately after CIPC application but that after storage, residues were not washed off as easily. This tends to indicate that CIPC may be bound to the plant surface. As the potatoes used in this study had been stored for 4 months, such a binding phenomena as they described might be occurring in potatoes.

Although no column contamination problems were encountered during the several months required for this study, a guard column with C_{18} Corasil packing, 37–50-μm particle size, could be used to prolong column life.

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