Role of Tobacco Stems in the Formation of N-Nitrosamines in Tobacco and Cigarette Mainstream and Sidestream Smoke

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Nine experimental cigarettes without filter tips were prepared with 70% of a defined blend of laminae and 30% of various types of stems, resulting in nitrate contents between 0.68 and 2.65%. N'-Nitrosonornicotine (NNN; 1.02–6.35 ppm) and the sum of tobacco-specific N-nitrosamines (TSNA; 1.95–8.57 ppm) were found to be strongly correlated with the nitrate content of tobacco (r^2 = 0.978 and 0.948). The yields of TPM, nicotine, CO, and CO_2 in mainstream smoke are not greatly influenced by the nitrate content; however, N-nitrosodimethylamine (NDMA) and NNN are correlated with the nitrate content of the tobacco (r^2 = 0.957 and 0.889). The sidestream smoke of six of the nine cigarettes delivered exceptionally high amounts of NDMA (>1000 ng/cigarette). The yields of NDMA and NNN in sidestream smoke were correlated with the nitrate content of the blend (r^2 = 0.501 and 0.767); however, other factors influence the nitrosamine yields in sidestream smoke also.

Stems constitute more than 20% of the total weight of the tobacco leaf. A few decades ago, most parts of the larger stems were discarded; however, advances in the threshing of total leaf and economic considerations have led to increased utilization of stems in the cigarette blend (Norman, 1982). Stems represent the richest source of nitrates (Neurath and Ehmke, 1964); thus, their use as well as other factors have elevated the nitrate content of the U.S.-blended cigarette (U.S. Surgeon General, 1979). During smoking, nitrate not only gives rise to nitrogen oxides, nitrogen, and ammonia but also serves as a precursor for the nitrogen atom in a number of organic compounds including N-nitrosamines (Hoffmann et al., 1979; Brunnemann and Hoffmann, 1982).

It was the objective of this study to examine the influence of tobacco stems on the yields of volatile N-nitrosamines (VNA), N-nitrosoproline (NPRO), and tobacco-specific N-nitrosamines (TSNA) in tobacco and in cigarette smoke. Experimental cigarettes for this study were made with a defined blend of laminae and with various types of stems in a ratio of 7:3. The cigarette tobaccos were analyzed for total nitrogen, nitrate, nicotine, proline, NPRO, and TSNA. The mainstream smoke (MS) analyses included FTC-TPM, nicotine, CO, CO_2, VNA, and TSNA; sidestream smoke (SS) was analyzed for VNA and TSNA. The resulting analytical data were evaluated for correlations between the concentrations of nitrate and several other tobacco components and for the yields of N-nitrosamines in tobacco and tobacco smoke.

MATERIALS AND METHODS

Apparatus. A 20-channel Phipps and Bird automatic smoking machine was employed for the determination of CO and CO_2 (Brunnemann and Hoffmann, 1974a) and TPM and nicotine (Pillsbury et al., 1969). VNA and TSNA were determined in MS generated with a 20-port automatic smoker with rotating head (H. Borgwaldt, Hamburg, West Germany). Every second port of the rotating head was connected with a nitrogen source in order to avoid the artifactual formation of nitrosamines in the headspace of the smoking machine and in the traps (Hoffmann et al., 1974). VNA and TSNA were also determined in SS produced in a smoke chamber (Brunnemann and Hoffmann, 1974b), which was connected with a single-puff piston-type smoking machine (H. Borgwaldt, Hamburg, West Germany).

A Model 543 thermal energy analyzer (TEA) from the Thermo Electron Corp. (Waltham, MA) was interfaced directly with a Model 700 gas chromatograph (Hewlett-Packard) by using modifications previously described (Brunnemann and Hoffmann, 1981).

Experimental Cigarettes. All cigarettes were 85 mm long, were without filter tips, and had the same cigarette paper. The blends used for the cigarettes consisted always of 70% laminae (50% Virginia, 30% Burley, and 20% Turkish tobacco; 0.82% NO_3^-) and 30% of various stems that were opened and cut. The stems were from regular bright leaves (1), low-nitrate bright leaves (2) and from bright leaves originating from Brazil (3), Georgia (4), and North Carolina (5). Stems from low-nitrate Burley leaves (6) and high-nitrate Burley leaves (7) were also used. For one experimental cigarette (8), 3% K_2CO_3 was added to the regular bright stems; another experimental cigarette (9) was made with additions of 0.1% ascorbic acid to low-nitrate bright stems. (The additives were introduced by treating the stems with aqueous solutions of K_2CO_3 or ascorbic acid, respectively). The cigarettes were several weeks old at the onset of this study. The cigarettes were stored for at least 24 h in a humidity chamber at 60% relative humidity and at 20–22 °C prior to tobacco analysis or smoking.

Analytical Methods. The tobacco blends were analyzed for moisture content by a modified Dean-Stark procedure (von Bethmann et al., 1961), for nitrate by using a NO_3-specific electrode (Jacin, 1970), and for nicotine by GC (Schmeltz et al., 1976). Nitric oxide was determined by GC–TEA (Adams et al., 1978). TSNA determinations were done by a GC–TEA procedure based on the method by Adams et al. (1983) but modified by using 20% Apiezon N on Chromosorb WHP, 100–120 mesh, washed with 1% CH_3ONa in methanol. Tobacco analysis for proline was carried out by N-nitrosation with subsequent GC–TEA (Brunnemann et al., 1983), for NPRO by GC–TEA (Brunnemann et al., 1983), and for total nitrogen by a Kjeldahl method (Association of Official Analytical Chemists, 1970). All the above analyses were done in duplicate.

For the smoke analyses the cigarettes were smoked individually under standard conditions. These were 1 puff/min with a duration of 2 s and a volume of 35 mL; the cigarette butt length was 23 mm. Three clearing puffs were taken after each cigarette was smoked.

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Table I. Moisture, Nicotine, Total Nitrogen, Nitrate, and Tobacco-Specific N-Nitrosamines in the Tobacco of Experimental Cigarettes[a,c]

cigarette[a]	static burning rate, mg/min	water, %	nicotine,[b] mg/g	total nitrogen,[b] % N	nitrate,[b] % stems	nitrate,[b] % blend	NNN,[b] ppm	NAT,[b] ppm	NAB,[b] ppm	NNK,[b] ppm	total TSNA,[b] ppm
1	69.2	12.1	12.0	2.17	2.01	1.07	1.86	0.81	0.06	1.23	3.95
2	70.7	5.8	12.8	2.24	0.39	0.76	1.17	0.22	n.d.	0.56	1.95
3	74.2	10.5	12.3	2.01	1.11	0.91	1.19	0.67	0.03	0.75	2.63
4	70.4	11.8	10.4	2.02	0.78	1.00	1.16	0.24	n.d.	0.54	1.94
5	69.7	13.7	13.9	2.14	0.92	0.88	1.02	0.40	0.03	0.54	1.98
6	68.2	11.7	11.6	2.13	3.16	1.35	2.60	0.39	0.06	0.79	3.83
7	83.2	10.7	9.9	2.37	6.82	2.65	6.35	0.67	0.08	1.48	8.57
8	72.8	10.8	12.9	2.24	0.84	0.79	1.24	0.42	n.d.	0.69	2.35
9	79.1	11.0	11.6	2.03	0.38	0.68	1.04	0.42	n.d.	0.48	1.94
av			11.9 ± 1.2	2.15 ± 0.12							

[a] Compositions of cigarette blends (70% laminae, 30% stems): 1, standard laminae plus bright stems (Virginia); 2, standard laminae plus low-nitrate bright stems; 3, standard laminae plus bright stems (Brazil); 4, standard laminae plus bright stems (Georgia); 5, standard laminae plus bright stems (North Carolina); 6, standard laminae plus low-nitrate Burley stems; 7, standard laminae plus high-nitrate Burley stems; 8, standard laminae plus bright stems (Virginia) containing 3% K_2CO_3; 9, standard laminae plus low-nitrate bright stems containing 0.1% ascorbic acid. [b] Moisture adjusted. [c] Abbreviations: NNN = N'-nitrosonornicotine; NAT = N'-nitrosoanatabine; NAB = N'-nitrosoanabasine; NNK = 4-(methylnitrosamino)-1-(3-pyridyl)-1-butanone; TSNA = tobacco-specific N-nitrosamines; PRO = proline; NRPO = N-nitrosoproline.

Table II. Analytical Data for the Tobaccos of Experimental Cigarettes[a,d]

cigarette	NO_3, %	nicotine, %	NNN, ppm	% nitrosation × 10^{-3} [b]	total TSNA, ppm	PRO, ppm	NPRO, ppm	% nitrosation × 10^{-2} [c]
1	1.07	1.20	1.86	14.20	3.95	1330	1.13	6.80
2	0.76	1.28	1.17	8.36	1.95	1450	0.55	3.03
3	0.91	1.23	1.19	8.85	2.63	2230	0.75	2.69
4	1.00	1.04	1.16	10.20	1.94	1610	1.00	4.97
5	0.88	1.39	1.02	6.71	1.98	1760	0.55	2.50
6	1.35	1.16	2.60	20.50	3.80	1390	2.60	15.0
7	2.65	0.99	6.35	58.70	8.57	1320	2.30	13.9
8	0.79	1.29	1.24	8.80	2.35	1500	0.29	1.54
9	0.68	1.16	1.04	8.20	1.94	1860	1.30	5.59

[a] For composition of tobacco blends of cigarettes, see footnote a of Table I (values moisture adjusted). [b] Percent of nitrosation of nicotine to NNN. [c] Percent of nitrosation of PRO to NPRO. [d] Abbreviations: NNN = N'-nitrosonornicotine; TSNA = tobacco-specific N-nitrosamines; PRO = proline; NPRO = N-nitrosoproline.

Table III. Statistical Correlations between Constituents in Tobacco Blends (r^2) of Experimental Cigarettes

	PRO	TSNA	NNN	NO_3	NONIC[a]	NOPRO[b]	NIC
NPRO	0.157	0.476[c]	0.519[c]	0.513[c]	0.499[c]	0.974[d]	0.449[c]
PRO		0.227	0.241	0.208	0.221	0.272	0.079
TSNA			0.970[d]	0.948[d]	0.960[d]	0.559[c]	0.381[c]
NNN				0.978[d]	0.996[d]	0.599[c]	0.419[c]
NO_3					0.979[d]	0.590[c]	0.447[c]
NONIC[a]						0.572[c]	0.457[c]
NOPRO[b]							0.427[c]

[a] NONIC = % nicotine (NIC) N-nitrosated to NNN. [b] NOPRO = % proline N-nitrosated to NPRO. [c] $P < 0.05$ using linear regression analysis. [d] $P < 0.005$ using linear regression analysis.

GC determinations required 2 × 4 cigarettes each for establishing CO and CO_2 (Brunnemann and Hoffmann, 1974a), moisture in TPM (Schultz and Spears, 1966), and nicotine in smoke (Rathkamp et al., 1973). Four times 4 cigarettes each were smoked for FTC-TPM determinations by the Cambridge filter method (Pillsbury et al., 1969), 2 × 10 cigarettes for VNA analysis by GC–TEA (Brunnemann et al., 1977), and 2 × 60 cigarettes each for TSNA determinations by GC–TEA (Adams et al., 1983).

For the determination of VNA and TSNA in SS, we used the standard smoking conditions for the generation of MS, and directed air at 1500 mL/min through the SS collection chamber and gas wash bottles containing the trapping solution (Brunnemann and Hoffmann, 1974b). VNA in SS were determined from 2 × 10 cigarettes by the GC–TEA method (Brunnemann et al., 1977) and TSNA from 60 cigarettes by the GC–TEA method (Adams et al., 1983).

As in our previous studies, labeled compounds were used as internal standards. These were nicotine-*methyl*-^{14}C for nicotine, N-nitrosodimethylamine-*U*-^{14}C for VNA, N-nitrosonornicotine-2'-^{14}C for TSNA and N-nitrosoproline-*U*-^{14}C for NPRO. Proline-*U*-^{14}C served as a monitor for the N-nitrosation reactions. In our studies with radioactive carcinogens we followed the regulations given in the New York State Department of Health Sanitary Code, Chapter 1, part 16, and the Safety Manual of the American Health Foundation.

RESULTS AND DISCUSSION

Tobacco Blends. Table I lists the data for the moisture content, nicotine, total nitrogen, nitrate, and tobacco-

Table IV. Smoking Parameters

cigarette	weight, mg	draw resistance, mm of H_2O	puffs, no.	nicotine, mg	FTC-TPM,[a] mg
1	971	54	7.19	1.01	16.5
2	960	54	7.00	0.92	16.9
3	911	48	6.69	1.16	16.4
4	1008	59	7.25	0.93	16.5
5	1003	60	7.56	0.99	15.8
6	969	53	7.38	0.92	16.3
7	988	51	6.88	0.88	16.9
8	943	53	7.19	0.98	17.3
9	987	59	7.31	0.88	16.9

[a] Wet TPM minus water minus nicotine.

Table V. Carbon Monoxide, Carbon Dioxide, Nitric Oxide, and Volatile *N*-Nitrosamines in Mainstream Smoke[a,b]

cigarette	CO, vol %	CO, mg	CO_2, vol %	CO_2, mg	NO, μg/cig	NDMA, ng	NEMA, ng	NPYR, ng	total VNA, ng
1	4.02	14.2	8.28	45.8	256	6.5	0.5	25	32
2	3.67	12.5	7.78	41.7	162	4.2	0.4	12	17
3	4.36	15.4	8.67	48.0	203	5.0	0.5	12	17
4	4.20	15.0	8.56	48.0	181	5.0	0.4	10	15
5	4.19	15.0	8.19	45.9	192	5.0	0.5	12	17
6	3.40	12.3	7.30	41.6	244	9.0	1.0	19	29
7	4.06	14.1	8.42	45.9	464	14.6	1.2	29	45
8	3.94	14.2	8.01	45.3	220	5.8	0.4	32	38
9	4.03	14.2	8.10	44.8	143	4.3	0.7	11	16

[a] For composition of tobacco blends of cigarettes, see footnote *a* of Table I. [b] Abbreviations: NDMA = *N*-nitrosodimethylamine; NEMA = *N*-nitrosoethylmethylamine; NPYR = *N*-nitrosopyrrolidine; VNA = volatile *N*-nitrosamines.

Table VI. Tobacco-Specific *N*-Nitrosamines (TSNA) in Mainstream Smoke (ng/Cigarette)[a]

cigarette	NNN	NAT	NAB	NNK	total TSNA
1	156	120	14	87	380
2	92	84	11	54	240
3	98	85	10	50	240
4	81	82	10	46	220
5	90	89	10	47	230
6	230	130	16	160	540
7	390	167	18	240	820
8	125	102	15	84	330
9	96	85	11	61	250

[a] For composition of tobacco blends of cigarettes, see footnote *a* of Table I; for abbreviations, see footnote *d* of Table II. Each value represents an average of two runs; the reported data are isolated amounts; recovery rate 70-80%.

specific *N*-nitrosamines of the nine cigarette tobaccos employed in this study. The values for nicotine varied between 9.9 and 13.9 mg/g of tobacco with an average of 11.9 ± 1.2 mg/g of tobacco. These results indicate that the differences in the TSNA values of the tobaccos and of the MS and SS of these cigarettes may be influenced not only by the variations in the nitrate content alone (0.68–2.65%) but also by those of the alkaloids. Table II presents results for NNN, TSNA, proline, and NPRO and the degree of N-nitrosation of nicotine to *N'*-nitrosonornicotine and proline to *N*-nitrosoproline. The correlations (r^2 values) between tobacco constituents revealed that the nitrate content of the cigarette blend, which is greatly influenced by the use of stems, is a highly significant factor for the yields of NNN and TSNA in the experimental blends (Table III). In an earlier study with 14 commercial products, we found that the nitrate content of the tobacco is less strongly correlated with the yields for NNN (r^2 = 0.592) and TSNA (r^2 = 0.621) (Brunnemann et al., 1983) than in this study with tobacco blends containing 30% of stems (r^2 = 0.978 and 0.948). This finding emphasizes the important role of the nitrate content of the stems in the formation of TSNA during tobacco processing.

Cigarette Mainstream Smoke. Tables IV–VI report the analytical data for the MS of the nine experimental cigarettes including values for nicotine, FTC-TPM, CO, CO_2, NO, volatile *N*-nitrosamines, and tobacco-specific *N*-nitrosamines. Figure 1 depicts a GC–TEA trace of the

Table VII. VNA and TSNA in Sidestream Smoke (ng/Cigarette)[a]

cigarette	VNA: NDMA[b]	VNA: NPYR	VNA: total VNA	TSNA: NNN	TSNA: NAT	TSNA: NAB	TSNA: NNK	TSNA: total TSNA
1	460	80	540	141	61	35	217	454
2	1000	140	1140	110	72	20	201	403
3	1110	160	1270	123	75	15	233	446
4	910	230	1140	119	84	22	278	503
5	1170	230	1400	137	91	22	293	543
6	1180	260	1440	114	94	22	228	458
7	1880	500	2380	390	220	40	540	1190
8	570	140	710	131	84	16	215	446
9	1210	240	1450	185	97	20	250	552

[a] For abbreviations, see Tables II and V. [b] The NDMA peaks in the chromatograms were too large for clearly resolving the NEMA peaks.

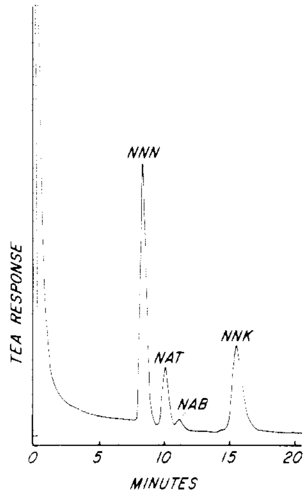

Figure 1. GC–TEA trace of the tobacco-specific nitrosamines isolated from the mainstream smoke of cigarette 7.

TSNA fraction from the MS of cigarette 7. From these results, it appears that the nitrate content of the stems does not have a major influence on the smoke yields for nicotine, FTC-TPM, CO, CO_2. However, as expected (Tso et al., 1975; Brunnemann and Hoffmann, 1982), the nitrate content of the tobacco blend is a major determining factor for the mainstream smoke yields of NO ($r^2 = 0.935$), volatile *N*-nitrosamines (NDMA; $r^2 = 0.957$) and the tobacco-specific N-nitrosamines (NNN; $r^2 = 0.922$). The high values for the individual TSNA in the smoke of the cigarette with the high-nitrate Burley stems (2.65%) are well in line with the overall findings.

Cigarette Sidestream Smoke. Table VII lists the analytical data for VNA and TSNA in sidestream smoke. We have chosen the high flow rate of 1500 mL/min through the chamber in order to obtain maximal differences in nitrosamine yields in SS (Rühl et al., 1980). In previous studies, the *N*-nitrosodimethylamine released into the sidestream smoke from cigarettes did not exceed 1000 ng/cigarette (Brunnemann et al., 1980; Rühl et al., 1980). In this study, however, five out of nine cigarettes delivered more than 1000 ng of NDMA/cigarette. This observation suggests that cigarettes that contain nitrate-rich stems can deliver SS that is rich in *N*-nitrosodimethylamine, a potent animal carcinogen (Magee et al., 1976). The nitrate contents of the tobaccos of the nine experimental cigarettes influenced the yields in sidestream smoke of VNA (NDMA; $r^2 = 0.501$) and TSNA (NNN; $r^2 = 0.767$). However, other factors, not determined in this study, have most likely also an impact on the SS yield of these *N*-nitrosamines. The addition of 0.1% ascorbic acid to tobacco stems (cigarette 9) did not inhibit the formation of either VNA or TSNA in the smoke. Ascorbic acid is a known inhibitor for the N-nitrosation of amines in solution (Mirvish et al., 1972).

The overall findings of this study suggest that the nitrate content of the stems in cigarette blends greatly influences the formation of volatile and tobacco-specific *N*-nitrosamines during the processing of tobacco and their pyrosyntheses during generation of mainstream smoke and sidestream smoke. The utilization of stems with low nitrate content in cigarette blends appears desirable. This may be achieved by selection of low-nitrate stems or by reduction of the nitrate content in the stems by special fermentation processes, water extraction, or other means.

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Registry No. NNN, 16543-55-8; nicotine, 54-11-5; NDMA, 62-75-9; NAT, 71267-22-6; NAB, 1133-64-8; NNK, 64091-91-4; PRO, 147-85-3; NPRO, 7519-36-0; CO, 630-08-0; CO_2, 124-38-9; NO, 10102-43-9; NEMA, 10595-95-6; NPYR, 930-55-2; nitrate, 14797-55-8.

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