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Fate of Diamidafos (Phenyl *N,N'*-Dimethylphosphordiamidate) in Tobacco, Cured Tobacco, and in Smoke

Richard W. Meikle

Tobacco was treated with diamidafos-^{14}C in transplant water at the rate of 0.33 lb/acre. The following radioactive substances—diamidafos, phenyl β-D-glucopyranoside, an unidentified glycoside of phenol, and nonextractable material, the latter three expressed as phenol—were found in mature fresh leaves at concentrations of 2.61, 0.72, 0.15, and 0.07 ppm, respectively. In cured leaves, the concentrations were 1.29, 0.67, 0.08, and 0 ppm, respectively, when adjusted to an equivalent fresh leaf-weight basis. Smoking effectively destroyed diamidafos and the phenol glycosides in the cigarette tobacco. The major breakdown product in the mainstream smoke was phenol which was already present in much larger quantities as a result of thermal degradation of tobacco.

Of the three groups of nematodes which cause problems in tobacco, root-knot (*Meloidogyne* spp.), lesion (*Pratylenchus* spp.), and stunt (*Tylenchorynchus claytoni*), the root-knot group is believed to be by far the most important in flue-cured tobacco and is responsible for about 80% of all losses caused by these organisms (Todd and Nusbaum, 1968). The use of chemical soil treatment plays a vital role in control of nematode disease of tobacco. Diamidafos (proposed common name), phenyl *N,N'*-dimethylphosphordiamidate has been shown to give excellent root-knot nematode control when applied in water solution (Youngson, 1959). The efficacy of this compound when used to control root-knot nematodes in a variety of crops has been reported (Youngson and Goring, 1963; Turner, 1963; Coleby et al., 1965; Thomason and Baines, 1969) and in tobacco (Todd and Nusbaum, 1968, 1970, 1971). The metabolism of this compound has been studied in cucumbers (*Cucumis sativus*)(Meikle, 1968) and in soil (Meikle and Christie, 1969).

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This investigation was designed to determine the fate of diamidafos, applied in transplant water, in green and cured tobacco leaves, and in the smoke resulting from smoking cigarettes made from this cured tobacco. Such information is a prerequisite to the establishment of recommendations for safe and effective use of pesticides and for development of meaningful analytical methods for determination of residues.

MATERIALS AND METHODS

Chemicals. The preparation of phenyl-*1*-^{14}C *N,N'*-dimethylphosphordiamidate (diamidafos-^{14}C) has been described by Meikle (1968). The specific activity of the compound used in this work was 0.060 mCi/mmol and the radiochemical purity was 100% as determined by thin-layer chromatography. Phenyl-β-D-glucopyranoside and phenyl-β-D-galactoside were purchased from Mann Research Laboratories, New York, N.Y.

Planting and Treatment Logistics. Three containers constructed of $^3/_4$ in. plywood and having dimensions of 31 × 31 × 99 cm were filled to a depth of 91 cm with a top soil from Contra Costa County, Calif. This soil had the following properties; pH 5.9, 0.65% organic carbon, 75%

sand, 12% silt, 13% clay, and the $^1/_3$ bar moisture tension value was 15%. The soil was brought to 100% of this moisture value and the surface was then allowed to dry until it was workable. A trench 15 cm long, 8 cm wide, and 8 cm deep was formed in the soil surface in each container. The transplant water formulation of diamidafos-^{14}C, 20.8 mg/100 mL of water for each container, was poured into the trench and allowed to soak into the soil—this required only a few seconds. The tobacco was transplanted immediately, one plant to each box, and the trench was filled with soil. The experiment took place in a greenhouse.

At the time of transplanting, the tobacco plants (*Nicotiana tobacum* L., var. NC-402) were at the six-leaf stage; the seed was germinated in the greenhouse, and the plants were selected for equal vigor.

The amount of chemical applied to each plant was equivalent to a treatment rate of 0.33 lb/acre, predicated on a plant density of 7100 plants/acre or 6.1 ft^2/plant. This is characteristic of the planting density in the northern tobacco district of the United States (Martin and Leonard, 1967).

The plants were grown under a 14-h photoperiod and were watered and fertilized on demand. The air and soil temperatures were continuously monitored by means of thermister temperature probes. The average temperature of the air was 25 °C with a range of 34 to 19 °C; the average temperature of the soil was 23 °C with extremes of 26 and 18 °C.

Sampling Plant Tissue. Samples were taken by cutting the plants at the soil line at 25, 45, and 65 days following transplanting. The leaves were collected by cutting at the stem, and terminal buds and flowers were removed. All plant parts were weighed immediately to get fresh weights. Except for 11 leaves from the 65-day sample, all plant parts were cut into small pieces, freeze-dried, and weighed again. The dry material was ground in a Wiley mill to pass a 20-mesh screen (0.6 mm). All subsequent analytical work was carried out on this dry, finely ground plant material. The 11 leaves from the 65-day tobacco were flue-cured.

Flue-Curing of Tobacco Leaves. Curing involves the processes of drying, decomposition of chlorophyll until the green color disappears from the leaf, changes in the nitrogen compounds including the release of ammonia, hydrolysis of starch into sugars, and respiration or fermentation of the sugars.

The procedure described by Martin and Leonard (1967) was used to flue-cure the tobacco leaves in this experiment. The fresh leaves were hung in a large oven immediately after priming. The oven was maintained at 32 to 39 °C with a relative humidity of 70 to 76% for 48 h. This was the yellowing stage. The temperature was next raised to and maintained at 56 to 57 °C for 30 h with a relative humidity of 30 to 32%. This was the drying stage. Following this, the oven temperature was increased to 68 to 71 °C, relative humidity 10 to 15%, and held there for 24 h. This was the killing stage. Finally, the leaves were transferred to a constant temperature room (22 °C) having a relative humidity of 80 to 90% where they were allowed to regain moisture for 42 h. This was the ordering stage. At this point, the leaves were very damp. They were hung in a laboratory hood until dry enough to shred. The leaves were crumbled into small particles and then put through a 10-mesh screen (1.65 mm). This tobacco was allowed to regain moisture in the constant temperature, high-humidity room to the extent of 15% of its dry weight. It was now suitable for analysis and preparation of experimental cigarettes.

Radioactive Assay of Dry Plant Material. All dried plant material and extracted tissue was assayed for radioactivity by catalytic combustion to $^{14}CO_2$ which was collected and counted using a scintillation counter. The combustion apparatus was a Beckman Biological Material Oxidizer (BMO) and the scintillation counter was a Packard Instrument Company Tri-Carb Liquid Scintillation Spectrometer, Model 3375.

Extraction Procedures. All leaf and stem samples were each combined and thoroughly mixed. The samples were continuously extracted for 8 h with chloroform containing 10% methanol by volume. This solvent system was recommended by Bowman et al. (1968) as being very effective for removing residues of phosphorus insecticides and metabolites from plant tissue.

A second, batch-type extraction was performed on the previously extracted plant tissue using ethanol containing 20% water by volume. The filtered extract was combined with the chloroform–methanol extract and evaporated to incipient dryness in vacuo at less than 40 °C. The residue was then redissolved in a small volume of methanol, ethanol, or combinations of these solvents with water as appropriate for further analysis.

It was determined that diamidaphos was stable in the presence of these solvents under the conditions of extraction and concentration employed here. It was further determined that an additional extraction with the aqueous ethanol solvent did not remove any additional significant quantity of radioactive material from the twice-extracted plant tissue.

The plant extract, after being redissolved, was assayed for radioactivity by solution counting. For this purpose, a toluene-based scintillation solution, or one designed to hold water in solution (Bruno and Christian, 1961), was used.

Chromatography. Chromatography was used for investigation of the radioactivity extracted from plant material. All extracts were submitted to cochromatography with authentic samples of diamidafos and its potential metabolites.

Paper chromatography (PC) was carried out using Whatman no. 3 paper, descending at ambient temperature. Visualization of known compounds on paper chromatograms was accomplished by means of iodine vapor (Block et al., 1958). Thin-layer chromatography (TLC) was performed using silicic acid as absorbent (Silica Gel F_{254} precoated plates, E. Merck, Darmstadt, Germany). This absorbent contained a fluorescent, UV-sensitive indicator.

All chromatograms were examined for radioactivity by liquid scintillation techniques. Segmented paper strips (1.5 cm) were treated with methanol, 1 mL per counting vial, to desorb radioactive compounds; this mixture was counted in a toluene-based scintillation solution. Thin-layer chromatograms were also assayed by incremental counting. Silica gel powder from the TLC plates was scraped into scintillation vials in $^1/_2$- or 1-cm segments and treated with methanol as above. The chromatography counting data were analyzed using a computer program which enabled us to describe the distribution of significant radioactive areas on the chromatograms, at the 95% level of confidence, as a percent of the total radioactivity on the plate.

The solvent systems for PC and TLC with peak R_f values for diamidafos, metabolites, and their derivatives are shown in Table I. Also included are the peak R_f values for the extracted radioactive compounds.

Preparation of Experimental Cigarettes. Experimental cigarettes were made from the radioactive tobacco as follows: Casings were prepared by carefully removing

Table I. Peak R_f Values of Diamidafos, Metabolites, and Their Derivatives, and Radioactive Compounds

Compound	Solvent system[a]						
	PC		TLC				
	I	II	III	IV	V	VI	VII
Diamidafos	0.90	0.34	0.75	1.00			
Phenyl-β-D-glucopyranoside	0.66	0.00		0.74			
Phenyl-β-D-galactoside	0.67	0.00					
Phenol					0.54		
2,4,6-Tribromophenol						0.39	0.56
Radioactive A	0.90	0.34	0.75	1.00			
Radioactive B	0.66	0.00		0.74			
Radioactive B hydrolyzed					0.54		
followed by bromination						0.39	0.56
Radioactive C	0.31	0.00		0.89			
Radioactive C hydrolyzed					0.54		
followed by bromination						0.39	0.56

[a] System I, 1-butanol saturated with 1.5 N ammonia; system II, benzene saturated with water; system III, 1-butanol-water-acetic acid (25:24:1, v:v); system IV, chloroform-methanol-water (65:38:8, v:v); system V, benzene; system VI, carbon tetrachloride; system VII, benzene-cyclohexane (1:1, v:v).

the tobacco from commercial cigarettes (Winston filter, 6-cm, R. J. Reynolds Tobacco Co.). The usable volume of the casing was 3.27 cm^3. The average packing density of this cigarette brand was 257 mg cm^{-3} with a standard error of ±3 for nine samples.

Experimental radioactive tobacco was put into an empty cigarette casing in five to six portions. After each portion, the tobacco was carefully packed by compressing it with a pencil which had not yet been sharpened. The length of the fill was 6.0 to 6.4 cm as required to give a packing density equivalent to a commercial cigarette. For example, the average packing density for six experimental cigarettes was 266 mg cm^{-3} with a standard error of ±7 for six samples. A comparison of this average with that for the commercial cigarettes gives a Student's t-value of 1.47. Therefore, the averages are not significantly different at the 90% level of confidence.

Smoking Procedure for Experimental Cigarettes. For smoking studies, the most widely used standard is the 35-mL puff of 2-s duration drawn once per minute to a butt length of 23 mm (Wynder and Hoffman, 1964). The smoking machine consisted of the following: The cigarette holder was fashioned from an 18/9 ball joint carrying 10-mm o.d. glass tubing 2.5 cm long and flared on the end to make cigarette placement easy. This led into the main body of a smoke condensate trap with the dimensions, 2.5 × 12 cm, and it was fitted with 18/7 socket joints. the "pump" was a 50-mL glass syringe fitted with a two-way check valve, and it was connected to the trap outlet by means of an 18/7 socket joint through the check valve.

The smoking machine was operated as follows: The smoke condensate trap, with its inlet closed, was immersed to about three-fourths of its length in a liquid nitrogen bath. The cigarette holder, with cigarette, was quickly attached to the trap inlet. Because of the restricted air flow through the cigarette, a partial vacuum was formed under these conditions and the cigarette holder stayed in place without clamping. The cigarette was lit and the prescribed regimen of smoking was set in operation as follows: The syringe plunger was withdrawn to 35 mL in 2 s time and held against the vacuum at that position until it would stay of its own accord—about 10 s. At this point, the cigarette holder was quickly replaced with a ball joint carrying a sealed tube to prevent air from entering the trap. At exactly 1 min after the first puff (syringe plunger withdrawal), the cigarette holder with cigarette was put back on the machine and another cycle (puff) started. This procedure was continued until the butt length was about 2 cm. From seven to ten puffs were required to meet these conditions. About 70% of the experimental tobacco was burned. In order to get sufficient combustion product to analyze, three cigarettes were smoked in sequence and the total combustion product was collected.

A substantial amount of the trapped combustion product was volatile at ambient temperature. In order to retain this material (other than CO and CO_2), it was transferred to a second trap. The additional trap was a microhydrogenator described by Cheronis (1954), but it was used without a condenser. It contained 10 to 15 mL of ether. This phase of the smoking experiment was carried out as follows: Immediately following the end of the smoking operation, the syringe and two-way valve were removed, this trap outlet was closed, and the second trap was attached to what was the inlet of the smoke condensate trap. The liquid nitrogen bath was quickly lowered, but only enough so as to prevent ether from backing up into the first trap. By adjusting the level of the liquid nitrogen bath, i.e., warming the smoke condensate trap, the volatile material was bubbled through the ether in the second trap at a slow controlled rate sufficient to keep spray to a minimum. When the first trap reached ambient temperature, nitrogen gas was put through the system at a slow rate for 20 min to purge the first trap of all remaining volatile material. At this point, the ether solution contained volatile ether-soluble material and the first trap retained the tar and nonvolatile compounds. These fractions could now be investigated separately.

Analysis of Tobacco Combustion Products. The contents of the trap containing nonvolatile material (referred to as tar, hereafter) were dissolved in methanol and this solution was assayed for radioactivity. After concentration, these extracts were analyzed for discrete radioactive compounds by TLC. The contents of the trap containing volatile material were also assayed for total radioactivity. In this case, however, it was not possible to investigate the volatile organic compounds because they were present in low concentration and could not be submitted to TLC, nor could the ether solution be concentrated without losing the volatile organic material.

After the cigarettes were smoked, both the unburned tobacco in the butt and the cigarette filters were analyzed for total radioactive content by combustion analysis. The cigarette filters were exhaustively extracted, first with three portions of ether, then with three portions of a methanol/water mixture (1:1) and, finally, once with water. These extractions were carried out by allowing the filters to soak in the solvents from 4 to 16 h. The dried, extracted

Table II. Concentration of Radioactivity in Tobacco Tissue

Days after transplant	ppm[a] as equivalents of diamidafos, wet-weight basis				
	Leaves	Stems	Terminal bud	Flowers	Seed
25	12.1 ± 0.09	0.45 ± 0.01			
45	7.3 ± 0.07	0.14 ± 0.005	0.17 ± 0.007		
65	4.6 ± 0.05	0.07 ± 0.002		0.06 ± 0.01	0.06 ± 0.02

[a] ± values are the standard error of the average of three determinations.

Table III. Distribution of Radioactivity in Extracts of Uncured Tobacco Tissue

Sample	Days after transplant	Percent of total radioactivity[a]		
		Chloroform-methanol extract	80% ethanol extract	Nonextractable
Leaves	25	95.1 ± 1.2	3.9 ± 0.2	1.0 ± 0.1
Leaves	45	80.7 ± 1.3	16.7 ± 0.6	2.6 ± 0.1
Leaves	65	77.8 ± 3.0	20.0 ± 0.6	3.2 ± 0.1
Stems	25	88.4 ± 3.7	2.6 ± 1.0	9.0 ± 0.7
Stems	45	77.2 ± 4.7	6.3 ± 2.2	16.5 ± 2.1
Stems	65	34.0 ± 2.0	40.4 ± 2.3	25.6 ± 1.8

[a] ± values are the standard error of the average of three determinations.

Table IV. Distribution of Radioactive Compounds in Fresh Tobacco Leaves and Stems

Sample	Days after transplant	Percent of total and ppm[a]			
		Diamidafos	Phenyl-β-D-glucopyranoside	Unknown glycoside of phenol	Nonextractable
Leaves	25	(88.6) 10.72	(9.5) 0.54	(0.8) 0.046	(1.1) 0.061
Leaves	45	(69.1) 5.04	(22.8) 0.78	(5.4) 0.18	(2.7) 0.094
Leaves	65	(56.7) 2.61	(33.1) 0.72	(7.0) 0.15	(3.2) 0.071
Stem	25	(92.0) 0.41	<0.005	<0.005	(8.0) 0.017
Stem	45	(60.0) 0.084	<0.002	(22.9) 0.015	(17.1) 0.011
Stem	65	(10.4) 0.007	(4.2) 0.001	(58.3) 0.019	(27.1) 0.009

[a] Concentration of phenol conjugates and nonextractable radioactivity is reported as equivalents of phenol. Parentheses enclose percent of total radioactivity in the tissue.

filters were then assayed for radioactivity. The filter extracts were also assayed for radioactivity. The identity of the radioactive entities in these extracts was decided by chromatographic procedures.

RESULTS AND DISCUSSION

Distribution of Radioactivity in Tobacco Plants. The concentration of radioactivity in the tobacco tissues, as equivalents of diamidafos, is shown in Table II. These data show that the concentration in leaf and stem tissue decreased with time. There was a low level of radioactivity in the terminal bud at 45 days, and the flowers and seed at 65 days showed only a minimally significant radioactivity content.

Identification of Radioactive Compounds in Fresh Tobacco Leaves and Stems. Extraction of fresh tobacco leaves and stalks resulted in removal of radioactivity with the distribution pattern shown in Table III. Chromatographic analysis of these extracts resulted in the array of radioactive compounds shown in Table IV.

Diamidafos was identified by chromatography, both PC and TLC, where the coincidence of the radioactive material and of the known compound were exact (Table I).

Phenyl β-D-glucopyranoside was identified by both PC and TLC, where the coincidence of the known compound and the radioactive material was exact in all chromatograms (Table I). In addition, the radioactive glycoside was separated from extracts by means of large-scale PC on Whatman no. 3 paper using solvent system I, followed by elution with water using the concentration technique described by Davis et al. (1962). Hydrolysis experiments were then carried out using this separated radioactive material. A portion of the material was submitted to emulsin hydrolysis (β-glucosidase from almonds obtained from Mann Research Laboratories, New York) exactly as described by Marco and Jaworski (1964). The hydrolysis was 92% complete in 23 h. The recovered radioactive phenol was identified by means of TLC (Table I). The coincidence of the radioactive material with nonradioactive phenol was exact. In addition, the radioactive hydrolysis product was fortified with a small amount of nonradioactive phenol, the 2,4,6-tribromo derivative was prepared (Shriner et al., 1965), and the derivative was submitted to TLC in solvent systems VI and VII (Table I). The radioactivity was exactly coincident with 2,4,6-tribromophenol in both solvent systems. In each case, there was no other radioactivity on the thin-layer plates. Base hydrolysis was attempted with 0.5 M potassium hydroxide in methanol solution at 37 °C for 4 h with shaking. The radioactive material was resistant to hydrolysis under these conditions: only 8% hydrolysis took place. The radioactive portion of the hydrolysate was phenol identified as described above. Acid hydrolysis was carried out in 1 M hydrochloric acid solution at 100 °C for 23 h. In this case, hydrolysis was 93% complete and the resulting radioactive hydrolysis product was again shown to be phenol.

The unknown radioactive glycoside was also separated from extracts by means of large-scale PC and it was submitted to hydrolysis experiments, enzymic, alkaline, and acidic, exactly as described for phenyl β-D-glucopyranoside. In this case, enzymic hydrolysis led to a 22% yield of radioactive phenol in 20 h. Acidic hydrolysis resulted in complete degradation of the unknown with resultant recovery of radioactive phenol. Alkaline hy-

Table V. Radioactive Compounds in Cured and Uncured Tobacco

Radioactive compd	% of total: Cured tobacco	% of total: Uncured tobacco
Diamidafos	44.5	56.7
Phenyl-β-D-glucopyranoside	49.2	33.1
Unidentified glycoside of phenol	6.3	7.0
Nonextractable	0	3.2

drolysis gave no radioactive phenol at all, only recovered starting material.

The behavior of this radioactive unknown as a result of these treatments was very similar to that of phenyl β-D-glucopyranoside except that the first-order rate constant for the enzymic hydrolysis of the unknown was only one-ninth that of the known glycoside, 0.012 and 0.110 h^{-1}, respectively. By means of PC in solvent system I, it was demonstrated that the unknown glycoside was not phenyl β-D-galactocide (Table I).

Radioactive, unconjugated phenol was not found in any of the plant extracts or in freeze-dry condensates. Therefore, it appears that free phenol was not present in detectable quantity as a result of decomposition of diamidafos in the tobacco plant.

Diamidafos, as a percent of the total radioactivity, decreased with time in both stem and leaf tissues. All other radioactive entities, judged on the same basis, increased with time in both tissues (Table IV).

On a concentration basis, parts per million fresh weight, diamidafos decreased with time in both stem and leaf tissue while the concentration of the other radioactive entities in leaf tissue peaked at 45 days. In stem tissue, however, the trend was not clear for these materials because the concentrations were rather low, ≤0.019 ppm (Table IV).

The extractive-free residue, that is, the nonextractable material in Table IV, contained radioactivity associated with lignin as demonstrated for diamidafos in cucumber plant tissue (Meikle, 1968).

Radioactive Compounds in Cured Tobacco. Assay of cured, humidified, experimental tobacco for its content of radioactive material gave a concentration of 26.1 ± 0.4 ppm (standard error of the average, three determinations) expressed as equivalents of diamidafos. In order to compare this value with that for the tobacco leaves before curing, it was necessary to convert 26.1 ppm to a value based on the weight of fresh leaves as follows: (26.1 × 32.40)/291.5 = 2.9 ppm, where 26.1 ppm is the concentration in the cured, humidified tobacco and 32.40 g is the weight of same, and 291.5 g is the weight of the equivalent quantity of fresh leaves before curing. The concentration in the cured tobacco, expressed as the diamidafos equivalent in fresh tissue, was only 63% of the corresponding value for the leaves before curing—4.6 ppm (Table II). This loss of radioactive material during the curing process could be due to enzymatic and/or chemical hydrolysis of both diamidafos and glycosides of phenol with concomitant loss of phenol by volatilization. These compounds, except for phenol, were shown to be present in the cured tobacco (see later discussion).

During the curing process, which has been reviewed by Forsyth (1957), there is immediate and rapid hydrolysis of protein, carbohydrate, glycosides, and many other conjugated compounds.

Extraction of the cured, humidified tobacco resulted in complete removal of its radioactive contents as determined by combustion of extracted tissue and assay of the resulting carbon dioxide for radioactivity. Distribution of the radioactivity in the extraction solvents was as follows: chloroform–methanol, 95%; 80% ethanol, 5%. Table V shows the radioactive products in the cured, humidified tobacco, and for purposes of comparison, the equivalent data for uncured tobacco.

Table VI. Radioactive Content of Mainstream Smoke Condensate from Experimental Cigarettes

Condensate	Radioactivity as a percent of total available in cured tobacco[a]
Nonvolatile[b]	3.7
Volatile[b]	0.3

[a] Total available for this experiment was 34 000 dpm, or 51 μg expressed as equivalents of diamidafos from three cigarettes. [b] At ambient temperature.

The data in Table V show that there was some reduction in the concentration of diamidafos relative to the other radioactive compounds as a result of the curing process. This suggests that, of the radioactive compounds present, diamidafos is most readily destroyed or, in any event, lost by whatever reactions are taking place during the curing process.

The identity of the radioactive compounds in the cured tobacco extracts was determined by cochromatography of these extracts with the extracts of uncured tobacco as well as with authentic samples of known compounds. The appropriate peaks were all coincidental for the two sets of extracts. Thus, the qualitative equivalence of the radioactive contents of the two sets of extracts was established.

Radioactive Compounds in Cigarette Smoke. Cigarette smoke is an aerosol having a discontinuous or particulate phase (about 8% of the total weight) and a continuous phase composed of vapor constituents (19%), excess nitrogen (15%), and air (58%) (Keith and Tesh, 1965).

In a burning cigarette, cigar or pipe, a sequential pattern of thermal changes occurs. The maximum temperature in the cigarette cone is about 880 °C and variable temperatures in cigars and pipes have been reported (Wynder and Hoffman, 1964). In cigarettes, a sharp thermal gradient is formed in a narrow region behind the burning cone (Harlow, 1956); this gradient may vary with the length of the remaining butt (Egerton et al. 1963). Volatilization, distillation, sublimation, pyrolysis, chemical interaction, and possibly mechanical cellular eruption (Stedman et al., 1966) occur chiefly within this region; and oxygen plays a minor role in the reactions (Hobbs, 1957). The formed aerosol proceeds rapidly down the cigarette during the "draw" and is mixed with air which permeates the cigarette paper. Partial deposition of the aerosol occurs during this passage and the condensed material is again subjected to heat and revolatilized as the cone moves down the cigarette. The compositional nature of the aerosol thus changes from puff to puff, giving higher concentrations of aerosol and components therein as smoking proceeds (Newsome and Keith, 1957).

What effect, then, does such a process have on the residual radioactive compounds shown to be present in the experimental cigarette tobacco (Table V)? Total radioactive content of the mainstream smoke condensate from the experimental cigarettes is shown in Table VI. Only 4% of the available radioactive material would have reached the smoker via the mainstream smoke. Assay of the filters for their radioactive content revealed that an additional 32% of the available radioactive material was intercepted by the filters. Thus, 89% of the radioactive organic matter in the mainstream smoke was trapped by the filters.

Table VII. Radioactive Compounds in Nonvolatile Portion of Mainstream Smoke Condensate from Experimental Cigarettes

Radioactive compd	Quant. as a percent of total avail. radioact. in tobacco[a]	μg per cigarette
Phenol	2.0	0.2
Phenyl-β-D-glucopyranoside	1.3	0.1[b]
Unidentified glycoside of phenol	0.4	0.03[b]
Diamidafos	<0.01	$<2 \times 10^{-4}$ [c]

[a] Total available for this experiment was 34 000 dpm, or 51 μg expressed as equivalents of diamidafos from three cigarettes. [b] Expressed as phenol. [c] Expressed as diamidafos.

Table VIII. Radioactive Compounds in Filters after Smoking Experimental Cigarettes

Radioactive compd	Quant. as a percent of total radioact. avail. in tobacco[a]	μg per cigarette
Phenol	27.8	2.2
Phenyl-β-D-glucopyranoside	3.5	0.3[b]
Unidentified glycoside of phenol	0.7	0.06[b]
Diamidafos	<0.05	$<8 \times 10^{-4}$ [c]

[a] Total available for this experiment was 34 000 dpm, or 51 μg expressed as equivalents of diamidafos from three cigarettes. [b] Expressed as phenol. [c] Expressed as diamidafos.

It was calculated by difference that 64% of the available radioactivity in the experimental cigarette tobacco was lost: in the sidestream smoke, as carbon dioxide and carbon monoxide in both the sidestream and mainstream smoke, and as residual material in the butt.

The identity and quantity, both relative and absolute, of radioactive compounds in the nonvolatile portion of the mainstream smoke condensate from the experimental cigarette tobacco appear in Table VII.

The quantity of radioactive phenol passing through the filter, per cigarette, was 0.2 μg. Eight micrograms of phenol is commonly found in the mainstream smoke from an unfiltered cigarette (Carruthers and Johnstone, 1960). Allowing for a filter efficiency of 92% for phenol, this still allows 0.6 μg to reach the smoker. Therefore, the amount of additional phenol getting through the filter to the smoker is about one-third of what is already indigenous to the cigarette mainstream smoke. Likewise, the total amount of phenols in mainstream smoke per cigarette is reported to be 95 μg (Carruthers and Johnstone, 1960). With a filter efficiency of 92% for phenols, this means that about 8 μg of phenolic compounds may reach the smoker. The 0.2 μg of phenol added to the total phenolic material, as a result of residual compounds derived from diamidafos in the experimental tobacco, is of little significance.

Attempts to identify the radioactive compounds in the volatile portion of the mainstream smoke condensate were unsuccessful. The high volatility of the radioactive constituents precluded successful TLC or PC analysis. Analytical techniques not dependent on radioactivity were not sensitive enough to detect the tiny amount of radioactive material present.

The identity and quantity, both relative and absolute, of residual radioactive compounds in the filters of the experimental cigarettes are shown in Table VIII.

In spite of the fact that diamidafos comprises nearly one-half of the radioactive content of the cured tobacco, it is noteworthy that none of this compound is present in the mainstream smoke from the tobacco.

Both diamidafos and phenyl-β-D-glucopyranoside are thought to be the precursors of phenol for the following reason: Differential thermal analysis of these compounds gave endotherms at their melting points and at 315 and 303 °C, respectively. These latter represent thermal decomposition of the molecule. In the case of diamidafos, the odor of phenol permeated the apparatus and phenol could be wiped from the inside of the bell jar which housed the sample. Clearly, thermal decomposition of diamidafos gave phenol. It has been reported (Irvine and Oldham, 1925; Pictet and Sarasin, 1918) that thermal decomposition of glucosides gives the anhydro sugar, 1,6-anhydro-β-D-glycopyranose, and presumably the aglycone, in this case, phenol. Thus, phenol is probably also formed as a result of thermal degradation of the glycosides, but perhaps not so readily as with diamidafos.

The quantitative relationship of the radioactive compounds derived from diamidafos to the sequence of events, uncured tobacco, cured tobacco, filter and tar, is given in Table IX. One-half of the diamidafos present in the fresh tobacco leaves was lost during the flue-curing process, and the remaining half disappeared during smoking. Very little (7%) phenyl-β-D-glucopyranoside was lost during the curing process, but 90% was lost during smoking. About one-half of the unidentified glycoside of phenol was lost while curing the tobacco, and most of that remaining (90% overall) disappeared during smoking. Finally, no unconjugated phenol was present in the experimental tobacco, cured or uncured; but it was present in the mainstream smoke. Most of the phenol (92%) was trapped by the filter. Apparently the tobacco plant conjugates phenol readily. The burning process that takes place during smoking effectively destroys diamidafos and phenol glycosides in the tobacco. The major breakdown product in the mainstream smoke is phenol which is already present in much larger quantities as a result of thermal degradation of the tobacco itself. It is felt, therefore, that the residual compounds in tobacco, which arise as a result of the use of diamidafos as a transplant water treatment, will not be a health hazard to the smoker.

Table IX. Quantitative Relationship of Radioactive Compounds to the Sequence: Uncured Tobacco, Cured Tobacco, Filter, Tar

	μg of compound per cigarette			
Sequential events	Diamidafos	Phenyl-β-D-glucopyranoside[b]	Unidentified[b] glycoside of phenol	Phenol[b]
Uncured tobacco[a]	15.2	4.2	0.9	0
Cured tobacco	7.6	3.9	0.5	0
Filter	0	0.3	0.06	2.2
Tar	0	0.1	0.03	0.2

[a] The quantity of uncured tobacco is that amount required to generate the cured tobacco actually pyrolyzed in smoking one cigarette. [b] Expressed as phenol.

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Transfer of Exogenously Applied and Endogenous Alkaloids and Sterols from Tobacco to Its Smoke Condensate

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Exogenously applied alkaloids were transferred from treated cigarette tobacco to its smoke condensates in quantities relative to that of endogenous alkaloids, although there was a linear trend toward reduced transfer percentage as alkaloid levels were increased. In contrast to the alkaloids, exogenously applied sterols were not transferred to the condensate in quantities relative to the endogenous sterols. Transfer percentages were higher for the increased levels of added sterol. The alkaloid × sterol interaction was significant for sterol recovered from the cigarette tobacco and that transferred to the condensate, but not transfer percentage. This would indicate that the added alkaloid resulted in greater quantities of sterol being adhered to the shredded leaf surface. There were no significant differences among treatments for total particulate matter. These results emphasize the need for quantification of chemical constituents in the tobacco and its smoke condensate in chemical additive studies.

The major alkaloids and sterols that occur in the leaf have been identified in tobacco smoke condensate (Stedman, 1968; Tso, 1974). The influence of total alkaloid and smoking variables on their delivery into smoke condensate has been examined more extensively than any other tobacco constituent (Bogen, 1929; Newsome and Keith, 1957; Wynder and Hoffmann, 1967; Kaburaki et al., 1965; Stedman, 1968; Bush et al., 1972; Housemann, 1973; Jenkins et al., 1975). Sterol transfer from the leaf to condensate has been examined by several investigators (Johnstone and Plimmer, 1959; Kallianos et al., 1963; Stedman, 1968; Grunwald et al., 1971; Cheng, 1973; Schmeltz et al., 1975). The information on quantitative transfer of specific compounds needs to be expanded to include exogenously applied compounds. The results would be valuable in determining the effect of changes in levels of leaf components on condensate levels especially in view of the possible use of additives to nontobacco materials. Also, the use of additives could provide a practical means of study for biological activity if levels in condensate could be altered by adding compounds to tobacco prior to smoking. For the latter studies to be meaningful, the transfer of the exogenously applied compounds should be similar to that in the leaf. We studied alkaloids and sterols, major groups of compounds which are present as natural products in tobacco. The major objectives were: (1) to ascertain the influence of exogenously applied alkaloids and sterols on smoke condensate levels of these compounds and (2) to further examine the transfer of endogenous quantities of these two groups of compounds into the smoke condensate.

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MATERIALS AND METHODS

Chemicals. The alkaloids, nicotine (99%) and anabasine (70%) with 30% anatabine contamination, used in this research were obtained from K & K Laboratories, Inc.,