# A Study on Precursors of Nitric Oxide in Sidestream Smoke\*

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## SUMMARY

By a method of thermal analysis and smoke analysis of cellulose cigarettes treated with various nitrogenous compounds, it was found that nitric oxide in sidestream smoke is formed not only from nitrates but also from amino acids and water-insoluble protein, while little of the nitric oxide in mainstream smoke is formed from these organic nitrogenous compounds.

# ZUSAMMENFASSUNG

Mittels thermischer Analyse und Rauchanalyse wurde an Cellulosezigaretten, die mit verschiedenen Stickstoffverbindungen behandelt worden waren, festgestellt, daß im Nebenstromrauch enthaltenes Stickoxid nicht nur aus Nitraten, sondern auch aus Aminosäuren und aus in Wasser nicht löslichem Eiweiß entsteht, während das Stickoxid des Hauptstromrauches nur in geringem Maße von diesen organischen Stickstoffverbindungen herstammt.

## RESUME

Au moyen d'une méthode d'analyse thermique et d'une analyse de la fumée sur des cigarettes de cellulose ayant été traitées auparavant avec différents composés azotés, on a établi que le monoxyde d'azote contenu dans le flux de fumée secondaire est créé non seulement à partir des nitrates mais encore à partir d'acides aminés et de protéines insolubles dans l'eau, tandis que l'oxyde d'azote présent dans le flux de fumée principal n'est formé qu'en faible quantité à partir de ces constituants organiques azotés.

# INTRODUCTION

It is well known that a large portion of the nitrogen oxides in the mainstream smoke (MS) and sidestream smoke (SS) of cigarettes is nitric oxide (NO) (1, 2). Since the amount of NO in mainstream smoke is quantitatively related to the level of nitrate in tobacco (1-4), nitrate has been regarded as a major source of NO in mainstream smoke. On the other hand, it has been suggested that NO in sidestream smoke might be pro-

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duced from other sources besides nitrate (1, 4, 5). Recently, NORMAN et al. have demonstrated that there remained a significant amount of NO in both mainstream and sidestream smoke which could not have arisen from nitrate, and suggested that part of the residual NO probably arose from the oxidation of atmospheric nitrogen (4). In sidestream smoke, however, atmospheric nitrogen contributes to only part of the residual NO. Therefore, the nitrogen source of NO in sidestream smoke has not yet been completely clarified.

In order to identify other nitrogen sources, our investigation was carried out by a method of thermal analysis and smoke analysis of cellulose cigarettes to which various nitrogen compounds were added.

## MATERIALS AND METHODS

## Cigarette Samples

Unblended non-filter cigarettes differing in nitrate content were prepared. Weight and nitrate content of the samples are shown in Table 1. Additionally, handmade cellulose cigarettes composed of filter paper shreds treated with  $K_2CO_3$  as a burning additive were used. A given amount of various nitrogenous compounds such as potassium nitrate, asparagine, glutamic acid, proline and nicotine, or the water-insoluble residue of tobacco powder was added to the filter paper to examine the possibility of their acting as a source of NO in smoke. All cigarettes were 70 mm in length and 8 mm in diameter and were wrapped with ordinary cigarette paper with a permeability of 10 cm<sup>3</sup>/min/cm<sup>2</sup>/10 cm H<sub>2</sub>0. The nitrate content was measured with a nitrate ion selective electrode<sup>\*</sup>.

\* Orion Research Inc., Cambridge, Mass. (Model 901).

\*\* Monitor Labs. Inc., San Diego, Calif. (Model 8440).

Figure 1.

Schematic diagram of measurement of nitrogen oxides in cigarette smoke.



#### Measurements of Nitrogen Oxides in Cigarette Smoke

Figure 1 illustrates the scheme for the simultaneous measurement of NO and NO, in the sidestream smoke (SS) and NO<sub>x</sub> in the mainstream smoke (MS) of cigarettes. Cigarettes were lit by electric heater and smoked to a 30 mm butt in a smoking chamber (volume: about 1000 ml) by means of a syringe-type smoking machine. The flow of air into the chamber was held constant at 2000 ml/min, which was necessary to ensure the same number of puffs as under standard smoking conditions without the chamber, and to prevent sidestream smoke from accumulating in the chamber. The sidestream smoke diluted with air was passed through a Cambridge filter pad attached to the outlet end of the chamber to remove particulate matter. Part of the sidestream gas phase flowing out of the chamber was continuously passed through a chemiluminescence NO/ NO, analyzer\*\* at a constant sampling rate. On the

Cigarette		Nitrate content (dry basis)	Cigarette weight	Puff count	Nitrogen oxides (µmol/cigarette)			
					Mainstream smoke (MS)	Sidestream smoke (SS)		SS/MS ratio of NO
		(%)	(g)		NOx	NO	NOx	·
Flue-cured	A	0.075	1.07	10.5	2.41	17.0	17.8	7.1
	в	0.057	0.98	9.7	2.49	29.9	30.9	12.0
	С	0.11	0.92	8.0	1.43	18.3	17.1	12.8
Domestic	D	0.41	0.73	7.0	2.93	25.2	24.8	8.6
	Е	0.69	0.86	8.3	5.25	29.0	29.1	5.5
	F	1.57	0.65	4.8	6.66	23.0	24.9	3.5
Burley	G	0.58	0.79	6.5	3.79	21.2	21.5	5.6
	н	0.26	0.79	6.0	3.04	22.5	21.1	7.4
	I	0.48	0.76	6.0	2.14	19.8	19.8	9.3
	J	0.41	0.77	5.8	2.00	18.9	18.7	9.5
	к	3.32	0.81	5.3	17.50	65.2	66.5	3.7

		Tab	le 1.		
Amount of	nitrogen	oxides in	n unblended	cigarette	smoke

#### Figure 2.

Relationship between NO yield of mainstream smoke and nitrate content in tobacco.



\* The µmol/puff values are obtained from the values as shown in Table 1.

other hand, individual puffs of mainstream smoke were passed through a Cambridge filter pad into the sampling stream (air: 1000 ml/min) of a chemiluminescence NO<sub>x</sub> analyzer<sup>+</sup>. NO and NO<sub>x</sub> levels were determined from the output peak areas on the basis of a calibration curve.

It could be experimentally ascertained by this instrument that the measurement of  $NO_x$  was only slightly interfered with by CO,  $CO_2$  and nitrogen compounds, such as ammonia and hydrogen cyanide.

As shown in Table 1, no significant difference in the amounts of NO and NO<sub>x</sub> could be observed in sidestream smoke. Therefore, nitric oxide in sidestream smoke was determined in the condition of NO mode. As for the mainstream smoke, little difference in the values determined was observed between NO<sub>x</sub> and NO mode in the NO/NO<sub>x</sub> analyzer, which agreed with the findings by other workers (2, 6). Hence, hereafter NO<sub>x</sub> in mainstream smoke will be referred to as NO.

## Formation Profile of NO

Temperature-yield profiles of NO and weight loss (derivative thermogravimetry (DTG)) during the thermal decomposition of tobacco and cellulose containing various nitrogenous compounds were measured simultaneously to elucidate the process by which NO is

+ Yanaco (Model E11-77).

Figure 3.

Relationship between NO yield of sidestream smoke and nitrate content in tobacco.



formed. With a thermobalance<sup>++</sup>, about 10 mg of a ground sample were heated at a constant heating rate of 5 °C/min in an atmosphere of air or helium with a flow rate of 100 ml/min. After removal of particulate matter with a Cambridge filter pad, the resulting gas was passed continuously through the sampling stream of a chemiluminescence NO/NO<sub>x</sub> analyzer<sup>\*\*</sup> at the rate of 500 ml/min for analysis.

#### **RESULTS AND DISCUSSION**

Nitrogen Oxides in Mainstream and Sidestream Smoke of Unblended Cigarettes

As shown in Table 1, the ratio of the NO level in sidestream smoke to that in mainstream smoke (SS/MS ratio) was in the range 3.5 to 12.8. This ratio is significantly higher than that of the weight of tobacco consumed between puffs to that during puffs, reported in the range 1.1 to 3.6 (7–9). This indicates that the rate of NO formation (the ratio of NO yield formed to the amount of tobacco consumed) between puffs is higher than that during a puff.

Figures 2 and 3 show mainstream NO yield per puff and sidestream NO yield per unit time as a function of the nitrate content in tobacco. These plots show that both mainstream NO yield per puff and sidestream NO yield per unit time are linearly related to nitrate content. In Figures 2 and 3 the extension of the line back to zero yields a value of 0.09  $\mu$ mol/puff for main-

<sup>++</sup> Rigaku Denki Co. (Model TG-DSC-8085D1).

<sup>\*\*</sup> Monitor Labs. Inc., San Diego, Calif. (Model 8440).

#### Figure 4.

Typical temperature-yield profiles for NO and weight loss (derivative thermogravimetry (DTG)) curves of tobacco (domestic tobacco: F).



stream smoke and 2.15 µmol/min for sidestream smoke. This suggests that a certain amount of NO is formed from nitrogen sources other than nitrate, particularly for NO in sidestream smoke.

#### Figure 6.

influence of nitrate content on the amount of NO evolved from tobacco heated at 5 °C / min in air atmosphere.



NO evolved in the lower temperature region.
 NO evolved in the higher temperature region.

#### Figure 5.

Influence of nitrate content on the amount of NO evolved from tobacco heated at 5 °C / min in helium atmosphere.



# Formation Profiles of Nitric Oxide by Thermal Analysis

Figure 4 illustrates temperature-yield profiles for NO and weight loss (derivative thermogravimetry (DTG)) curves obtained by heating tobacco at 5 °C/min in an atmosphere of air or helium with a flow rate of 1000 ml/min. In the helium atmosphere, NO was produced in the temperature range of about 210-400 °C, showing a peak at about 315 °C. This temperature range approximately coincides with that of the rapid weight loss of tobacco due to thermal decomposition (10, 11). The amount of NO formed in this region is related to the nitrate content of the tobacco as shown in Figure 5. It is reasonable to conclude that in such an inert atmosphere NO is formed by the thermal decomposition of nitrate in the tobacco. In an atmosphere of air, NO was formed in the two temperature regions of 210-360 °C and 360-550 °C as shown in Figure 4. The lower temperature region corresponds to the thermal decomposition of the tobacco. The amount of NO formed in this region is dependent on the nitrate content as shown in Figure 6. On the other hand, a large amount of NO was produced in the higher temperature region, where carbonaceous residue left after the thermal decomposition of the tobacco was oxidized leaving ash (11, 12). As can be seen in Figure 6, the amount of NO formed in this region was independent of the nitrate content of the tobacco. These results imply that a considerable amount of NO can be formed by the oxidation of nitrogen sources other than nitrate in the presence of oxygen. To clarify this point, cellulose powders to which various organic nitrogen compounds had been added and, in addition, water-insoluble tobacco residue were subjected to EGA (evolved gas analysis) and weight loss (derivative thermogravimetry (DTG)) analysis. The results are shown in Figure 7. From the potassium nitrate added to cellulose, a considerable amount of NO was produced in both air and helium atmospheres (Figure 7 (a and b)). The temperature region of NO formation showing a peak at about 350 °C closely corresponds to the region of major weight loss of cellulose due to thermal decomposition (10, 11). In an air atmosphere, however, the region of NO formation shifts to lower temperatures showing a peak at about 310 °C. From the cellulose containing proline, glutamic acid or asparagine, NO was produced only in the presence of oxygen (Figure 7 (c, d and e)). The production of NO from these amino acids occurs over a wide temperature range (400-600 °C). Since the thermal decomposition temperature of these amino acids is much lower than 400 °C (13), the formation of NO from these amino acids seems to be caused by the oxidation of nitrogen-containing substances left after the complicated thermal reactions of the amino acids. Formation of NO from nicotine added to the cellulose was not observed even in an oxygen-containing atmosphere. Figure 8 shows NO temperature-yield profile and weight loss (derivative thermogravimetry (DTG)) curve for the water-insoluble residue prepared from Burley tobacco. The water-insoluble residue is free from nitrates and contains water-insoluble introgen compounds such as proteins. The production of NO from the residue was observed only in the presence of oxygen in the temperature region of 360-530 °C, showing a peak at 468 °C and having a profile similar to those obtained for amino acids. The conversion efficiencies of nitrogen into NO obtained from the temperature-yield profiles shown in Figures 7 and 8, are summarized in Table 2. The conversion efficiencies of amino acids and water-insoluble nitrogen compounds were lower than that of nitrate.

# Nitric Oxide Formation in Cellulose Cigarettes

Table 3 shows nitric oxide formation from cellulose cigarettes to which were added various organic nitrogen compounds. An appreciable amount of NO was found in both mainstream and sidestream smoke of cellulose cigarettes, even when nitrogen free. These values correspond to 0.09 umol/puff for mainstream smoke and 0.30 µmol/min for sidestream smoke. The NO yield per puff in the mainstream smoke was the same as that obtained from extension of the calculated values to zero nitrate (Figure 2). It seems to be caused by the oxidation of atmospheric nitrogen, as suggested by NOR-MAN et al. (4). However, the NO yield per unit burning time in the sidestream smoke from the pure cellulose cigarette was no more than one seventh of that obtained from tobacco cigarettes as shown by the extrapolation back to zero in Figure 3. This suggests that the NO in sidestream smoke is formed by a process other than thermal decomposition of nitrate and/or oxidation of atmospheric nitrogen.

The addition of potassium nitrate to cellulose cigarettes enhanced the formation of NO in both mainstream and sidestream smoke, while nicotine hardly contributed to NO formation of either smoke stream. The addition of amino acids and nitrate-free water-insoluble residue of tobacco increased the NO yield of sidestream smoke significantly, but it had no noticeable effect on the NO yield of mainstream smoke. From the results and thermal analysis, it can be assumed that NO in sidestream smoke was formed not only by thermal decomposition of nitrate but also by oxidation of organic nitrogenous compounds such as amino acids and proteins.

BAKER and co-workers have reported on the difference in the contours of oxygen concentration in the burning coal for a puff and for static smouldering, indicating an oxygen deficiency in the interior region during a puff (14-17). In addition, it has been reported by JOHNSON that incorporation of atmospheric oxygen into the var-

Samples*	Atmosphere	Nitrogen content (%)	Amount of NO (µmol/g)	Conversion efficiency** (%)			
2.9% KNO <sub>3</sub> + cellulose	helium air	0.406	112.8 118.0	39.0 40.0			
2.7% asparagine + cellulose	air	0.506	25.6	7.1			
4.5% glutamic acid + cellulose	air	0.432	22.4	7.2			
4.2% proline + cellulose	air	0.516	51.0	14.0			
Water-extracted residue $\begin{cases} (A) \\ (F) \end{cases}$	air air	2.08 2.58	127.0 199.0	8.5 11.0			
L (G)	air	1.82	115.2	8.9			

 Table 2.

 Conversion of nitrogen sources into NO when heated at the rate of 5 °C/min.

\* Percentages of compound added to the actual weight basis of cellulose.

\*\* (NO evolved / nitrogen added) × 100.





a: 2.9% KNO3 + cellulose (in air).

- b: 2.9% KNO<sub>3</sub> + cellulose (in helium).
- c: 2.7% asparagine + cellulose (in air).
- d: 4.5% glutamic acid + ceilulose (in air).
- e: 4.2% proline + cellulose (in air).

Sample weight: 10 mg. Heating rate: 5 °C/min.



#### Figure 8.

Temperature-yield profile for NO and weight loss (derivative thermogravimetry (DTG)) curve of nitrate-free water-extracted residue of tobacco (Burley: G).



ious components of sidestream smoke was higher than that into the components of mainstream smoke (18). From these facts, it seems reasonable to conclude that a considerable amount of the NO in sidestream smoke is formed by the oxidation of organic nitrogenous compounds. This also explains the high SS/MS NO ratio observed for cigarettes with a low nitrate content as shown in Table 1.

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	Nitrogen	Cigarette weight (g)	Puff count	Nitric oxide (µmol/cigarette)		
Cellulose cigarette *	(%)			Mainstream smoke (MS)	Sidestream smoke (SS)	
Nitrogen free	0	0.56	3	0.29	0.91	
1.2% KNO3	0.17	0.74	5	4.22	8.92	
2.0% asparagine	0.42	0.62	4	0.05	3.38	
4.0% glutamic acid	0.38	0.62	4	0.13	3.58	
3.4% proline	0.41	0.56	4	0.10	2.31	
3.7% nicotine	0.63	0.63	3	0.02	0.52	
37% water-extracted residue (flue-cured)	0.77	0.90	5.4	0.08	5.09	
36% water-extracted residue (domestic)	0.92	0.90	6	0.08	10.82	
36% water-extracted residue (Burley)	0.66	0.90	5.6	0	5.72	

 Table 3.

 Yield of nitric oxide from cellulose cigarettes containing nitrogen compounds added.

\* Percentages of compound added to the actual weight basis of cellulose.

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