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Decomposition and Metabolism of the Ethylene-releasing Compound CGA-15281 in Peach Orchard Soils

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Abstract. **Decomposition and metabolism of the ethylene-releasing plant growth regulator, (2-chloroethyl)methylbis(phenylmethoxy)silane (CGA-15281), was studied over a 14-month period in 3 peach orchard soils. The molecule decomposed rapidly when exposed to a soil environment. Major decomposition products were: ethylene, benzyl alcohol, and low levels of (2-chloroethyl)methyl(phenylmethoxy)chlorosilane and (dichloromethyl)-(chloroethylmethylphenylmethoxy)disiloxane which appear to be formed in side reactions of CGA-15281 breakdown. The compounds have molecular ions of 247 and 341 and display molecular ion isotope patterns consistent with molecules containing 2 and 3 chlorine atoms, respectively. Methoxy positions on the parent molecule were largely metabolized to CO_2, while the ethyl group was primarily liberated as ethylene. The methyl group on the parent molecule remained bound to the silicon atom, forming insoluble organosilicates; little was metabolized by soil microorganisms to CO_2. Soil type had a small but statistically significant effect on degree of release of ethylene from the chloroethyl positions; however, it did not affect metabolism of the methoxy group or degree of partitioning of the methyl group into insoluble compounds. This difference in ethylene release could not be explained by differences in pH, percentage of organic matter, or cation exchange capacity of the soils tested. Soil pH had a pronounced effect on the rate of breakdown of the parent molecule with the most rapid decomposition at low pH.**

We recently have reported on metabolism of CGA-15281 in young peach fruits (7) and uptake and transport of the compound in vegetative and reproductive tissue (3). The parent molecule breaks down readily after application with subsequent formation of benzyl alcohol, benzylmethylglucoside, benzylglucoside, and a number of volatile hydrocarbons (e.g., ethylene, CO_2, methane, ethane, propylene, butane, and others). Little of the compound is taken up by either vegetative tissue or young fruits, reflecting the low solubility of the parent molecule in water. The growth regulator appears to act primarily through the release of ethylene and penetration of the gas into the tissue rather than the uptake of the paretn molecule with subsequent release (3).

Significant portions of agricultural chemicals applied as foliar sprays are deposited either directly or indirectly in the soil. Nontarget placement of chemicals may be due to drift, misdirected sprays, rain or irrigation washing from the plant, volatilization, and leaf fall. The magnitude of nontarget placement varies widely with field conditions, chemical applied, and method of application. Estimates of 17% to 61% nonplant placement on annuals (8) are, however, not uncommon. Because of the potentially significant volume of material entering the soil zone, it is of considerable ecological interest to ascertain the fate of these compounds in orchard soils. We report the decomposition and general metabolism of CGA-15281 in 3 types of peach orchard soils.

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Materials and Methods

General metabolism. The metabolism of CGA-15281 was studied in 3 soils (Tifton, Greenville, and Cecil sandy loams) selected from representative peach orchards in northern, central, and southern Georgia (Table 1). Four replications of each soil type were analyzed for pH, phosphorus, potassium, calcium, and magnesium (5), cation exchange capacity (6), and percentage of organic matter (4) using standard techniques. A 50-g, air-dried sample of soil was placed in individual 250-ml Erlenmeyer flasks and brought to 80% field capacity by adding a predetermined amount of deionized distilled water. Each flask contained 2 small vials, one containing 7.5 ml of 10% KOH and a filter paper wick for trapping CO_2 and the other 0.25 M mercuric perchlorate for trapping ethylene produced during the experiment. To each flask was added formulated CGA-15281 from a 3000-ppm stock solution to give a final concentration of 10-ppm active CGA based on the air-dried weight of the soil. In addition, technical [^{14}C]-CGA-15281, labeled by Pathfinder Laboratories, St. Louis, Mo., was added to each flask to give a final concentration of 1×10^6 dpm. [^{14}C]-CGA-15281 was labeled in either the ethyl, methyl, or methoxy position on the parent molecule and had specific activities of 15.0, 19.5, and 20.1 mCi/mM, respectively. The labeled technical CGA-15281

Table 1. Characteristics of the 3 soils used in the study, selected from representative peach orchards in northern, central, and southern Georgia.

Soil type	pH	P (kg/ha)	K (kg/ha)	Ca (kg/ha)	Mg (kg/ha)	CEC[z] (meq/100 g)	OM[z] (%)	Description
Tifton sandy loam	5.7 ± 0.2	60 ± 2	40 ± 6	577 ± 68	68 ± 7	3.1 ± 0.1	0.4 ± 0.1	Fine loamy, siliceous, thermic, plinthic, paleudults
Greenville sandy loam	6.3 ± 0.1	90 ± 6	362 ± 19	1338 ± 10	191 ± 16	4.1 ± 0.1	2.01 ± 0.1	Clayey, kaolintic, thermic, rhodic, paleudults
Cecil sandy loam	6.2 ± 0.1	9 ± 0	123 ± 24	686 ± 53	73 ± 6	4.3 ± 0.3	1.0 ± 0.1	Clayey, kaolinitic, thermic, typic, hapludulto

[z]Cation exchange capacity (CEC), organic matter (OM).

was formulated with the solvent and emulsifiers used in the formulated product. The flasks then were sealed using a serum stopper and stored at 21°C during the test period. Samples for analysis were removed at 0, 0.5, 1, 3, 6, 9, and 14 months. O_2 and CO_2 concentrations within the flasks during the experimental period were monitored by withdrawing a 1-ml aliquot of the headspace gas and measuring by gas chromatography. Samples were separated on a 6.4-mm × 1.8-m column of 30% DEHS on Columnpak (60–80 mesh) and a 4.8 mm × 2.0 m column of Molecular Sieve 13× (42–60 mesh) and measured with a Hamilton Fisher Model 29 gas chromatograph equipped with thermal conductivity detectors. When the internal oxygen concentration dropped to 15%, the flask was aerated and the enclosed test tubes were replaced with new KOH and mercuric perchlorate. The amount of labeled [^{14}C]-CO_2 was measured by placing half of the KOH from each vial in a 125-ml Erlenmeyer flask containing a small test tube with 2 ml of ethanol : ethanolamine (2:1). The flask was sealed with a serum vial stopper and 4 ml of 6N H_2SO_4 injected from a syringe and hyperdermic needle through the stopper into the bottom of the flask releasing the trapped CO_2. After 24 hr, the ethanol : ethanolamine was transferred to a scintillation vial to which 10 ml of scintillation fluid was added (6 g PPO + 0.06 g POPOP/liter toluene) and 1 ml of ethanol. The samples were counted using a Beckman LS-100C counter (76% efficiency) and corrected for dilution.

At 0-, 0.5-, 1-, 3-, 6-, 9-, and 14-month intervals, 4 replications of flasks of each soil type and label position were extracted with organic solvents to remove extractable metabolities. Each sample was agitated on a wrist action shaker for 1 hr with 50 ml of hexane after which the solvent was decanted off and filtered. Two additional hexane extractions were made; the extracts were combined and concentrated *in vacuo* to 500 µl. The same procedure was then repeated using methanol. An aliquot of each solvent was then counted to determine the level of radioactivity (6 g PPO + 0.06 g POPOP/liter toluene).

To purify the major radiolabeled peaks, individual thin-layer chromatography (TLC) peaks were removed from the silica gel plates, eluted with hexane, and concentrated. Collective peaks were separated on a 3-mm × 1.8-m glass column of 3% OV 17 on Superco (100–120 mesh) using a Tracor 550 gas chromatograph with flame ionization detector. Conditions were: inlet temperature 290°C, detector temperature 230°, outlet temperatuer 282°, and oven temperature programmed from 200° to 230° at 5°/min. After characterizing the separation, a sample was collected from a 1.5 × 10-mm stainless steel tube collection port in a glass capillary tube (2.5-mm × 1.2-m) held in liquid nitrogen. This technique, however, proved to be unsatisfactory due to the low recovery of label. Direct injection of crude hexane and methanol extracts gave a large number of peaks and insufficient separation. Similarly, separation on a 10-m OV 1 capillary column, although providing better resolution, was insufficient for adequate identification of many peaks due to the large number of apparent background peaks in the samples. From mass spectral analysis, a number of these background peaks appeared to be hydrocarbons present in the soil prior to the test. Eugenol was identified and appeared, along with many of the other background peaks, to have accumulated in the orchard soils from dormant season oil sprays applied in the respective peach orchards.

After solvent extraction, unextractable label remaining in the soil was removed by digestion with sodium hydroxide. Ten grams of wet soil was placed in a 125-ml Erlenmeyer flask; 30 ml of 0.5N NaOH was added and agitated for 60 min on a wrist-action shaker. After agitation, the slurry was placed in a 50 ml centrifuge tube and centrifuged at 10,000× *g* for 15 min. A 0.1-ml aliquot of the supernatant was placed in a scintillation vial, 10 ml of scintillation fluid (100 g naphthalene + 5 g PPO/liter dioxane) added and counted 24 hr later. The procedure was repeated a 2nd time on the same soil sample, after which the soil was allowed to air-dry and weighed to determine the percentage of the original 50-g sample it represented. Data were corrected for instrument counting efficiency, quenching, and dilution with the corrected counts for the 2 extractions combined.

Ethylene released from the parent molecule or produced by microorganisms within the flasks was trapped in a vial containing 7.5 ml of 0.25 M mercuric perchlorate and a filter paper wick. Labeled ethylene was measured by placing 2 ml of the trapping solution in a 125-cc flask containing a test tube with 3 ml of 0.1 M mercuric acetate. Ethylene was released by injecting 2 ml of 10 M LiCl into the flask containing the mercuric perchlorate and trapping overnight. The mercuric acetate was transferred to a scintillation vial and counted with a dioxane scintillator (100 g naphthalene + 5 g PPO/liter dioxane).

CGA-15281 decomposition products. Due to the problem of high levels of background organic compounds in soil extracts, the decomposition of technical CGA-15281 was studied in washed sea sand. Although very low in cation exchange sites, this medium provided a silicon dioxide environment similar to what would be found in many orchard soils. Washed sea sand was additionally washed with hexane (6× and air-dried; 0.5 g were placed in reaction vials to which was added 150 µl of citric acid-sodium dibasic phosphate buffer solution (pH 5.0). The volume of buffer solution was just sufficient to wet the sand medium. Twenty µl of technical CGA-15281 (95% purity) were applied to the sand-buffer medium, agitated for 20 sec with a test tube Vortex shaker, and allowed to stand for varying inter-

vals up to 11 days. Decomposition products were then partitioned into 0.5 ml of methylene chloride containing a hexidecane internal standard; an aliquot was removed and chromatographed using a Hewlett Packard 5840A gas chromatograph interfaced with a Hewlett Packard 5895 mass spectrometer. Samples were separated on a 20-m × 0.3-mm ID capillary column of methylphenyl silicon (SE-54), 0.5 μm thickness (2) with the oven temperature programed from 80 to 280°C at 6°/min, 30 cm/sec helium flow, split injection mode (100:1 split ratio), MS scan rate of 400 AMU/sec, ion source temperature 200°, and electron multiplier voltage 2000.

Effect of pH. Similar sand samples were prepared using citric acid-sodium dibasic phosphate buffers with pH of 3.0, 4.0, 5.0, 6.0, 7.0, and 8.0 with 20 μ of CGA-15281/reaction vial. The samples were briefly agitated, capped, and allowed to stand at room temperature (21°C) for 48 hr, after which time 0.5 ml of methylene chloride was added, and the samples were reagitated and an aliquot was removed for GLC. Three μl of the methylene chloride phase were chromatographed using a Hewlett Packard 5720A gas chromatograph with flame ionization detector interfaced with a Texas Instruments 700 ASR electronic data terminal. Samples were separated on the capillary column (SE 54) programed from 100 to 280° at 8°/min.

Results and Discussion

General metabolism. Ethylene was released from the chloroethyl position rapidly upon exposure to the soil environment (Fig. 1). By 4 weeks, 77% of the total label evolved during the 14-month period had been liberated. After 4 to 6 weeks, there was a slow but continued release of ethylene, probably reflecting the formation of secondary haloethylsilane compounds with enhanced stability of the chloroethyl carbon-silicon bond. Soil type had a small but significant effect on the total amount of ethylene liberated, although the general rates after the first month were similar. The Cecil sandy loam resulted in a greater total release of ethylene than the Tifton sandy loam which was followed by the Greenville sandy loam. The effect on the cleavage of the chloroethyl-silicon bond did not appear to be correlated with the pH, cation exchange capacity, or percentage of organic matter of the 3 soils tested.

A portion of the label in each of the 3 positions on the parent molecule was liberated as [^{14}C]-CO_2 (Fig. 2). The methoxy position represented the major source of CO_2 reflecting both the availability of this portion of the parent molecule and the relative ease at which it was utilized by microorganisms as a carbon substrate. The ethyl group, although metabolized at a substantially lower rate than the methoxy portion of the molecule, was also converted to a limited extent to CO_2. The rapid utilization of ethylene by soil microorganisms has been shown previously (1). This lower metabolism rate may in part reflect the high ethylene concentration gradient created between the soil environment and the atmosphere by the use of an ethylene trap. Little of the methyl position was converted to CO_2, indicating that either the methyl group does not represent a readily assimilated carbon base for soil microorganisms or, more likely, that the silicon-carbon bond of the methyl position is quite resistant to cleavage in the soil environment. The initial rate of [^{14}C]-CO_2 formation is rapid for each of the 3 groups on the parent molecule, declining thereafter to a steady but lower rate.

Labeled carbon which was found to be insoluble in organic solvents, water, and mild alkaline solutions was largely from the methyl position (Fig. 3). Over 75% of the total methyl label applied was recovered in the insoluble residue after 14 months. Initially the rate of conversion to insoluble organic compounds was quite rapid, decreasing somewhat with time, but still forming at a significant rate over the duration of the experiment. Labeled insoluble carbon in the methoxy or chloroethyl position represented only around 10% of the total label of each. This

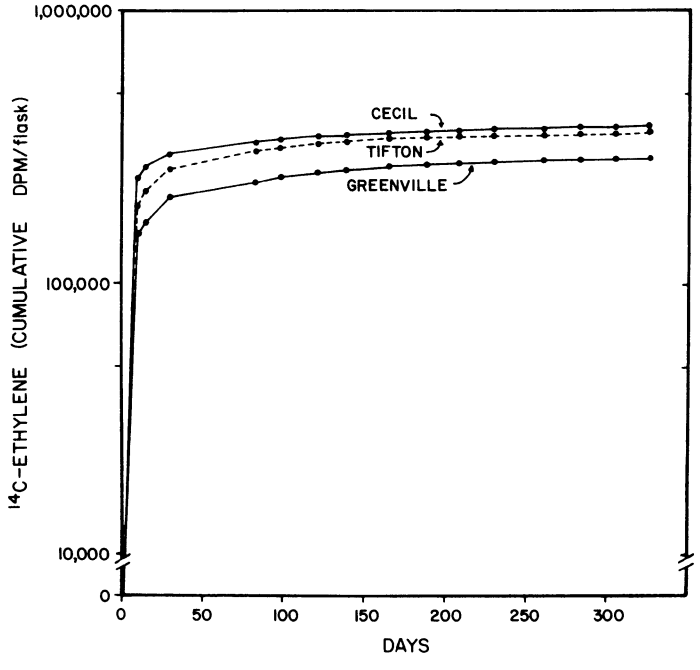

Fig. 1. The effect of soil type on the release of [^{14}C]-ethylene from [^{14}C]-ethyl-labeled CGA.

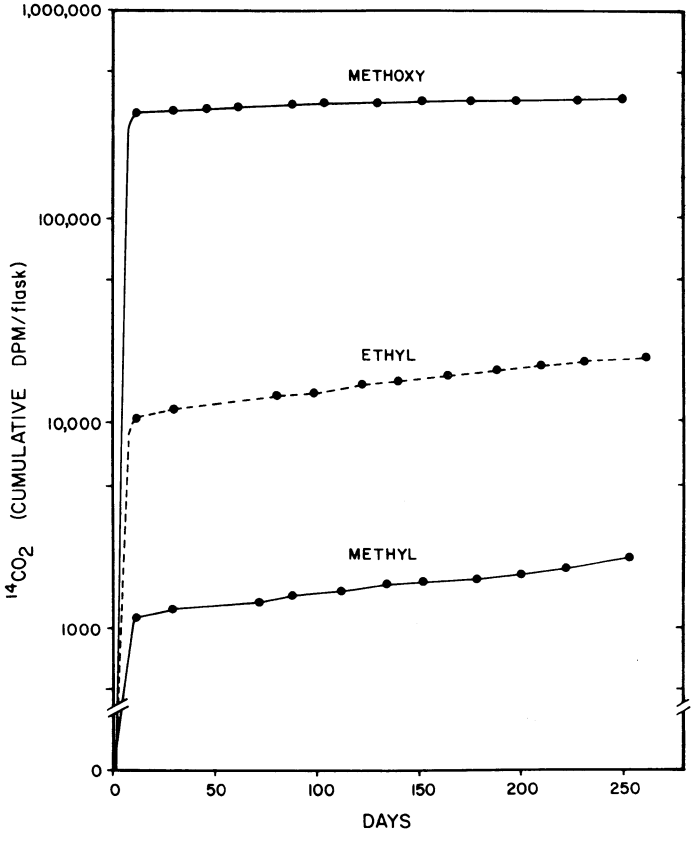

Fig. 2. The metabolism of [^{14}C]-methoxy-, ethyl-, and methyl-labeled CGA to CO_2 in peach orchard soils.

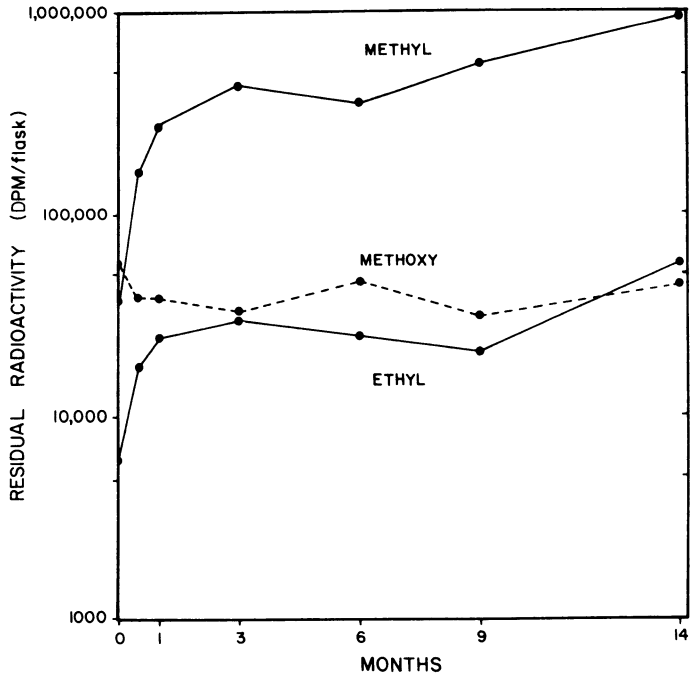

Fig. 3. The rate of incorporation of [^{14}C]-methyl-, methoxy-, and ethyl-labeled CGA into insoluble organsilicate compounds in orchard soils.

conversion was rapid after initial exposure to the soil, with little if any increase with time.

Distribution of the label from the methoxy, methyl, and chloroethyl positions on the parent molecule after 14 months is illustrated in Fig. 4. The methoxy group was largely converted to CO_2 and the chloroethyl group to ethylene. The methyl position appeared to remain attached to the silicon atom, forming insoluble silicon compounds in the soil. The ethyl label was the most widely dispersed into various fractions of the 3 labeled positions on the parent molecule. Ethyl-labeled compounds found in organic solvents were more polar than the original parent molecule, indicated by the lower recovery of label in the hexane fraction.

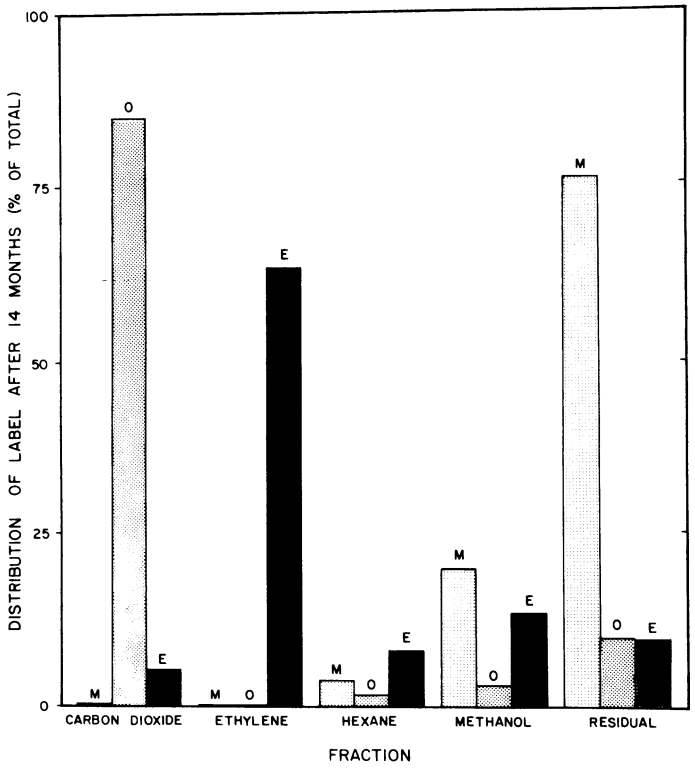

Fig. 4. The general fate of [^{14}C]-labeled methoxy (O), ethyl (E), and methyl (M) groups on CGA-15281 after 14 months in a soil environment.

Decomposition products. The parent molecule of CGA-15281 decomposed readily in a modified soil system at pH 5.0 (Fig. 5). By day 11, all of the parent molecule had disappeared and only 3 peaks had appeared or increased in size. The major decomposition product was benzyl alcohol, which represents about ⅔ of the molecular weight of the original parent molecule. Two new peaks appeared and were identified by GC-MS as (2-chloroethyl)methyl(phenylmethoxy)chlorosilane and (dichloromethyl)(chloroethylmethylphenylmethoxy)disiloxane.

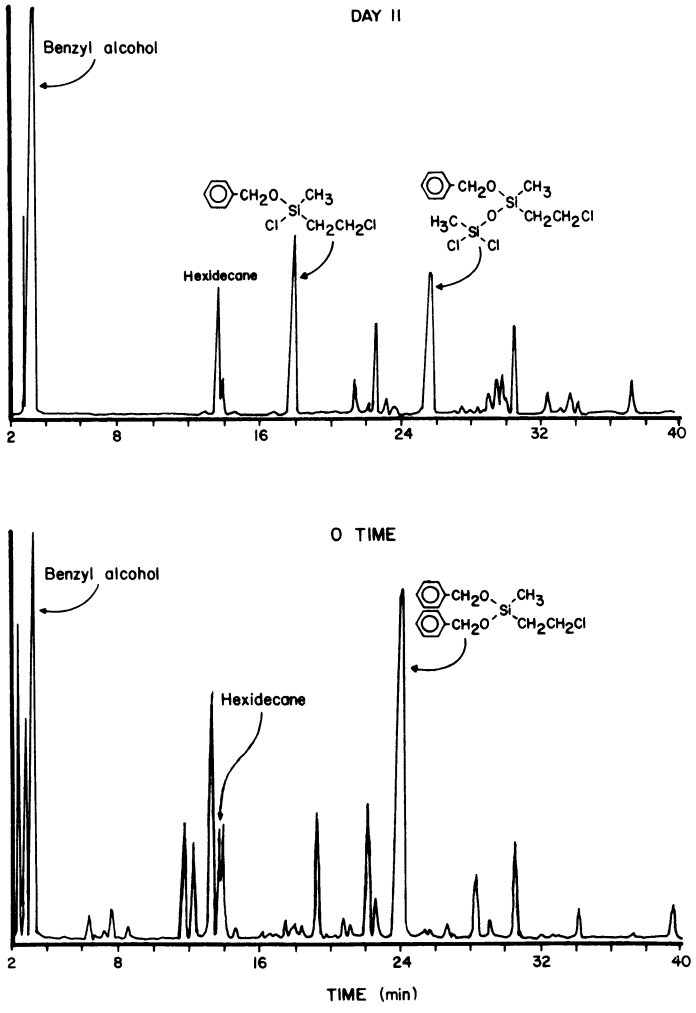

Fig. 5. Total ion current chromatograms of capillary GLC/MS analysis of the decomposition of CGA-15281 and the formation of benzyl alcohol, (2-chloroethyl)methyl(phenylmethoxy)chlorosilane (top center), and (dichloromethyl)(chloroethylmethylphenylmethoxy)-disiloxane (top right) in a modified soil environment over 11 days at 21°C, pH 5.0. At time 0, peaks for the parent molecule (CGA-15281), benzyl alcohol, and the internal standard (hexidecane) are identified. By day 11, the parent compound is not detectable; the benzyl alcohol peak has increased substantially; and 2 additional decomposition products are present.

The mass spectral data of (2-chloroethyl)methyl(phenylmethoxy)chlorosilane and (dichloromethyl)(chloroethylmethylphenylmethoxy)disiloxane showed molecular ion isotope patterns consistent with molecules containing 2 and 3 chlorine atoms, respectively, and fragmentation patterns consistent with the proposed structures. The molecular ions, 247 and 341 AMU, respectively, were the most abundant ions, indicating increased stability of these molecules over the parent molecule, CGA-15281, which gave a very weak molecular ion. The capillary GLC elution order of the 2 compounds (Fig. 5, upper chromatogram) is logical with respect to the original CGA-15281 molecule (Fig. 5, lower chromatogram). Since (2-chloroethyl)methyl(phenylmethoxy)chlorosilane and (dichloromethyl)-(chloroethylmethylphenylmethoxy)disiloxane are not present in the original CGA-15281 sample (Fig. 5, 0 time), they undoubtly are produced in side reactions of CGA-15281 breakdown and ethylene release.

Both the chlorosilane and disiloxane compounds contained the chloroethyl group. This, along with the more diverse distribution of the ethyl group (Fig. 4), suggest that the loss of one of the methoxy groups typically may precede the removal of the chloroethyl portion of the parent molecule or at least occur at a reasonable frequency in the soil environment.

Effect of pH. The pH of the modified soil environment had a pronounced effect on the rate of breakdown of the parent molecule (Fig. 6). Low pH especially below pH 6.0, rapidly accelerated the disappearance of the parent molecule and the appearance of benzyl alcohol. Breakdown products formed were similar for all pH with the exception of pH 3.0, where several additional peaks were found. Since this hydrogen ion concentration is not within the range that is conducive for the production of most agricultural crops, no attempt was made to identify these additional products.

In summary, we have shown that CGA-15281 is broken down rapidly in peach orchard soils. The ethyl position on the parent molecule is liberated primarily as ethylene; the methoxy group is predominately converted to carbon dioxide; the methyl group in general remains bound to the silicon atom, forming insoluble organosilicates. In the soils tests, soil type has a small but significant effect upon the release of ethylene from the chloroethyl position, but not on the fate or rate of cleavage of the methoxy or methyl groups. The major decomposition products formed were: ethylene, benzyl alcohol, and low levels of (2-chloroethyl)methyl(phenylmethoxy)chlorosilane and (dichloromethyl)-(chloroethylmethylphenylmethoxy)disiloxane; however, a number of insoluble silicon compounds are no doubt also present. The ease of formation of disiloxane and subsequent insoluble complexes in a largely silicon environment is apparent. The ecological significance of these compounds, although not established, is likely to be minimal because of their insolubility. Clearly, acid soils lead to a more rapid breakdown of CGA-15281.

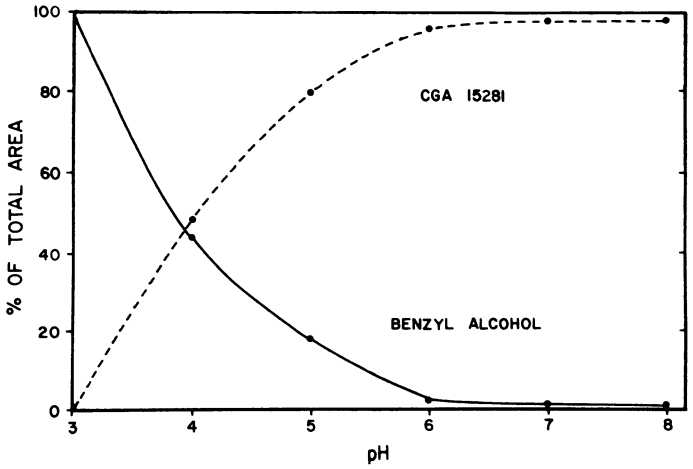

Fig. 6. The effect of pH on the rate of decomposition of CGA and the formation of benzyl alcohol after 48 hr in a modified soil environment; 21°C expressed as the percentage of the total area of peaks present.

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