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Simulation of Smoking Conditions by Pyrolysis

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A rapid, economical method was developed for the evaluation of the potential of different tobacco varieties to produce possibly hazardous smoke compounds. This controlled pyrolysis method produces pyrolyzate fractions very nearly identical with corresponding cigarette smoke fractions, as determined by analyses of their polynuclear aromatic hydrocarbons, neutral constituents, and phenolic contents.

Extensive research efforts are currently underway to develop safer smoking products which are still flavorful and aromatic. Thus, new varieties of tobacco and new methods of harvesting and curing tobacco must be evaluated. Typically, the evaluation involves the preparation of tobacco into cigarettes, the smoking of the cigarettes, and the subsequent testing of the cigarette smoke condensate. Such an evaluation is time consuming and expensive. We have been studying the formation of cigarette smoke components by the controlled pyrolysis (thermal decomposition) of tobacco leaf, tobacco extracts, and individual tobacco leaf compounds. With this improved pyrolysis method, we are able to rapidly evaluate the potential of a tobacco to form deleterious compounds, specifically, the tumor-promoting phenols and the carcinogenic polynuclear aromatic hydrocarbons (PAH) (Schmeltz et al., 1974).

Pyrolysis has been used frequently in studies to establish precursor–product relationships between tobacco components and cigarette smoke constituents (Schlotzhauer and Schmeltz, 1968; Bell et al., 1966; Schlotzhauer et al., 1976). The studies were made so that the smoke-forming process might be understood, and so that leaf constituents responsible for biologically active smoke compounds might be determined. However, the studies have been criticized since the pyrolytic conditions differed from those within a burning cigarette (Jenkins et al., 1970). We have adapted a method of pyrolysis that simulates those conditions closely, and the composition of the pyrolyzate formed resembles closely that of cigarette smoke. We used recent methods of analyzing smoke PAH's (Severson et al., 1976) to determine pyrolytic conditions that produce pyrolyzate with a PAH profile nearly identical with that of smoke PAH's. We now report the developed methodology and results.

EXPERIMENTAL SECTION

Materials. The tobacco used in the pyrolysis experiments was removed from 1R1 University of Kentucky reference cigarettes and was equilibrated at 60% relative humidity for 48 h.

Pyrolytic Apparatus. The pyrolysis apparatus was similar to one previously described (Smith et al., 1975) and is pictured in Figure 1. The Vycor pyrolysis tube was positioned horizontally through a moveable oven containing a 5.08-cm long heating core. The temperature of the oven was monitored and controlled by a temperature controller (±20 °C). Temperature range was ambient to 800 °C. Movement of the oven from 0.16 cm/min to 116.84 cm/min was controlled by a speed control unit. Two timers were used—one controlling operative puffing time and the other, nonoperative time (the intervals between puffs). When the operative timer was in control, puffing conditions were simulated in that nitrogen flowed and the oven moved at a preset rate along the pyrolysis tube. In the nonoperative mode, there was neither flow nor oven movement. Puffing time varied from 5 to 60 s, and the intervals between puffs were the differences between 60 s and puffing time. Nitrogen flow was controlled by a solenoid valve in relay with the timers; and the nitrogen flow rate, controlled by a needle valve, was monitored by an in-line gas flow meter.

Pyrolysis Procedure. A Vycor glass wool plug was placed about 30 cm from the outlet of the pyrolysis tube (1.22-m × 2.54-cm o.d.) and about 30 g of tobacco were loosely packed into the tube to yield a 60.96-cm column of tobacco. A Vycor glass wool plug was then inserted to close the column. Nitrogen flow was adjusted to the desired rate, and the oven temperature was equilibrated over an empty portion of the tube. The oven was then moved to the beginning of the tobacco column to initiate pyrolysis, and then allowed to move over the column at a preset rate, either continuously or in a pulsating motion. The trapping system consisted of a 3-L, uncooled expansion flask, three uncooled traps half-filled with ether, and a gas bubbler containing ether and 0.5% aqueous NaOH.

Pyrolysis Fractionation. The pyrolyzate was quantitatively removed from the traps and fractionated into neutral, basic, acidic, and phenolic fractions by solvent partitioning and pH adjustments (Higman et al., 1970). Residue weights for each fraction, as shown in Table I, were determined by reducing 10% aliquots of the indi-

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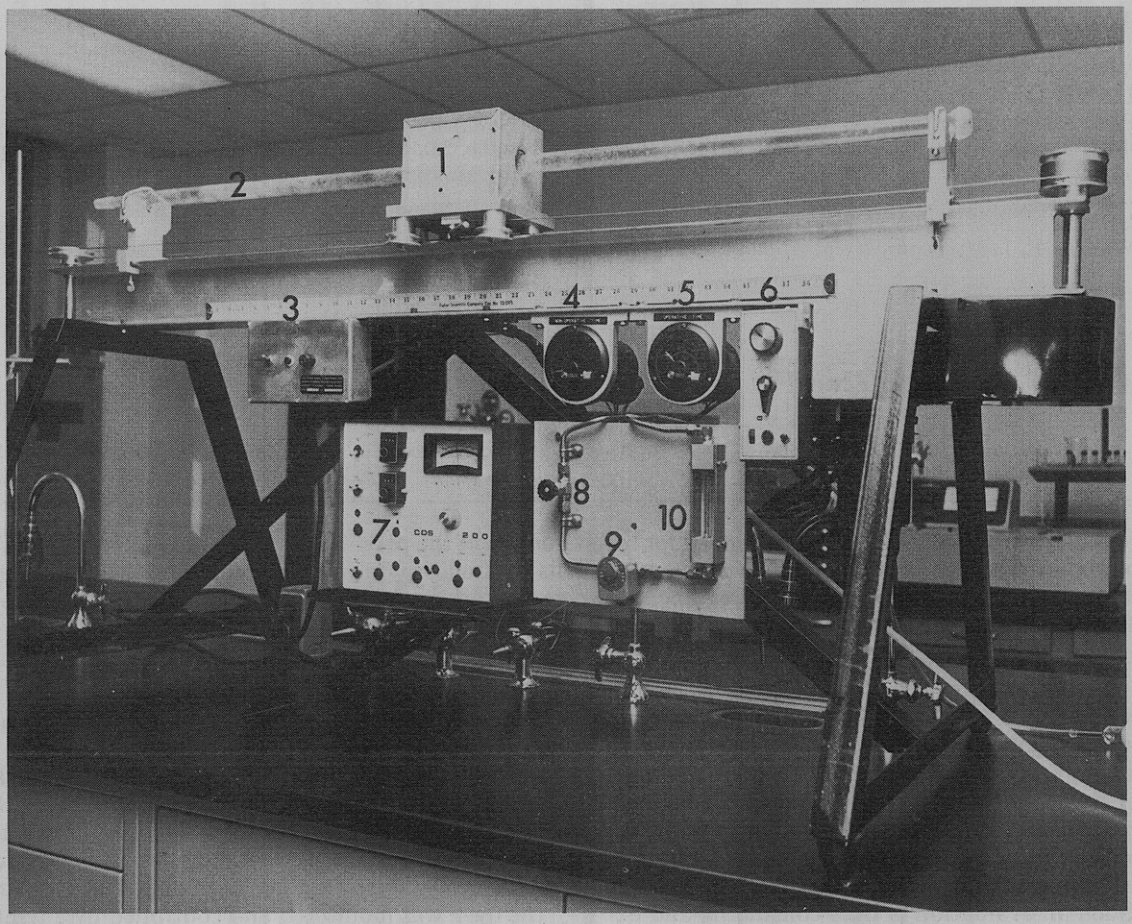

Figure 1. Pyrolysis apparatus (1, moveable oven; 2, pyrolysis tube; 3, cycle control unit; 4, non-operative timer; 5, operative timer; 6, speed control unit; 7, temperature controller; 8, needle valve; 9, solenoid valve; 10, gas flow meter).

Table I. Weights of Pyrolysis Fractions of 1R1 Cigarette Tobacco

Material pyrolyzed[a]	Quantity, g	N[b]	B[c]	A[d]	Ph[e]	Ash wt, g
		g/g pyrolyzed				
1R1	31.863	0.0529	0.0204	0.0046	0.0137	14.623
1R1 (750 °C)	30.800	0.0603	0.0218	0.0048	0.0126	10.656
1R1 (750 °C 5-s "puff")	31.078	0.0475	0.0134	0.0056	0.0115	9.930
1R1 (5-s "puff")	28.930	0.0413	0.0127	0.0049	0.0113	11.960
1R1 (20-s "puff")	34.135	0.0435	0.0125	0.0047	0.0165	14.458
1R1 (100 mL N_2, 20-s "puff")	30.970	0.0437	0.0168	0.0066	0.0127	10.734
1R1 (4 in., 60-s "puff")	30.983	0.0519	0.0179	0.0045	0.0118	13.953
1R1 (1 in., 60-s "puff")	32.605	0.0532	0.0177	0.0045	0.0090	12.986

[a] Unless otherwise noted, conditions were 700 °C, 200 mL/60-s N_2 flow, and oven movement of 5.08 cm/60 s. [b] Neutrals. [c] Bases. [d] Acids. [e] Phenols.

vidual fractions to constant weight with rotary evaporators.

To isolate and profile the PAH constituents of the neutral fraction, we chromatographed the neutral fraction on a column of silicic acid and eluted with solvents ranging from petroleum ether (PE) through benzene (B) to methanol (MeOH) (Figure 2) (Schlotzhauer et al., 1976). The PAH's were eluted in fraction B-PE and were purified by gel filtration chromatography on Bio-Beads SX-12 (Severson et al., 1976).

Gas Chromatographic (GC) Analyses. PAH profiles were obtained by use of a 4.57-m × 0.32-cm stainless steel column of 3% Dexsil 300 GC on 100/120 mesh Chromosorb W-AW. Chromatographic conditions were: oven temperature at 90 °C for 5 min, then programmed at 2

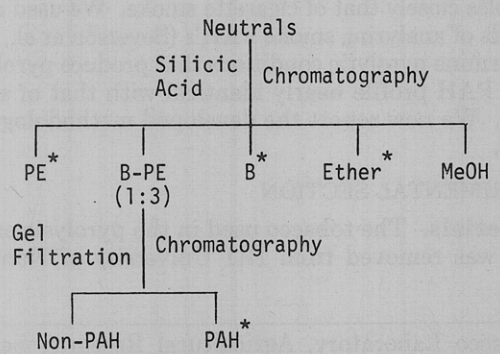

Figure 2. Fractionation scheme for pyrolysis neutrals. [Starred (*) fractions were compared to corresponding CSC fractions.]

Table II. Comparison of Relative Yields of PAH in 1R1 Tobacco Pyrolyzates and 1R1 CSC

PAH	Relative yields[a] 700 °C pyrolyzates 60[b] 200[e]	5[b] 200[e]	20[b] 200[e]	20[b] 100[e]	60[c] 200[e]	60[d] 200[e]	CSC
Phenanthrene-anthracene	3.02	3.22	2.27	2.44	2.09	2.19	3.17
Fluoranthene	0.93	0.72	0.67	0.77	0.57	0.56	1.06
Acephenanthrylene	0.97	0.42	0.64	0.54	0.64	0.52	0.63
Pyrene	1.00	1.00	1.00	1.00	1.00	1.00	1.00
1,2-Benzanthracene-chrysene-triphenylene	0.66	0.58	0.73	0.53	0.56	0.57	0.62
Benzo(*a*,*b*,*j* & *k*)fluoranthenes	0.31	0.20	0.38	0.23	0.09	0.18	0.26
Benzo(*a* & *e*)pyrenes	0.16	0.17	0.23	0.15	0.07	0.10	0.18

[a] Relative to pyrene. [b] Puff = oven movement in 60 s over 5.08-cm increments. [c] Puff = oven movement in 60 s over 10.16-cm increments. [d] Puff = oven movement in 60 s over 2.54-cm increments. [e] N_2 flow in mL/min.

°C/min to 325 °C and held at 325 °C for 30 min; injector port and detector were 290 and 350 °C, respectively; helium flow rate was 48 mL/min, measured at room temperature. GC analyses were obtained on a Hewlett-Packard Model 5830 flame ionization gas chromatograph. GC profiles of the PE, B, and E fractions were obtained on a 45.72-cm × 0.32-cm 5% Dexsil 300 column, with a temperature program of 100–300 °C at 4 °C/min. GC profiles of the phenol fractions were obtained on a 1.83-m × 0.32-cm glass column of Tenax, programmed from 150–300 °C at 2 °C/min.

RESULTS AND DISCUSSION

Our objective was to develop a pyrolytic procedure that would duplicate cigarette smoking especially with respect to the formation of PAH's. We varied the conditions of pyrolysis (temperature, gas flow rate, and oven movement) to optimize formation of PAH's in the same quantitative ratio as in CSC. Continuous oven movement and gas flow vs. a "stop and go" movement of the oven to simulate cigarette puffing were both examined. Previous pyrolysis work (Severson et al., 1977) indicated that the PAH profiles of tobacco pyrolyzed at temperatures ranging from 700 to 750 °C (±20 °C) resembled those of CSC. Therefore, we used those temperatures.

The effects of the various pyrolytic parameters on the yields of neutrals (N), bases (B), acids (A), and phenols (Ph) are given in Table I. The term "puff" is defined as the operative cycle of the pyrolysis system and refers to the time during which the oven moved 5.08 cm/60 s, unless otherwise indicated, with nitrogen flow. At 700 °C, with continuous flow and movement, defined as the 60-s puff, the yield of neutrals was 52.9 mg/g of tobacco pyrolyzed. The yields of neutral and basic fractions and of ash fell as the puff shortened. The same trends were observed at 750 °C. However, no such trends were observed in the yields of acids and phenols. The table shows that variations in puff time, temperature, oven rate, and nitrogen flow affected fraction yields.

Changes in puff duration and nitrogen flow also affected PAH distributions in the pyrolyzate. Table II shows the relative levels of selected PAH's in tobacco pyrolyzate and CSC. At 700 °C, 60- and 5-s puffs, both with nitrogen flow of 200 mL/min, produced the selected PAH's at levels which most closely resembled those in CSC. The decrease in nitrogen flow seemed to selectively increase pyrene levels, whereas the 20-s puff favored the production of higher molecular weight PAH. Table III compares the PAH levels of tobacco pyrolyzed in 5- and 60-s puffs at 750 °C with CSC PAH. The PAH levels of the 60-s puff more closely reproduced those in CSC.

The close relationship of pyrolyzate and cigarette smoke condensate fractions can best be illustrated by comparisons

Table III. Comparison of Relative Yields of PAH in 1R1 Tobacco Pyrolyzates and 1R1 CSC

PAH	Relative yields[a] 750 °C pyrolyzates 60[b] 200[c]	5 200	CSC
Phenanthrene-anthracene	3.47	2.59	3.17
Fluoranthene	0.90	0.75	1.06
Acephenanthrylene	0.74	0.28	0.63
Pyrene	1.00	1.00	1.00
1,2-Benzanthracene-chrysene-triphenylene	0.90	0.43	0.62
Benzo(*a*,*b*,*j* & *k*)fluoranthenes	0.30	0.28	0.26
Benzo(*a* & *e*)pyrenes	0.21	0.20	0.18

[a] Relative to pyrene. [b] Puff = oven movement in seconds over 5.08-cm increments. [c] N_2 flow in mL/min.

of their GC profile chromatograms. Figure 3 compares the gas chromatograms of the low molecular weight PAH from both 1R1 CSC and 700 °C pyrolyzate (60-s puff, 200 mL/min nitrogen). The lower levels of naphthalene and methylnaphthalenes in the CSC PAH's were due to the analysis of CSC from only 90 cigarettes and thus result from losses of these volatile components during solvent reductions. The higher amounts of PAH's produced in the pyrolyzates allow a more quantitative recovery. Overall, the quantitative agreement between the PAH profiles of pyrolyzate and CSC was good. The peak height ratios of dimethylnaphthalenes and acenaphthylene to methylfluorenes for CSC were almost identical with those of the pyrolyzates.

The remainder of the GC profiles are compared in Figure 4; these higher molecular weight PAH's are recovered quantitatively (Severson et al., 1976). The relationships between respective peak heights in the two chromatograms agreed well. Agreement of the following ratios was especially good: phenanthrene–anthracene/methylphenanthrenes–methylanthracenes, pyrene/methylpyrenes, and chrysene/methylchrysenes. The only observed differences were the fluoranthene levels. In previous pyrolytic experiments of a tube furnace (Schlotzhauer et al., 1976; Severson et al., 1977), the sample remained in or was drawn through the hot zone and mainly nonalkylated PAH's were formed then. In the present study, which involved the use of a moving oven over a stationary sample, the PAH profiles showed alkylated compounds similar in identity and distribution to condensate PAH.

The marked agreement between the PAH profiles of pyrolyzate and CSC prompted us to compare the distributions of the other products. Consequently, the PE, B,

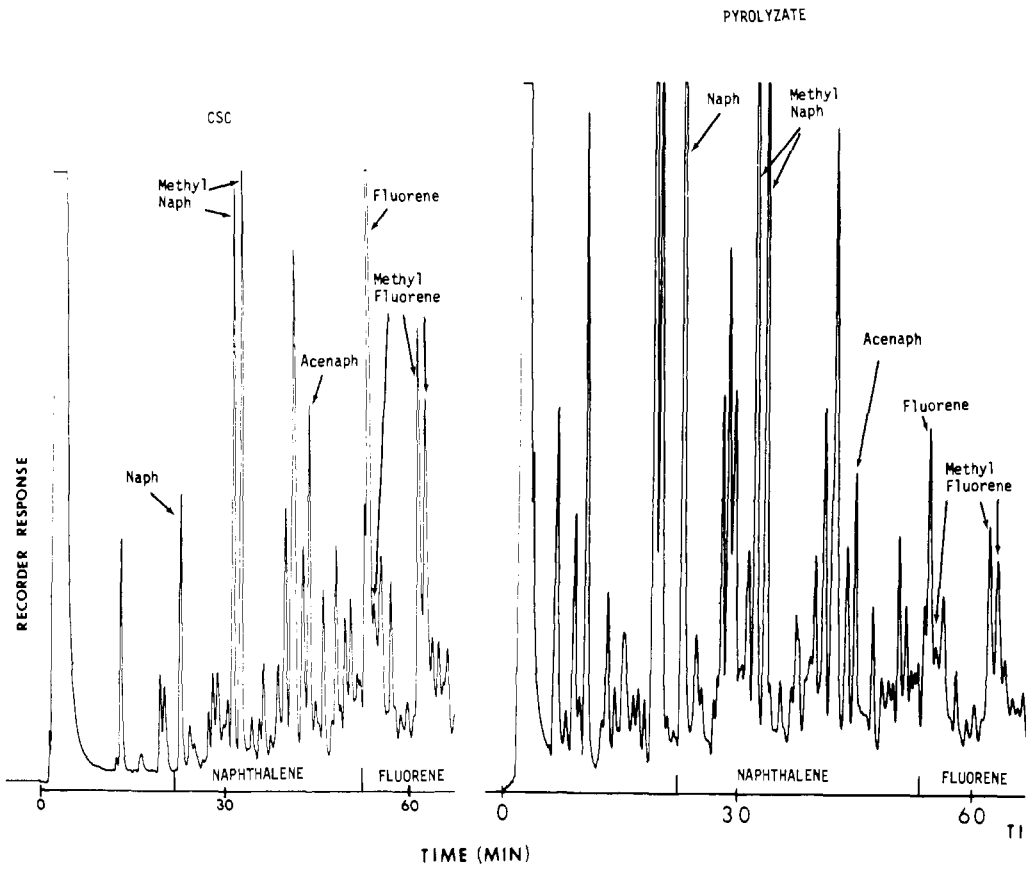

Figure 3. GC chromatograms of the low molecular weight PAH in CSC and 700 °C pyrolyzate.

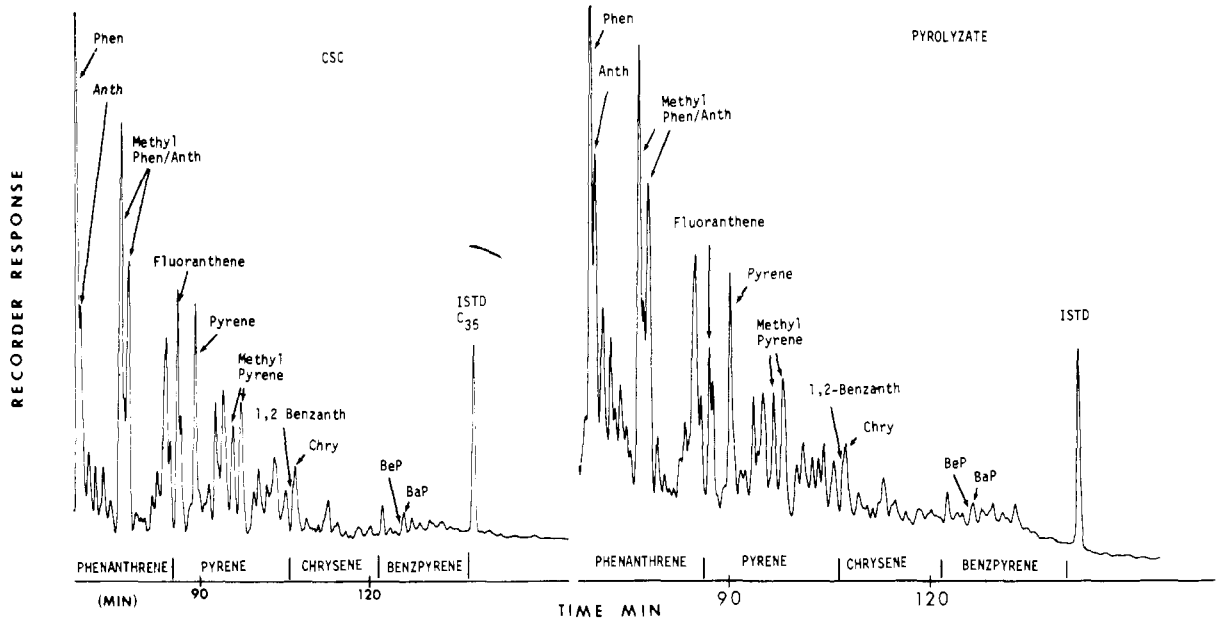

Figure 4. GC chromatograms of the high molecular weight PAH in CSC and 700 °C pyrolyzate.

and E fractions from the silicic acid chromatography of the neutrals (Figure 2) were also compared. Fraction PE has been shown to contain neophytadiene and C_{16} to C_{35} aliphatic hydrocarbons and waxes (Chortyk et al., 1975). The GC profiles of the PE fractions from 1R1 smoke condensate and 700 °C pyrolyzate (60-s puffs, 200 mL/min nitrogen) were very similar (Figure 5).

Fractions E of CSC and pyrolyzate were silylated with *N,O*-bis(trimethylsilyl)acetamide in dimethylformamide, and their GC chromatograms compared (Figure 6). The profile for CSC was very similar to chromatograms of tobacco leaf lipids (Ellington et al., 1976) and to the profile for the pyrolyzate. Fraction E contained acids, sterols, and some solanesol, a C_{45} terpenoid. However, in the pyrolyzate, a reduction in solanesol was observed.

The agreement between the GC profiles of fractions B from 1R1 smoke condensate and 700 °C pyrolyzate was also good (Figure 7).

The phenolic fraction of CSC has been shown to contain known tumor promoters (Schmeltz et al., 1974) and is therefore of significance in the total evaluations of a new tobacco variety for a safer cigarette. Examination of the

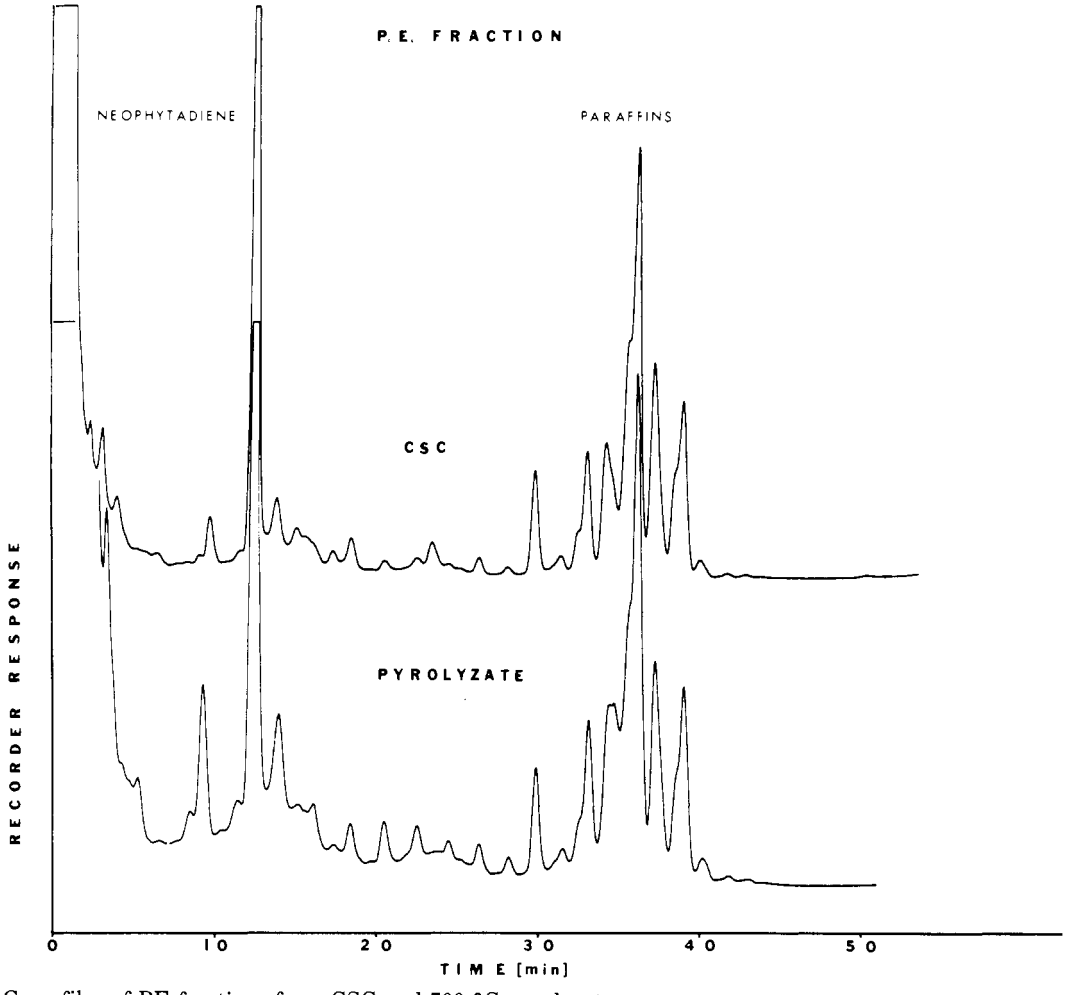

Figure 5. GC profiles of PE fractions from CSC and 700 °C pyrolyzate.

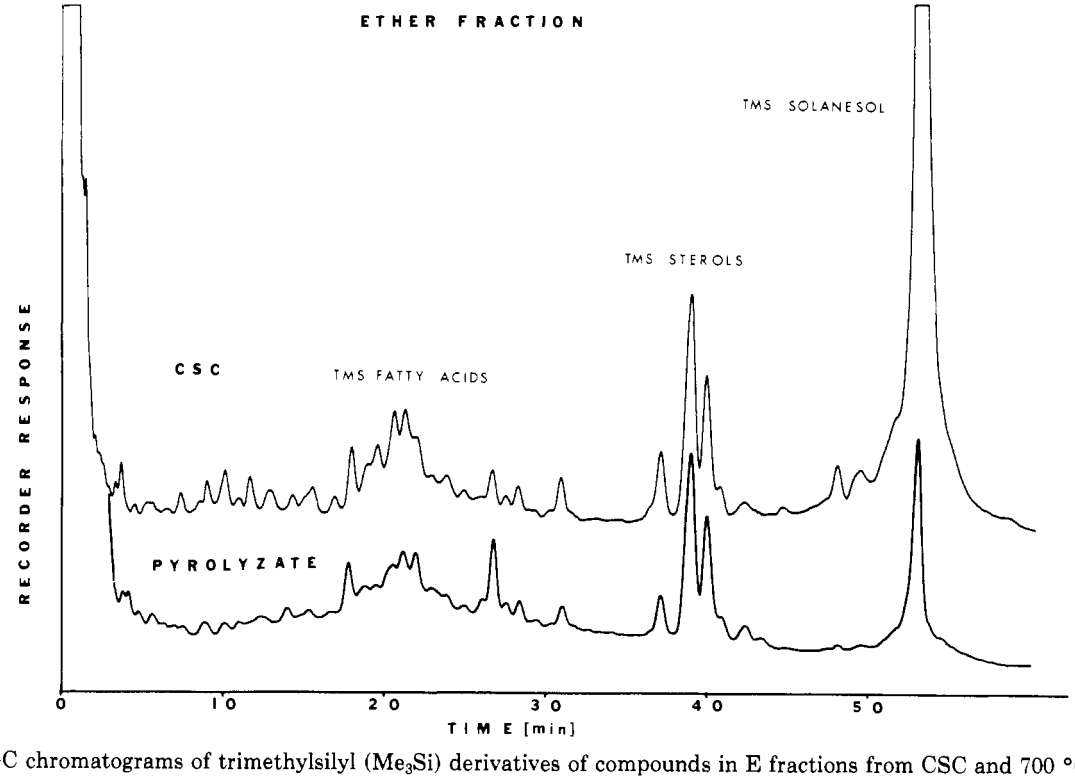

Figure 6. GC chromatograms of trimethylsilyl (Me_3Si) derivatives of compounds in E fractions from CSC and 700 °C pyrolyzate.

corresponding GC profiles of the phenol fractions also showed good agreement (Figure 8). The presence of pyridine and nicotine, as identified by GC–MS analysis, indicates the failure of the solvent partitioning and acid–base extraction methods to quantitatively separate different classes of compounds.

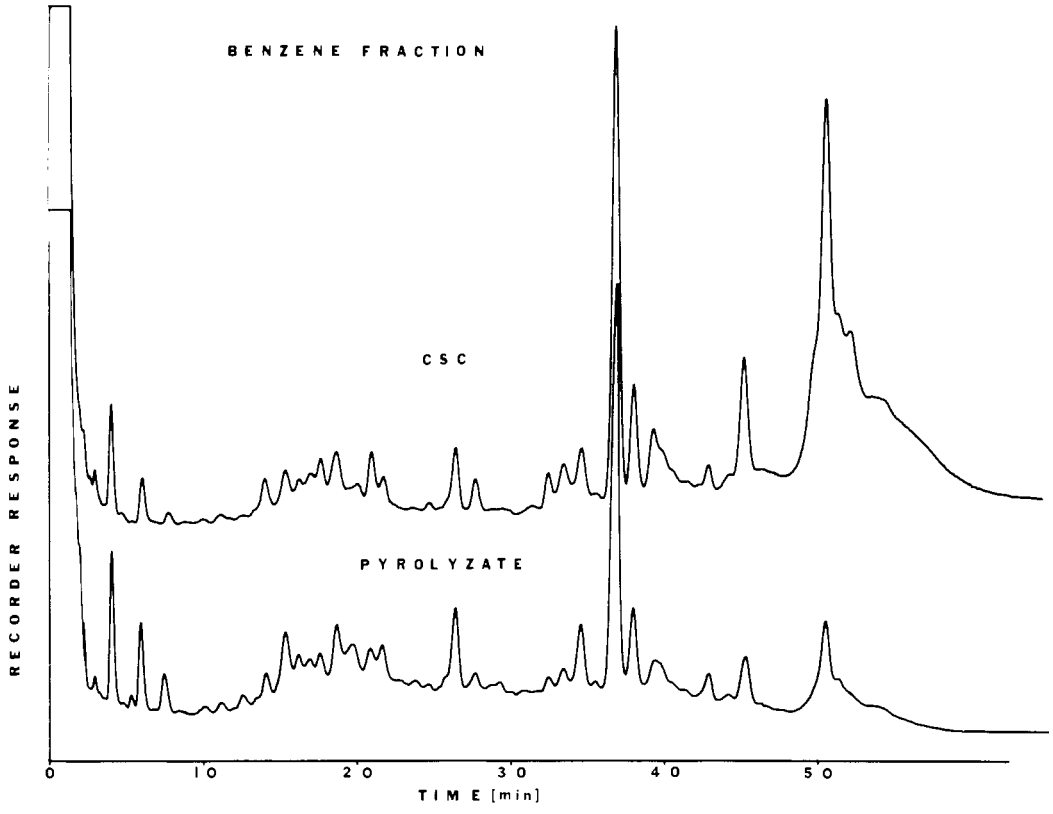

Figure 7. GC chromatograms of B fractions from CSC and 700 °C pyrolyzate.

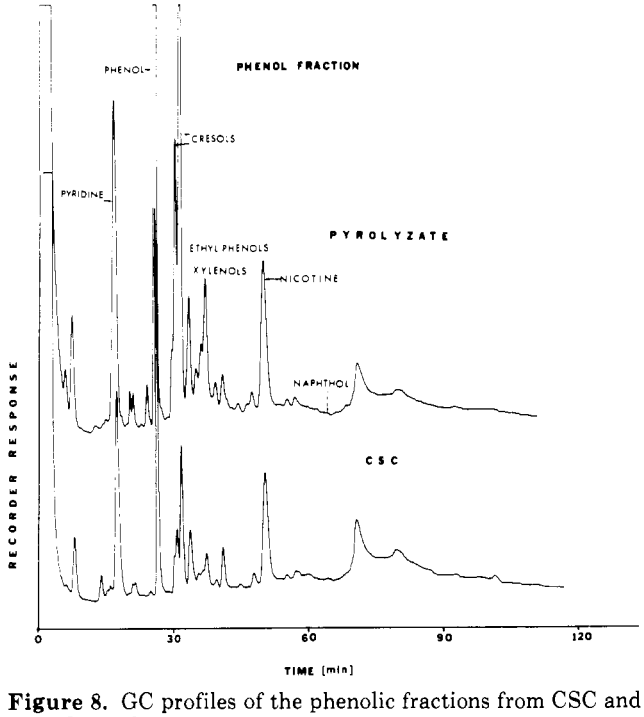

Figure 8. GC profiles of the phenolic fractions from CSC and 700 °C pyrolyzate.

Since the pyrolysis apparatus we used is intended to enable evaluation of tobacco samples for selected smoke constituents without the fabrication and the smoking of cigarettes, we studied the reproducibility of the system (Table IV). We conclude on the basis of PAH analyses that the pyrolysis and the chromatographic fractionation methods are reproducible. The developed procedure for pyrolyzing a column of tobacco along its length at 5.08 cm/min, 700 °C, and 200 mL/min constant nitrogen flow yielded a pyrolyzate almost identical with CSC, as determined by GC analyses of their PAH's, other neutral constituents, and phenolics.

Table IV. Reproducibility of Pyrolytic PAH Yields from 1R1 Tobacco (Continuous Oven Movement, 700 °C, 200 mL/min N_2)

PAH	Amount, μg/g	
	Run 1	Run 2
Phenanthrene-anthracene	4.48	4.58
Fluoranthene	1.39	1.39
Acephenanthrylene	1.41	1.50
Pyrene	1.46	1.54
1,2-Benzanthracene-chrysene-triphenylene	0.98	1.00
Benzo(*a,b,j* & *k*)fluoranthenes	0.44	0.47
Benzo(*a* & *e*)pyrenes	0.25	0.24

The similarity between pyrolyzate and CSC indicates that the heated zone of tobacco in the pyrolysis unit resembles the cone of a burning cigarette. Thus, as occurs during cigarette smoking, the advancing burning zone in the unit would cause distillation and condensation of materials onto the unpyrolyzed tobacco. The condensed pyrolyzate could then further distill or repyrolyze with additional tobacco; and the latter action could lead to possible important secondary reactions between the pyrolyzate and the tobacco.

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COMMUNICATIONS

Synthesis of 1-Amino-3,7,8-trichlorodibenzo-*p*-dioxin and 1-Amino-2,3,7,8-tetrachlorodibenzo-*p*-dioxin as Haptenic Compounds

The chemical syntheses and characterization of 1-amino-3,7,8-trichlorodibenzo-*p*-dioxin and 1-amino-2,3,7,8-tetrachlorodibenzo-*p*-dioxin are reported. These compounds can be used to couple with carrier proteins affording antigens for radioimmunoassay methodology.

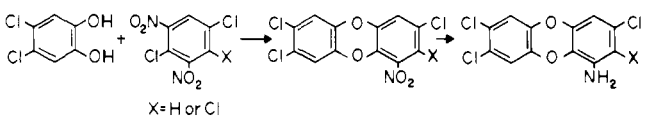
The high toxicity of certain polychlorinated dibenzo-*p*-dioxins has been demonstrated in a number of recent reports (Environmental Health Perspectives, 1973; McConnell and Moore, 1976). The most toxic member in this group is 2,3,7,8-tetrachlorodibenzo-*p*-dioxin (TCDD) which is a common contaminant of certain trichlorophenols and related compounds including the herbicide 2,4,5-T (2,4,5-trichlorophenoxyacetic acid). Because of its high chemical stability and lipophilicity, TCDD that is released into the environment could accumulate in the food chain. There is a need for a sensitive analytical method able to detect TCDD in biological tissue samples at levels well below its toxic dose. The common instrumental methods for analyzing TCDD in environmental samples are gas chromatography with an electron-capture detector or direct probe mass spectrometry. Electron-capture gas chromatography generally does not have the required high sensitivity and specificity for a single-method analysis. These methods, for most samples, require extensive sample cleanup and are relatively expensive to perform.

Radioimmunoassay (RIA) often permits specific measurement of compounds in the nanogram to picogram range. Since TCDD exhibits teratogenicity (Courtney et al., 1970; Courtney and Moore, 1971; Sparschu et al., 1971) and effects host resistance (Thigpen et al., 1975) even at sublethal doses, a RIA method would be useful for detecting minute quantities of the compound in biological systems as well as in various other environmental samples. In addition, RIA is readily adaptable to routine assay of large numbers of samples without extensive cleanup.

The TCDD and other chlorinated dibenzo-*p*-dioxins do not have functional groups in their molecule to bind with carrier proteins to form hapten–protein complexes (antigens). Therefore, it is necessary that they should be derivatized with such reactive groups as amino or carboxy groups. In this paper, the syntheses of 1-amino-3,7,8-trichlorodibenzo-*p*-dioxin and 1-amino-2,3,7,8-tetrachlorodibenzo-*p*-dioxin are described. These derivatized dioxins can be covalently linked to carrier proteins affording antigens for RIA method development.

Certain nitro-substituted dioxins have been obtained by condensation of catechol dianions with various nitrohalobenzenes (Tomita, 1945; Loudon and McCapra, 1959).

Scheme I



Pohland and Yang (1972) also used this method to prepare halogenated dioxins. This reaction was successfully applied in the present study. The condensation of 4,5-dichlorocatechol dianion with di- or trichlorodinitrobenzene formed tri- or tetrachloronitrodibenzo-*p*-dioxin. The nitro derivatives were then reduced to the corresponding amino dioxins either by zinc dust or stannous chloride in hydrochloric acid (Scheme I). These products were purified by chromatographic techniques and characterized as dioxin derivatives by means of their spectral properties. The position of the amino groups was determined by deamination via the diazonium salts to the corresponding 2,3,7-tri- and 2,3,7,8-tetrachlorodibenzo-*p*-dioxin.

The relative toxicities of these nitro and amino dioxin derivatives have been demonstrated in guinea pigs and mice (McConnell and Moore, 1976). *Although these compounds are generally less toxic than their corresponding parent chlorodioxins, they are still sufficiently toxic to justify handling with extreme caution.*

EXPERIMENTAL SECTION

Elemental analyses were performed by Atlantic Microlab, Atlanta, Ga. Melting points were taken with a Hoover Uni-Melt melting point apparatus. The proton NMR spectra were obtained with Varian FT XL-100 instrument. Gas chromatography (GC) was done on a Varian Aerograph Series 2100 instrument using an electron-capture detector (Sc^3H). A 6 ft × 2 mm i.d. glass column containing 3% OV-210 on 80–100 mesh Gas Chrom Q was used. The column temperature was maintained at 200 °C, the injector and detector temperature at 240 °C. The carrier gas flow was nitrogen at a rate of 40 mL/min. The relative retention time is expressed based on the retention time of standard 2,3,7,8-tetrachlorodibenzo-*p*-dioxin. The gas chromatography–mass spectrometric (GC–MS) analysis was performed using a Finnigan Model 9500 GC interfaced by a glass jet separator to a Finnigan