Decreased Concentrations of *N*-Nitrosodiethanolamine and *N*-Nitrosomorpholine in Commercial Tobacco Products

Klaus D. Brunnemann* and Dietrich Hoffmann

Naylor Dana Institute for Disease Prevention, American Health Foundation, Valhalla, New York 10595

In 1981, the carcinogens *N*-nitrosodiethanolamine (NDELA) and *N*-nitrosomorpholine (NMOR) occurred in U.S. tobacco products in concentrations up to 6800 and 700 ppb, respectively. Since then, use of the sucker-growth inhibitor maleic hydrazide–diethanolamine, the precursor for NDELA, has been gradually reduced and morpholine, the precursor for NMOR, has been eliminated from the manufacture of smokeless tobacco. Consequently, a gradual reduction of NDELA in tobacco products to less than 100 ppb and NMOR to less than 40 ppb has been observed. The effectiveness of these measures demonstrates that practical steps can be taken toward the reduction of carcinogenic *N*-nitrosamines in tobacco products.

INTRODUCTION

Secondary and tertiary amines give rise to *N*-nitrosamines during harvesting and processing of tobacco as well as during smoking (Hoffmann et al., 1984; Burton et al., 1989). The precursors for nitrosamines in tobacco products are either *Nicotiana* alkaloids, or proteins, or agricultural chemicals and contaminants with secondary or tertiary amino groups. Many *N*-nitrosamines are animal carcinogens (Preussmann and Stewart, 1984; Hecht and Hoffmann, 1989). Their metabolic activation occurs in human tissues as well as in vivo in animals. The resulting metabolites are highly active species that bind to DNA (Preussmann and Stewart, 1984; Hecht and Hoffmann, 1989). On the basis of these findings it is desirable to reduce the precursor amines in tobacco products. In the case of agricultural chemicals and contaminants containing secondary amines, this can be achieved by eliminating the use of such agents during the cultivation of tobacco and/or during manufacturing of tobacco products. In September 1981, the U.S. Environmental Protection Agency mandated the ban of maleic hydrazide–diethanolamine as a sucker-growth inhibitor for tobacco. In 1982, it was reported that U.S. snuff is contaminated with morpholine (Brunnemann et al., 1982). During processing and/or packaging of tobacco products, diethanolamine gives rise to *N*-nitrosodiethanolamine (NDELA), while morpholine is nitrosated to *N*-nitrosomorpholine (NMOR). According to the International Agency for Research on Cancer, both NDELA and NMOR are animal carcinogens and are "possibly carcinogenic to humans" (IARC, 1987).

Since 1981, we have monitored the concentrations of NDELA in the tobacco of three leading cigarettes, one leading snuff, and one leading chewing tobacco. We have also routinely determined the concentrations of NMOR in a leading snuff brand on the U.S. market. It was our goal to document the effectiveness of the ban of the diethanolamine formulation in respect to NDELA in U.S. tobacco products and to create awareness of the fact that morpholine is a precursor of NMOR and must therefore be removed.

MATERIALS AND METHODS

All chemicals and solvents were analytical reagents of the highest purity from J. T. Baker Chemical Co., Phillipsburg, NJ, and Fischer Scientific Co., Fair Lawn, NJ. Acetonitrile and *N,O*-bis(trimethylsilyl)trifluoroacetamide (BSTFA) with 1% trimethylchlorosilane (TMCS) were purchased from Pierce Chemical Co., Rockford, IL. *N,N*-Bis(2-hydroxypropyl)nitrosamine (2-HOPNA) was purchased from the NCI Chemical Carcinogen Repository, Midwest Research Institute, Kansas City, MO.

All tobacco products were purchased on the open market in Westchester County, NY. We ascertained that brand name products purchased at various times derived from different production batches. The tobacco products were stored in a cold room and were analyzed within 3 months of purchase.

Moisture in the tobacco products was determined according to the procedure of von Bethmann et al. (1961), and NDELA (until August 1988) and NMOR contents were determined according to earlier published methodology by GC–TEA (Brunnemann and Hoffmann, 1981; Brunnemann et al., 1982).

As of February 1989, the NDELA procedure was modified as follows: to 15–20 g of tobacco was added 400 mL of ethyl acetate containing 1 g of α-tocopherol (as nitrosation inhibitor) and 1 mL 2-HOPNA (5 ppm, as internal standard). The mixture was extracted overnight, filtered over Celite 545, dried with anhydrous sodium sulfate, and concentrated by rotary evaporation to approximately 3 mL. This concentrate was then chromatographed on a small column containing 50 g of silica gel (40–140 mesh), prewashed with cyclohexane, dichloromethane, ethyl acetate, and methanol. The column was first eluted with 100 mL of ether–hexane (70:30), yielding a fraction that contained mostly chlorophyll. The column was then eluted with 250 mL of ethyl acetate–methanol (96:4), resulting in a fraction containing NDELA and 2-HOPNA. Both fractions were then analyzed separately according to an earlier published method (Brunnemann and Hoffmann, 1981). The gas chromatographic conditions were as follows: 12 ft × 1/4 in. o.d. (2 mm i.d.) glass column packed with 3% OV-225 on Supelcoport (80–100 mesh); injection port 190 °C, oven 120 °C for 1 min, then programmed to 180 °C at 5 °C/min; a thermal energy analyzer (TEA) served as detector. Under these conditions, 2-HOPNA and NDELA had retention times of 14.0 and 15.4 min, respectively. All determinations were done in duplicate.

RESULTS AND DISCUSSION

The concentrations of NDELA in the tobacco of three leading cigarettes, a popular moist snuff, and a popular chewing tobacco sold in the United States at 15 time points between February 1981 and February 1990 are listed in Table I. Although major fluctuations are observed in the NDELA concentrations between some time points, there is a major trend of reduction of NDELA in U.S. products. The fluctuation in the NDELA values from year to year, especially with respect to the February 1989 values (Table I), is not due to the analytical procedure, but is possibly related to the utilization of tobacco that had been in storage. Specifically, NDELA in cigarette tobaccos was

0021-8561/91/1439-0207$02.50/0

Table I. NDELA in Tobacco (on the Basis of Dry Tobacco Weight)

	yield, ppb				
date	cig A, NF, 85 mm	cig B, F, 85 mm	cig C, F, 85 mm	fine-cut snuff	chewing tobacco
Feb 1981	115	194		6840	224
Feb 1983	99	132	232	3035	151
Nov 1983	81	215	248	982	168
Aug 1984	95	187	154	4260	165
Feb 1985	91	138	175	2900	83
Aug 1985	94	150	68	2040	166
Feb 1986	135	360	136	5460	116
Aug 1986	60	246	185	1690	236
Feb 1987	28	72	56	788	145
Aug 1987	75	139	117	253	128
Feb 1988	39	79	75	114	75
Aug 1988	66	90	51	287	92
Feb 1989	300	732	279	426	348
Aug 1989	66	127	102	189	66
Feb 1990	53	94	84	94	74
% change since 1981	-54	-52	-64[a]	-99	-67

[a] Percent change since Feb 1983 (232 ppb).

Table II. NMOR in Fine-Cut Snuff (on the Basis of Dry Tobacco Weight)

year	yield, ppb	year	yield, ppb
1981	690	1986/87	nd[a]
1984	29.4	1987	43
1984/85	238	1990	nd[a]
1985	29		

[a] nd, not detected (<2 ppb).

reduced by 52–64%; NDELA in smokeless tobaccos was reduced by 67–99%. In eight commercial snuff brands analyzed in 1986, the levels of NDELA had already decreased to 30–1100 ppb (Hoffmann et al., 1987). The decrease of NDELA and NMOR in tobacco products is not necessarily caused by a decrease in precursor amines alone but also may be associated with changes in the processing of tobacco. This may also be one reason for the reduction of the tobacco-specific *N*-nitrosamines in the two leading U.S. moist snuff brands (≃90% of the market) since 1980 without concurrent changes in the concentration of the alkaloids (Hoffmann et al., 1990). The presence of up to 100 ppb of NDELA in tobacco products sold in 1990 may be, at least partially, due to contamination with diethanolamine from sources other than MH-30. Diethanolamine is a major industrial chemical (U.S. production in 1988 ≃ 276 000 tons) that is utilized in paints, plastics, packagings, and cosmetics (U.S. International Trade Commission, 1989).

In the past, NMOR has primarily been found in U.S. snuff. Its major precursor is the morpholine in wax layers of the cardboard that was used for snuff boxes (Brunnemann et al., 1982). Between 1981 and 1990, the level of NMOR in a leading U.S. moist snuff brand decreased from about 700 ppb to an undetectable level (≤2 ppb) in 1990 (Table II). In eight commercial snuff brands analyzed in 1986 we found only three products with traces of NMOR (9–39 ppb; Hoffmann et al., 1987). The significant decrease of NMOR in U.S. snuff brands is most likely due to modifications of the materials used for packaging since most snuff products today come in plastic containers that have no wax coatings.

The major reductions of the levels of the carcinogenic NDELA and NMOR (Preussmann and Stewart, 1984) in U.S. tobacco products have been achieved by changes in the cultivation of tobacco and/or packaging methods. Methods should also be developed to achieve a substantial reduction in tobacco of the precursors for volatile nitrosamines, proteins, and the tobacco-specific nitrosamines to reduce levels of carcinogens in smokeless tobacco and in tobacco smoke.

ABBREVIATIONS USED

NDELA, *N*-nitrosodiethanolamine; NMOR, *N*-nitrosomorpholine; 2-HOPNA, *N*,*N*-bis(2-hydroxypropyl)nitrosamine; MH-30, maleic hydrazide–diethanolamine; GC–TEA, gas chromatography–thermal energy analyzer.

ACKNOWLEDGMENT

This study was supported by Grant CA-29580 from the U.S. National Cancer Institute. The paper is No. XC of "Chemical Studies on Tobacco Smoke".

LITERATURE CITED

Brunnemann, K. D.; Hoffmann, D. Assessment of the carcinogenic N-nitrosodiethanolamine in tobacco products and tobacco smoke. *Carcinogenesis* **1981**, *2*, 1123–1127.

Brunnemann, K. D.; Scott, J. C.; Hoffmann, D. N-Nitrosomorpholine and other volatile N-nitrosamines in snuff tobacco. *Carcinogenesis* **1982**, *3*, 693–696.

Burton, H. R.; Bush, L. P.; Djordjevic, M. V. Influence of temperature and humidity on the accumulation of tobacco-specific nitrosamines in stored burley tobacco. *J. Agric. Food Chem.* **1989**, *37*, 1372–1377.

Hecht, S. S.; Hoffmann, D. The relevance of tobacco-specific nitrosamines to human cancer. *Cancer Surv.* **1989**, *8*, 273–294.

Hoffmann, D.; Brunnemann, K. D.; Adams, J. D.; Hecht, S. S. Formation and analysis of *N*-nitrosamines in tobacco products and their endogenous formation in tobacco consumers. *IARC Sci. Publ.* **1984**, *57*, 743–762.

Hoffmann, D.; Adams, J. D.; Lisk, D.; Fisenne, I.; Brunnemann, K. D. Toxic and carcinogenic agents in dry and moist snuff. *J. Natl. Cancer Inst.* **1987**, *79*, 1281–1286.

Hoffmann, D.; Djordjevic, M. V.; Brunnemann, K. D. New brands of oral snuff. *Food Chem. Toxicol.* **1990**, in press.

IARC. Overall Evaluations of Carcinogenicity. An Updating of IARC Monographs 1–42. *IARC Monogr., Suppl.* **1987**, *7*, 40–74.

Preussmann, R.; Stewart, B. W. N-Nitroso carcinogens. In *Chemical Carcinogens*; Searle, C. E., Ed.; ACS Monograph 182; American Chemical Society: Washington, DC, 1984; 182, pp 643–828.

U.S. Environmental Protection Agency. Maleic hydrazide. Notification of issuance of intent to suspend pesticideregistrations. *Fed. Regist.* **1981**, *46*, (179), 45999–46000.

U.S. International Trade Commission. *Synthetic Organic Chemicals. United States Production and Sales*; Publication No. 2219; U.S. Government Printing Office: Washington, DC, 1989; p 15.

von Bethmann, M.; Lipp, G.; van Nooy, H. *Beitr. Tabakforsch.* **1961**, *1*, 19–29.

Received for review June 22, 1990. Accepted August 15, 1990.

Registry No. NDELA, 1116-54-7; NMOR, 59-89-2